Chemical Pulping 1
The influence of xylan on the sensitivity towards fiber damage

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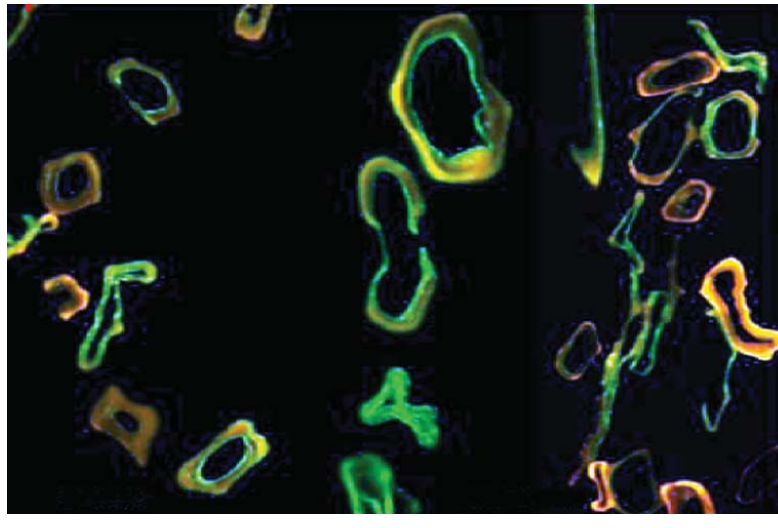

Intern rapport nr 2 (begränsad spridning)

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 determine if the presence and position of xylan in the fiber wall are of importance for the degree of damage introduced into fibers during mechanical action in the cook. Kraft pulps from spruce with different amounts of xylan have been produced in the laboratory, either by adding birch xylan in different positions in the cook or by redistribution of spruce xylan. At the end of the cook, fiber damages were introduced by subjecting the fibers to shear and compression forces.

The extra birch xylan had adsorbed on the fiber surfaces, the outer fiber walls (presumably S_1/primary wall) as well as on the fiber cell lumen wall. Xylan penetration into the fiber wall was very low. A large variation in coverage of surface xylan within the fibers and between fibers was noted. No significant difference between pulps produced in the different ways or between the pulps produced with or without mechanical treatment could however be observed.

The extra xylan added resulted as expected in an improved tensile strength development for these pulps.

No direct indications were seen that the extra xylan added during the cook resulted in a lower amount of introduced damaged areas. But some positive tendencies could be noted for the pulps produced with extra xylan added including:

- a lower kink/mm and lower amount of cleavage/fiber measured by the HCL method; and
- the zero-span level and tear-tensile relationship were not inferior compared to the reference despite the higher xylan content.

The removal and subsequent re-introduction of xylan into the cook seemed to negatively influence the strength properties, i.e. the tear-tensile relationship was inferior compared to the reference pulp. The redistribution procedure may have drained the fiber wall of xylan negatively influencing the strength properties.

Contents

1 Background

Increased pulp yield is desirable partly because of economical reasons and partly because of some physical properties, i.e. tensile strength properties are improved by increased pulp yield. However, whether an increased yield could also contribute to an improved resistant towards mechanical damage has not yet been determined.

Increased pulp yield can be accomplished by optimal use of existing xylan in the wood by for example optimization of cooking conditions, by redistribution of liquors rich in xylan during the cook and by adding xylan from an external source.

The aim with this study was to determine if the presence of xylan and the position of the xylan in the fiber wall are of importance for the degree of damage introduced in the 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 cook process or by redistribution of spruce xylan. At the end of the cook, fiber damages were introduced by subjecting the fibers to shear and compression forces.

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

Four different pulp types were produced in the laboratory. The different variants are described in *Table 1*. Birch xylan was used to introduce extra xylan either in the beginning or at the end of the cook in two pulps. The birch xylan used was obtained from Sigma Aldrich. Carbohydrate composition, molecule weight and degree of substitution for the birch xylan are shown in *Appendix 1A*. The concentration of birch xylan was 10 g/L in the liquor when added. In the case of redistribution of spruce xylan, a xylan rich liquor was withdrawn early in the cook and added back at the same concentration at a later stage. 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 described by Salmén and Lundqvist (Salmén & Lundqvist 2009). All four pulp types were produced with and without mechanical damage at the end of the cook (*Table 1*).

Table 1. Pulp variants included in the study

	Without mechanical damage introduced	**With mechanical damage introduced**
	Description	
Reference	Ref-N	Ref-S
Extra xylan added early in the cook	XY-0-N	XY-0-S
Extra xylan added late in the cook	XY-late-N	XY-late-S
Xylan removed early in the cook, added back late in the cook	PR-N	PR-S

The cooking conditions used are shown in *Table 2.* All pulps were cooked to about the same kappa number of around 30. For each pulp type, exactly the same conditions have been used for the pulp produced with- and without mechanical damage, i.e. half of the pulp in the digester was subjected to mechanical forces and half was not. To obtain enough pulp, four cooks were performed and merged. Primary data for all cooks are shown in *Appendix 1B*. Most of the analysis on the pulps were performed on never-dried pulps, however part of the produced pulps were dried prior to analysis. The drying procedure was done at Smurfit Kappa Kraftliner and the procedure is outlined in *Appendix 1C*.

Table 2. Cooking conditions

	Point of time[1]	Amount of xylan added, g/L	Compr, %	EA, %	H-factor	Cooking time[2] min	Residual OH- g/L	Kappa no N[3], S[4]
Ref	-	-	47	21	2000	197	4,0	30.1, 30.1
XY-0	0	10	45	21	2000	197	3,8	30.9, 30.1
XY-late	148	10	45	21	2000	197	3,8	29.0, 28.7
PR	40, 162	-	45	21	2150	211	3,9	31.9, 31.4

[1]*for withdrawal or addition of xylan*
[2]*at 165 ºC*
[3]*N cooks without mechanical treatment*
[4]*S cooks with compression and shearing forces during 2 minutes, 15 minutes before end of cook*
All data are average values from 4 cooks

The four pulps produced with mechanical treatment introduced at the end of the cook were further bleached in a DEDED-sequence and evaluated regarding composition, bulk and surface charge and fiber and strength properties as never-dried. Bleaching conditions in the DEDED-sequence are shown in *Table 3*.

Table 3. Bleaching conditions used in the DEDED-sequence

Stage	Conc. %	Time, min	Temp, ºC
D0	10	45	60
E	10	90	60
D	10	180	70
E	10	90	60
D	10	180	75

2.1 ANALYSIS

The chemical composition was analyzed on never-dried unbleached and bleached pulps after acidic hydrolysis and HPLC-analysis using electrochemical detection. The analyses were performed by Stora Enso.

Bulk and surface charge analysis were performed by Stora Enso on never-dried unbleached and bleached pulps.

Surface composition was analyzed on surface material produced during mechanical peeling in a disintegrator after 200 000 revolutions at 3% pulp concentration. More details on the procedure can be found in *Appendix 1D*. These analyses were performed by Södra on unbleached never dried pulps.

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 on the unbleached and bleached pulps as never-dried and on the unbleached pulps after being dried. 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 on whole fibers and on fiber cross-sections was analyzed using immunofluorescence microscopy. More details can be found in *Appendix 3*.

Visualization of adsorbed xylan on whole fibers was also done with SEM. More details can be found in *Appendix 4*. Distribution of xylan on fiber surfaces and overall composition was analyzed with FTIR microscopy. More details can be found in *Appendix 5*.

Measurements on the number of weak points in fibers were analyzed using the HCl-method. More details are found in *Appendix 6*.

3 Results and discussion

The aim of the study was to investigate if xylan could make the fibers less sensitive towards mechanical damage introduced during cooking and if the position of the xylan in the fiber wall was of importance.

Pulps with different amounts of xylan were produced in the laboratory by adding extra birch xylan or by redistribution of spruce xylan. At the end of the cook, fiber damages were introduced to half of the pulp by subjecting the fibers to a combination of shear and compression forces.

3.1 INVESTIGATED PULPS

Results after cooking are shown in *Table 4*. All pulps had about the same kappa number of around 30. Pulps produced with mechanical treatment at the end of the cook (S) had a higher dry content compared to their respective pulps produced without mechanical treatment (N), as analyzed after a standardized centrifugation. The yield for the reference pulp and pulp produced with redistribution of the xylan during the cook were about 48%. The two pulps produced with addition of extra birch xylan, either early or late in the cook had yields of 50.2 and 49.5% respectively (*Table 4*).

Table 4. Cooking results

		N		S			
	Residual OH- g/L	**Kappa no**	**Dry content %**	**Kappa no**	**Dry content %**	**Shives %**	**Tot. yield %**
Ref	4.0	30.1	25.8	30.1	31.4	1.7	48.1
XY-0	3.8	30.9	25.0	30.1	30.2	2.5	50.2
XY-late	3.8	29.0	24.6	28.7	30.3	1.8	49.5
PR	3.9	31.9	25.0	31.4	31.5	1.7	47.9

3.2 XYLAN DISTRIBUTION WITHIN THE FIBER WALL; VISUALIZATION

To determine if and where the xylan had been adsorbed and whether it had penetrated the fiber wall, visualization of adsorbed xylan were done on cross sections of fibers with fluorescence labeling. Indications were given that xylan had adsorbed on the outer fiber walls (presumable S_1/primary wall) as well as on the fiber cell lumen wall. The xylan had not penetrated deep, but were seen on the surfaces. The adsorption pattern were the same on both XY-0 (-N and -S) and on XY-late (-N and -S) and on both early- and late wood fibers. No conclusions on difference between those four pulps could be drawn. With this method primarily birch xylan was detected. Areas with

adsorbed birch xylan is green (*Figure 1)*. More results and more details from this study can be found in *Appendix 3*.

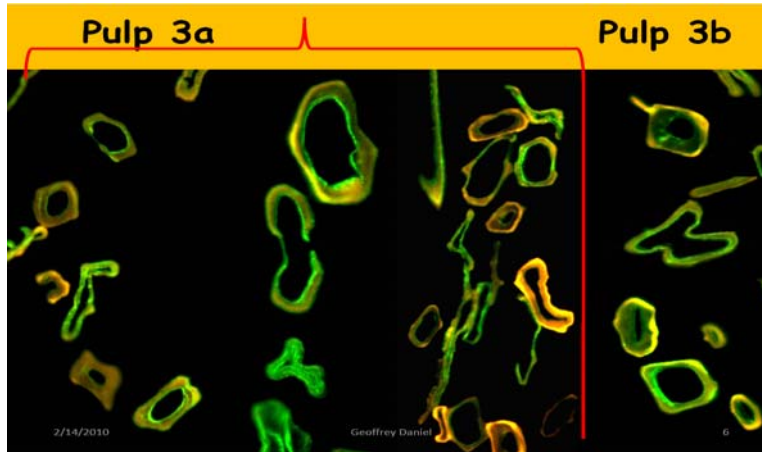

Figure 1. Visualization of adsorbed xylan for pulp XY-late-N (3a) and XY-late-S (3b).

To determine if there were any differences in distribution of the xylan on the surface between the different pulps, visualization of adsorbed xylan were done on whole fibers. As was seen from the cross section-analyses, xylan was adsorbed on the surfaces of all 4 pulps treated with birch xylan. However, the variability on the surface coverage was considerable between fibers in the same pulp sample. This was observed both between latewood and earlywood and between morphological structures on the fiber surfaces. Latewood fibers seem to have less absorbed xylan on the surfaces. No differences between the pulps produced without and with mechanical treatment could be seen with the fluorescence labeling technique. It is indicated that xylan once adsorbed on the surface seems to remain bound.

Also analysis with SEM and FTIR shows that the surface coverage of xylan differs considerably between fibers and that the xylan coverage is not evenly distributed along the fiber (more results and more details from these studies can be found in *Appendix 4* and *5* respectively). Areas with high and low xylan content could not be clearly connected to any structure seen in the microscope. FTIR images however, indicate that the mechanically treated pulps had more xylan compared to the non mechanically treated pulps. Also, the earlywood fiber surfaces had in general more xylan than the latewood fibers surfaces, consistent with the results with fluorescence labeling. The

differences between the pulps were only indications; the differences were not statistically significant.

The xylan seems to be distributed as small aggregates on the surfaces when analyzed with FTIR. However, conventional SEM observations did not reveal the adsorbed xylan as aggregates on the surfaces. Instead the surfaces of all pulps seemed to have a very smooth structure, which could indicate that any xylan present on the surface will be found as bound in the pores of the outer fiber cell wall. Also with SEM the morphological appearances were very similar between all pulps and no morphological effects from the mechanical treatment on the fiber structure were apparent.

3.3 CARBOHYDRATE COMPOSITION AND FIBER CHARGES

The content of xylan was about the same in the reference pulp and the pulp with redistributed xylan (*Table 5*). The pulp with extra xylan added early in the cook has somewhat higher content than the pulp with extra xylan added late in the cook, presumably due to a longer adsorption time. No difference in total amount of xylan could be seen between respective pulps produced with and without mechanical treatment. However, when comparing the amount of surface xylan, pulp produced with mechanical treatment had a lower amount compared to the pulp without mechanical treatment (*Table 5*). Tables with complete carbohydrate compositions can be found in *Appendix 1E*.

Table 5. Summary of total xylan content in the lab. pulps, fiber charges, as well as surface xylan content analyzed on the removed material from mechanical surface peeling. Tables with complete carbohydrate compositions can be found in Appendix 1E

		Ref		XY-0		XY-late		PR	
		N	S	N	S	N	S	N	S
Xylan	Rel %	8.2	8.1	12.1	12.2	10.6	11.1	8.5	8.4
Tot. acid groups	mmole/kg	95	92	108	104	107	103	94	90
Surf. Charge (neg)	meqv/kg	8.9	7.2	8.0	7.3	9.2	10.6	9.4	8.6
Surface xylan	Rel %	16.9	13.6	21.4	20.8	22.2	21.2	19.3	16.1

The pulps with higher amounts of surface xylan seem to loose less xylan during the shearing and compression forces of the mechanical treatment compared to Ref- and PR-pulps (*Table 5*). This could be interpreted as fibers with higher xylan or surface xylan withstand the mechanical forces to a greater extent. The same trend can be seen when comparing the surface charge of the pulps produced with and without mechanical treatment. The pulps subjected to mechanical treatment had a lower surface charge (with

one exception, XY-late). The pulp with extra xylan added late in the cook compared to early and the pulp with redistributed xylan compared to the reference pulp had a higher content of surface xylan and surface charge.

The same relative trend regarding xylan content and charge as for the unbleached pulps was also shown with the pulps after bleaching. The carbohydrate content and charge analyzed for the bleached pulps as well as the bleaching results for the different pulps can be found in *Appendix 1F*.

Quantification of adsorbed amount of xylan was done with ELISA, enzyme-linked immunosorbent assay (ELISA). ELISA indirectly quantifies the amount of xylan present on fiber surfaces of a relatively large number of fibers (about 1.7 mg). Results showed that the pulps could be divided into three groups (*Figure 2)*. The pulps XY-late-S and XY-0-S having the highest amount followed by those pulps cooked without mechanical treatment (XY-late-N and XY-0-N). According to this analysis, the mechanical shearing and compression forces seem to open up the fiber wall. More details from the quantification of xylan with ELISA can be found in *Appendix 2*.

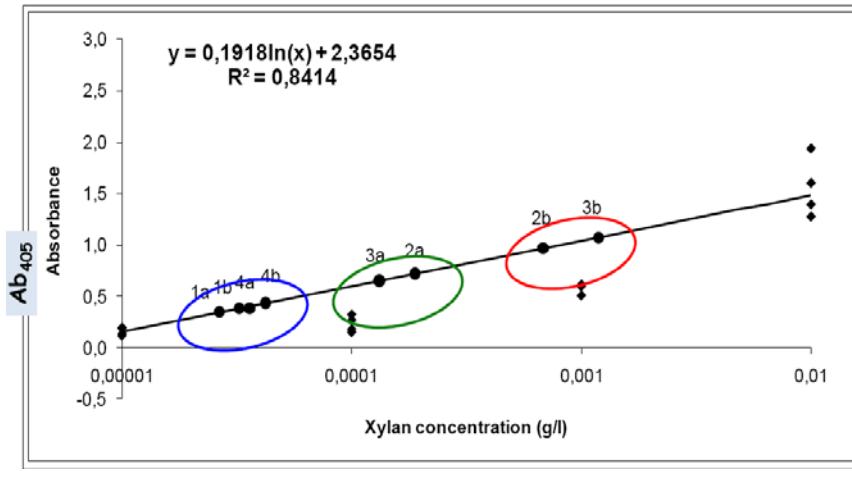

Figure 2. Xylan concentration estimated with ELISA for the investigated pulps.

3.4 INTRODUCTION OF FIBER DAMAGE

The mechanical treatment introduced kinks to about the same extent for all pulps. In *Table 6* the data for the unbeaten pulps are shown. A tendency was however, that pulps with increased xylan content (both pulps produced with and without mechanical treatment) showed a lower amount of kink/mm (*Figure 3*). In *Figure 4* the change in shape factor with increased refining is showed. All pulps had very straight fibers. Normally when the fibers are damaged the shape factor increases during PFI-refining. In *Appendix 1G* FiberMaster data for all pulps after beating is shown.

Table 6. Fiber length, width, shape factor (1.5-3.0 mm) and kink/mm for the investigated pulps after cook (unbeaten pulp)

		Ref		XY-0		XY-late		PR	
		N	S	N	S	N	S	N	S
Fiber length	mm	2.43	2.42	2.36	2.44	2.40	2.42	2.37	2.40
Fiber width	µm	32.6	31.0	32.6	31.8	33.0	31.4	32.6	30.4
Shape factor	%	92.6	91.8	93.6	92.6	93.6	92.5	93.3	92.2
Kink	no/mm	0.08	0.16	0.06	0.13	0.06	0.14	0.06	0.15

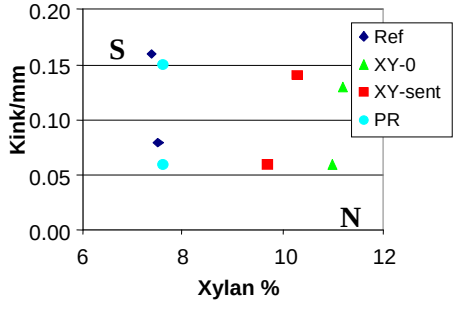

Figure 3a. Kink/mm vs total xylan content.

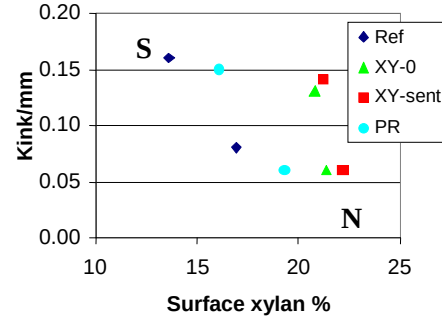

Figure 3b. Kink/mm vs surface xylan content.

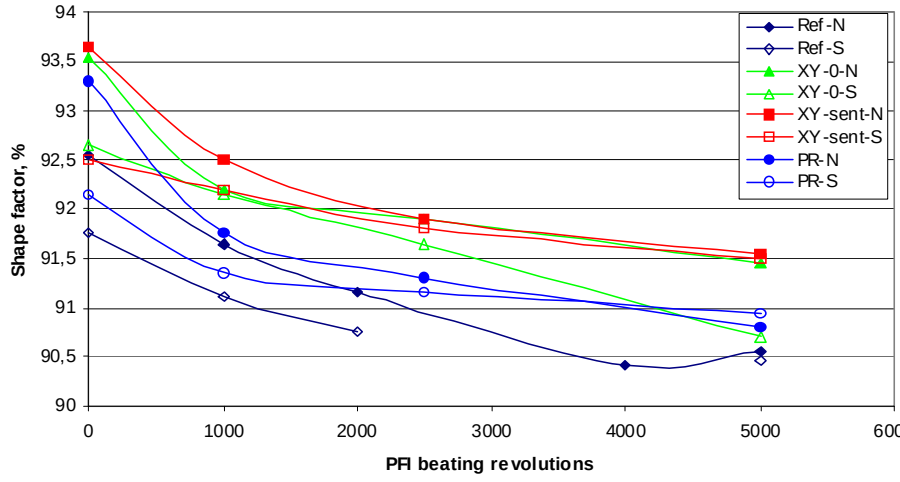

Figure 4. Shape factor vs PFI beating revolutions.

A method that cleaves fibers in damaged areas after HCl-treatment was used to quantify the amount of damaged areas and compare the different pulps. Based on the HCl-method, all four pulp types had a higher number of damaged areas after being subjected to mechanical treatment at the end of the cook as expressed as number of cleavages per fiber (*Table 7*). With non mechanical treated pulps, the amount of cleavage/fiber is lower with increased xylan content (*Figure 5a* and *b*) when compared with both total xylan and surface content of xylan. For pulps subjected to mechanical treatment, only the pulp with xylan added early in the cook had a lower amount of cleavage/fiber compared to the reference pulp and the other two pulps had a higher number. This could indicate that higher xylan content in the fiber wall could protect against damage.

Table 7. Length weighted fiber lengths (LWFL) and cleavage per fiber by HCl (standard method: 4h at 80 °C + 30 min final cleavage)

		Ref		XY-0		XY-late		PR	
		N	S	N	S	N	S	N	S
LWFL H2O	mm	2.48	2.49	2.45	2.43	2.38	2.50	2.41	2.46
LWFL after HCl	mm	1.36	0.94	1.56	1.04	1.44	0.85	1.34	0.85
Cleavage/fiber		0.82	1.64	0.56	1.35	0.65	1.96	0.80	1.91

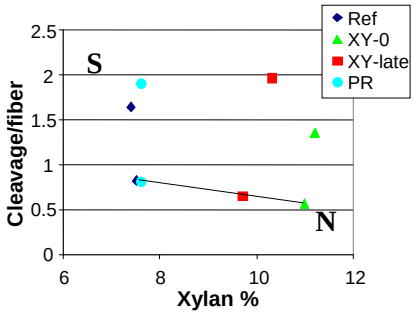

Figure 5a. Cleavage/fiber vs total xylan content.

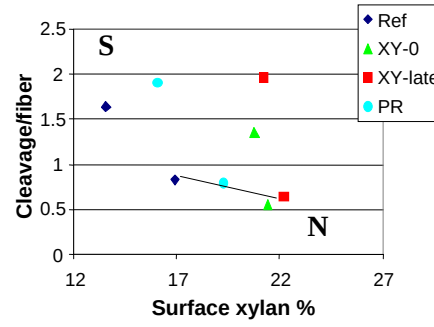

Figure 5b. Cleavage/fiber vs surface xylan content.

The zero-span level was slightly higher for pulps with extra birch xylan added in the range of 197-200 kNm/kg when compared to the other two unbleached never-dried pulps produced without mechanical treatment (i.e. 193-195 kNm/kg) and on the same or lower level when compared after mechanical treatment (*Figure 6a* and *Table 8*). From the zero-span measurements, the xylan does not seem to provide protection against fiber damage since the decrease in zero-span is somewhat higher due to the mechanical treatment for pulps with higher xylan content. The same pattern was seen when the pulps are compared after drying, (*Figure 6b* and *Table 9*). In *Appendix 1F* all zero-span values are shown.

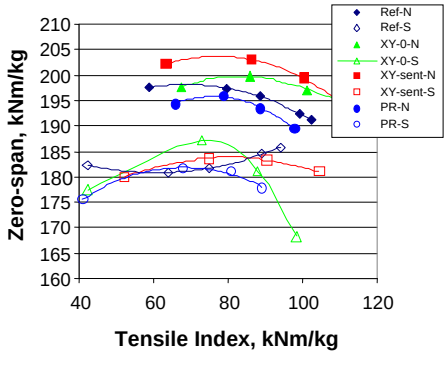

Figure 6a. Zero-span vs tensile index for never dried pulps.

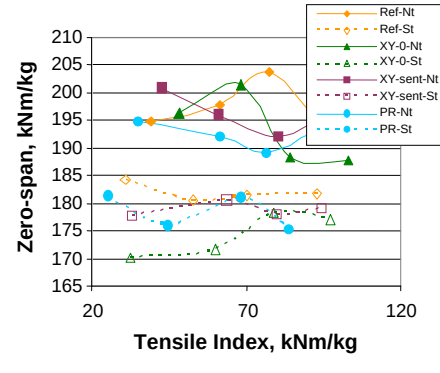

Figure 6b. Zero-span vs tensile index for dried pulps.

Table 8. Zero-span average values for never-dried pulp and change in zero-span values due to mechanical treatment

n.d.	Ref		XY-0		XY-late		PR	
	N	S	N	S	N	S	N	S
Average	195	180	197	173	200	182	193	179
Change N-S, abs		15		24		18		14
Change N-S, %		7.4		12.1		8.8		7.4

Table 9. Zero-span average values for dried pulp and change in zero-span values due to mechanical treatment

	Ref		XY-0		XY-late		PR	
Beating rev.	**N**	**S**	**N**	**S**	**N**	**S**	**N**	**S**
Average	197	181.8	193.4	174.3	196.4	178.8	192.2	178.4
Change N-S, abs		15.2		19.1		17.6		13.8
Change N-S, %		7.7		9.8		8.9		7.2

The tear-tensile relationships for never-dried and dried pulps with- and without mechanical treatment are shown in *Figure 7* and *Table 10*. The never-dried pulps not exposed to mechanical treatment, were all on the same tear index level when compared at tensile index 90. For pulps with higher xylan content (XY-0 and XY-late), the drop in tear index level was slightly higher due to the mechanical treatment. The pulp produced with redistribution of xylan during the cook (PR) had even lower tear index due to the mechanical treatment compared to the reference pulp. For the dried pulps, the reference pulp had the highest tear index when not exposed to mechanical treatment. After mechanical treatment the Ref-S, XY-0-S and XY-late-S are on the same level. Again the PR-pulp was the pulp with lowest tear index-level at tensile 90.

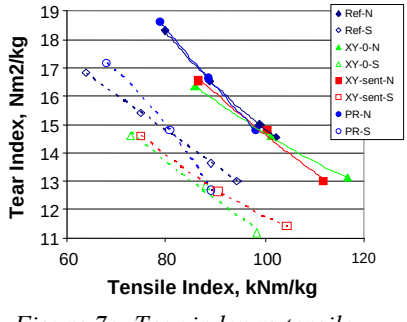

Figure 7a. Tear index vs tensile index for never-dried pulps.

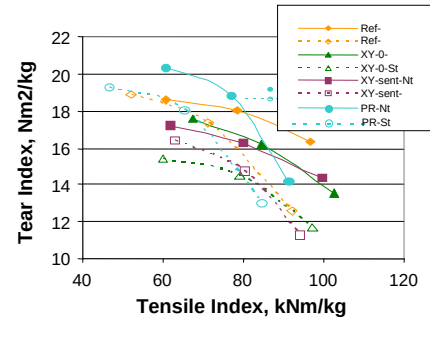

Figure 7b. Tear index vs tensile index for dried pulps.

Table 10. Interpolated tear index values at tensile index 90 for never dried pulp and change in tear@tensile90 due to mechanical treatment

n.d.	Ref		XY-0		XY-late		PR	
	N	S	N	S	N	S	N	S
Tear@tensile 90	16.4	13.5	15.8	12.7	15.6	12.7	16.4	12.6
Change N-S, abs		2.9		3.1		2.9		4.0
Change N-S, %		17.7		19.6		18.6		24.4

Table 11. Interpolated tear index values at tensile index 90 for dried pulp and change in tear@tensile90 due to mechanical treatment

dried	Ref		XY-0		XY-late		PR	
	N	S	N	S	N	S	N	S
Tear@tensile 90	17.0	13.2	15.6	12.9	15.6	12.4	14.6	11.4
Change N-S, abs		3.8		2.7		3.2		3.2
Change N-S, %		22.4		17.3		20.5		21.9

The extra xylan added during the cook contributed as expected to a better tensile strength development for these pulps (*Figure 8*) both in pulps with and without mechanical treatment during the cook. The same trends were seen for the dried pulps. All strength data are shown in *Appendix 1G*.

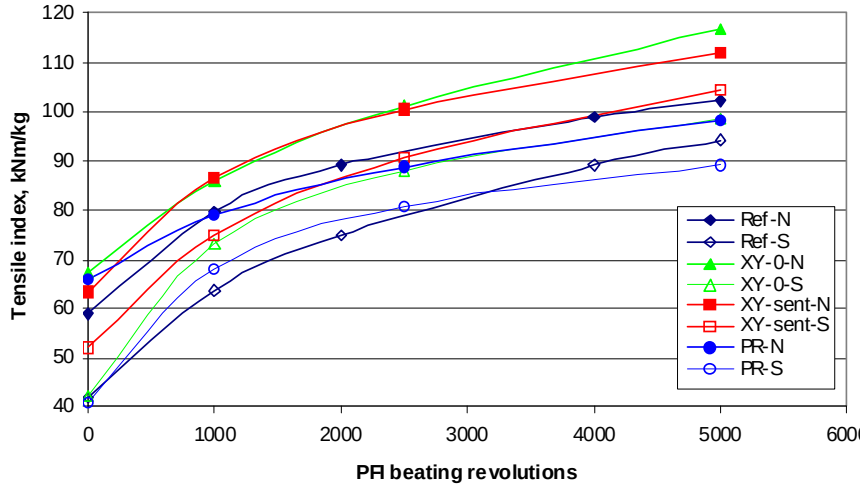

Figure 8. Tensile index vs PFI beating revolutions for never-dried pulps.

4 Conclusions

Visualization methods of surface xylan showed large variation in surface coverage within the fibers and between fibers. No significant differences between pulps produced in the different ways or between the pulps produced with or without mechanical treatment could however be seen.

The extra birch xylan had adsorbed on the outer fiber walls (presumably S_1/primary wall) as well as on the fiber cell lumen wall. Penetration into the fiber wall was very low.

The extra xylan added resulted as expected in an improved tensile strength development for these pulps.

No direct indications were seen that the extra xylan added during the cook resulted in a lower amount of introduced damaged areas. But some positive tendencies could be noted for the pulps produced with extra xylan added including a lower kink/mm and a lower amount of cleavage/fiber measured by the HCl-method. The zero-span level and the tear-tensile index relationship were not inferior compared to the reference, despite the higher xylan content.

The redistribution of xylan seemed to negatively influence the strength properties, i.e. the tear-tensile relationship was inferior compared to the reference pulp. The redistribution procedure may have drained the fiber wall of xylan, negatively influencing the strength properties.

5 Recommendation

Investigate if extra xylan introduced during oxygen delignification or bleaching can contribute to make the fibers less sensitive towards mechanical treatment, i.e. in stages when the fiber structure is more open and the xylan may penetrate into the fiber wall to a greater extent.

Further investigate the negative influence of xylan removal during cooking on the fiber sensitiveness towards mechanical damage and the negative influence on strength properties.

6 References

Salmén, L. and *Lundqvist, F.* (2009): Effects of mechanical forces for strength delivery in kraft cooking, ISWFPC, Oslo, Norway.

7 Appendices

APPENDIX 1A. ANALYSIS ON THE BIRCH XYLAN

Relative carbohydrate composition

Xyl	Glc	Man	Ara	HexA	Gal	4OMeGlcA	GlcA	Totalt
89,2	1,5	1,5	0,1	1,3	0,5	5,8	0,1	100,0

Absolute carbohydrate composition

Xyl	Glc	Man	Ara	HexA	Gal	4OMeGlcA	GlcA	Totalt
76,0	1,3	1,3	0,1	1,1	0,5	5,0	0,1	85,2

Mn	**Mw**	**Mw/Mn**
6000	6900	1.2

DS
4.6 : 100 4-O-MeGlcA : Xyl

APPENDIX 1B. COOKING CONDITIONS

Cook		Point of time[1]	Amount of xylan added, g/l	Compression, %	EA, %	H-factor	Cooking time at 165°C, min	Residual OH, g/l	Kappano top sheared	Pulp ad, g	Dry content % top sheared	Kappano bottom ref	Pulp ad, g	Dry content % bottom ref	Tot shive %	Tot yield %
CR.1	Ref		-	45	20	1800	177	3,7	37,0	117,0	31,0	37,1	111	25,1	3,8	49,39
CR.2	Ref		-	45	21	2000	197	3,8	31,1	123	31,8	30,6	112	26,4	1,6	48,57
CR.3	Ref		-	47	21	2000	197	4,1	30,3	142	30,6	29,4	88	25,0	1,8	47,95
CR.4	Ref		-	49	21	2000	197	4,2	29,4	132	31,3	30,1	97	25,8	1,9	47,83
CR.5	Ref		-	47	21	2000	197	3,9	29,7	121	31,8	30,3	112	26,0	1,5	48,16
									Ref-S			Ref-N				
Average mixture			-	47	21	2000	197	4,0	30,1	518	31,4	30,1	409	25,8	1,7	48,1
CR.6	XY-0	0	10	45	21	2000	197	3,2	32,0	134	30,2	32,1	102	25,1	3,0	50,19
CR.7	XY-0	0	10	45	21	2000	197	4,2	30,2	130	30,8	31,5	109	25,6	2,4	50,28
CR.8	XY-0	0	10	46	21	2000	197	4,0	29,1	159	30,0	29,4	82	24,6	2,2	50,41
CR.9	XY-0	0	10	45	21	2000	197	3,6	29,1	135	30	30,7	102	24,6	2,5	50,03
									XY-0-S			XY-0-N				
Average mixture			10	45	21	2000	197	3,8	30,1	558	30,2	30,9	395	25,0	2,5	50,2
CR.10	XY-late	148	10	44	21	2000	197	4,0	29,5	129	29,2	29,8	112	24,1	1,9	50,17
CR.11	XY-late	148	10	45	21	2000	197	3,9	29,2	134	30,2	29,3	106	24,7	1,8	49,87
CR.12	XY-late	148	10	45	21	2000	197	3,8	27,9	131	30,3	28,2	105	24,8	1,7	49,10
CR.13	XY-late	148	10	45	21	2000	197	3,6	28,3	139	31,5	28,5	96	24,6	1,7	48,84
									XY-late-S			XY-late_N				
Average mixture			10	45	21	2000	197	3,8	28,7	533	30,3	29,0	419	24,6	1,8	49,5
CR.14	PR	40 and 148		45	21	2000	197	3,9	31,0	135	32,3	32,8	97	25,2	1,9	48,4
CR.15	PR	40 and 148		45	21	2000	197	3,7	34,6	120	31,2	33,9	112	25,3	2,4	48,69
CR.16	PR	40 and 167		45	21	2200	216	3,6	31,7	132	30,7	31,7	98	24,2	1,7	47,85
CR.17	PR	40 and 167			21	interrupted due to leakage										
CR.18	PR	40 and 167		45	21	2200	216	3,3	33,8	136	30,9	34,1	92	24,8	2,3	47,98
CR.19	PR	40 and 167		45	21	2200	216	3,5	31,7	144	31,1	32,7	89	25,3	1,3	47,90
CR.20	PR	40 and 167			21	interrupted due to leakage										
CR.21	PR	40 and 167		45	21	2200	216	4,6	31,3	113	31,7	30,2	114	25,5	2,1	47,52
									PR-S			PR-N				
Average mixture									31,4	524	31,5	31,9	398	25,0	1,7	47,9

[1]for withdrawel or addition of xylan

APPENDIX 1C. DRYING PROCEDURE

Tillverkning av laboratorieark

Tillverkning av laboratorieark för fysikalisk provning enligt ISO 5269:1 med följande modifieringar

* Sätt virahållaren på plats
* Stäng mäldbehållaren genom att vrida reglaget för virapress till läge "**sänk**"
* Fyll arkformen med vatten upp till en nivå minst 50 mm ovanför omröraren med knapp "**Fyllning**"
* Tillsätt den mängd mäld som motsvarar 3,47 gram torrtänkt massa, vilket ger en ytvikt av 100 g/m^2 Gäller även 60 gram ark
* Tryck på knapp "**Tömning**". Mälden rörs om och arkformen töms på vatten
* Öppna arkformen genom att vrida reglaget för virapress till läge "**höj**"
* Lossa viran med vidhängande ark och placera den på plexiglasplattan
* Placera 2 st läskark över det våta arket på viraduken
* Lägg guskplattan ovanpå läsken och vänta i 20 sekunder.
* Tag försiktigt bort läsken och provarket från viran. Kasta det översta läskarket.
* Stapla enligt följande:

upp	glansplåt - släta sidan mot ark
	labark + guskläsk
	2 torra läskpapper
ned	pressplåt

Upprepa förfarandet till lämplig höjd.

* Pressa arkstapeln i laboratoriepressen vid 0,55 MPa i 5 minuter ± 15 sek.
* Stapla på nytt med nya läsk i omvänd ordning:

upp	pressplatta
	1 läskpapper
	labark
ned	glansplåt – släta sidan mot ark

Upprepa förfarandet

* Pressa arkstapeln vid 0,55 MPa i 2 min ± 15 s.

* Lossa läskarket från labarken. Spänn in glansplåtarna, med vidhängande labark, i för ändamålet avsedda torkramar inne i pappersprovningslab. *Kolla att arken sitter väl fästa mot glansplåten!!*

* Torka arken i konditionerat utrymme till nästa dag.

* Ta loss arken från glansplåtarna och konditionera dem i 24°C och 50%RH minst 4 timmar innan provning

APPENDIX 1D. MECHANICAL PROCEDURE FOR REMOVAL OF FIBER SURFACE MATERIAL

Figure A1d gives an overview of the procedure for mechanical peeling and fractionation. The primary fines, originally present in the pulp, and the material removed from the fiber surface, (i.e. secondary fines) were separated from the pulp by a sieving procedure. A nylon sieve of size 100 µm (Bigman AB, Gothenburg) was used. The fractionation procedure was repeated three times for each pulp using the same amount of water. The procedure recommended for removal of surface material is based on mechanical peeling in a disintegrator of a consistency of 3% and at 100.000-200.000 numbers of revolutions. The fines were collected by centrifugation

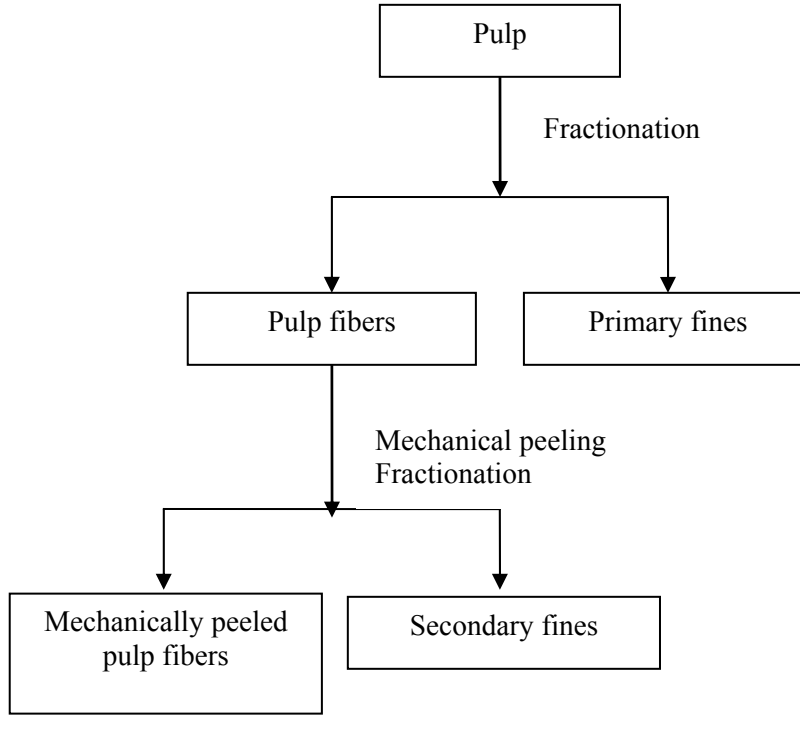

Figure A1d. Procedure for mechanical peeling and fractionation.

APPENDIX 1E. CARBOHYDRATE COMPOSITION

Carbohydrate content in the unbleached lab. pulps, as well as fiber charges

		Ref		XY-0		XY-late		PR	
		N	S	N	S	N	S	N	S
Galactos	Rel. %	0.4	0.4	0.5	0.4	0.4	0.4	0.5	0.5
Glucose	Rel. %	83.5	83.6	79.9	79.9	82.3	81.1	83.0	83.2
Mannose	Rel. %	7.1	7.2	6.9	6.9	6.1	6.8	7.3	7.3
Arabinos	Rel. %	0.7	0.7	0.6	0.6	0.6	0.6	0.8	0.7
Xylos	Rel. %	8.2	8.1	12.1	12.2	10.6	11.1	8.5	8.4
Galactos anhydro	%	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Glucose anhydro	%	77.9	78.7	74.3	75.1	77.1	76.7	76.0	77.1
Mannose	%	6.6	6.7	6.4	6.5	5.7	6.4	6.7	6.8
Arabinos anhydro	%	0.7	0.7	0.6	0.5	0.6	0.6	0.7	0.7
Xylos anhydro	%	7.5	7.4	11.0	11.2	9.7	10.3	7.6	7.6
Tot anhydo sugar	%	93.1	93.9	92.7	93.7	93.5	94.3	91.4	92.5
Hydrorest	%	4.3	4.2	4.9	4.1	5.2	3.4	4.9	5.5
Tot acid groups	mmole/kg	95	92	108	104	107	103	94	90
Surf. Charge (neg)	meqv/kg	8.9	7.2	8.0	7.3	9.2	10.6	9.4	8.6

Surface carbohydrate composition, rel amount of anhydrosugar, %, analyzed on the removed material from mechanical surface peeling

		Ref		XY-0		XY-late		PR	
		N	S	N	S	N	S	N	S
Galactos anhydro	%	1.3	1.1	1.1	1.0	1.3	1.0	1.2	1.1
Glucose anhydro	%	74.1	77.7	70.5	71.1	69.6	70.6	71.6	75.0
Mannose	%	6.3	6.4	5.9	6.2	5.8	6.2	6.4	6.5
Arabinos anhydro	%	1.4	1.2	1.1	0.9	1.1	1.0	1.6	1.3
Xylos anhydro	%	16.9	13.6	21.4	20.8	22.2	21.2	19.3	16.1
Tot anhydo sugar	%	100	100	100	100	100	100	100.1	100

APPENDIX 1F. BLEACHING RESULTS, CARBOHYDRATE COMPOSITION

Bleaching results after DEDED-bleaching

		Ref-S	XY-0-S	XY-late-S	PR-S
	K no	31.4	30.2	28.7	31.6
D0	Charge, kg ClO2/t	23.9	23.0	21.8	24.0
	Final-pH	1.7	1.6	1.6	1.5
	Cons., kg ClO2/t	23.9	23.0	21.8	24.0
E1	Charge, kg NaOH/t	13	13	13	13
	Final-pH	11.0	10.7	10.9	11.1
	Kappa no	6.0	5.8	6.5	6.7
D1	Charge, kg ClO2/t	11.8	11.4	13.2	14.2
	Final-pH	2.6	2.6	2.4	2.6
	Cons., kg ClO2/t	11.6	11.3	13.1	13.9
E2	Charge, kg NaOH/t	5.0	5.0	5.0	5.0
	Final-pH	11.2	11.0	10.9	11.2
D3	Charge, kg ClO2/t	5.9	5.7	6.6	7.1
	Final-pH	3.2	3.5	3.2	3.5
	Cons., kg ClO2/t	3.9	4.1	4.4	4.9
	Brightness, % ISO	90.1	89.8	90.1	90.6

Carbohydrate content in the bleached laboratory pulps as well as fiber charges

		Ref-S	XY-0-S	XY-late-S	PR-S
Galactos	Rel. %	0.3	0.3	0.3	0.3
Glucose	Rel. %	84.3	80.4	81.6	84.2
Mannose	Rel. %	6.9	6.7	6.7	6.8
Arabinos	Rel. %	0.5	0.4	0.4	0.5
Xylos	Rel. %	8.0	11.9	11.0	8.2
Galactos anhydro	%	0.3	0.3	0.3	0.3
Glucose anhydro	%	81.5	78.4	79.1	81.1
Mannose	%	6.7	6.5	6.5	6.6
Arabinos anhydro	%	0.5	0.4	0.4	0.4
Xylos anhydro	%	7.6	11.3	10.5	7.7
Tot anhydo sugar	%	96.5	96.8	96.6	96.1
Hydroresidual	%	<0.05	<0.05	<0.05	<0.05
Tot acid groups	mmole/kg	50	55	61	50
Surf. Charge (neg)	meqv/kg	7.2	83	10.0	8.0

APPENDIX 1G. FIBERMASTER RESULTS, UNBLEACHED AND BLEACHED PULPS

Unbleached, never dried pulps

	Beat rev	Fiber lenght mm	Fiber width µm	Shape factor %	Kink angle °	Kink/mm	Kink/fiber	Mean segment
Ref-N	0	2,43	32,55	92,55	53,26	0,08	0,15	2,310
	1000	2,39	31,95	91,65	52,99	0,11	0,20	2,236
	2000	2,38	32	91,15	53,30	0,12	0,24	2,210
	4000	2,34	32,45	90,4	54,74	0,15	0,28	2,153
	5000	2,36	32,55	90,55	54,09	0,15	0,28	2,168
Ref-S	0	2,42	31	91,75	51,99	0,16	0,30	2,176
	1000	2,42	31,05	91,1	50,93	0,15	0,29	2,159
	2000	2,38	31,45	90,75	52,23	0,16	0,30	2,173
	4000							
	5000	2,36	31,9	90,45	54,01	0,16	0,30	2,190
XY-0-N	0	2,36	32,65	93,55	52,78	0,06	0,12	2,277
	1000	2,34	32,55	92,2	53,44	0,09	0,17	2,208
	2500	2,32	32,7	91,9	55,05	0,10	0,18	2,211
	5000	2,33	33,5	91,45	55,31	0,11	0,20	2,206
XY-0-S	0	2,44	31,75	92,65	51,61	0,13	0,26	2,238
	1000	2,41	31,7	92,15	51,23	0,12	0,21	2,256
	2500	2,39	32,35	91,65	52,69	0,11	0,22	2,248
	5000	2,35	32,9	90,7	53,92	0,14	0,27	2,170
XY-late-N	0	2,40	33	93,65	53,03	0,06	0,11	2,320
	1000	2,38	32,65	92,5	53,25	0,08	0,16	2,260
	2500	2,39	32,75	91,9	55,05	0,09	0,17	2,253
	5000	2,36	33	91,55	56,98	0,10	0,19	2,234
XY-late-S	0	2,42	31,45	92,5	52,08	0,14	0,27	2,210
	1000	2,38	31,35	92,2	51,17	0,11	0,21	2,227
	2500	2,37	31,9	91,8	53,54	0,11	0,20	2,232
	5000	2,36	32,35	91,5	54,64	0,12	0,22	2,215
PR-N	0	2,37	32,55	93,3	52,72	0,06	0,12	2,275
	1000	2,34	32,2	91,75	52,26	0,11	0,20	2,186
	2500	2,34	32,25	91,3	54,29	0,11	0,21	2,19
	5000	2,31	32,6	90,8	54,48	0,14	0,25	2,139
PR-S	0	2,40	30,75	92,15	50,84	0,15	0,30	2,173
	1000	2,36	30,75	91,35	51,61	0,15	0,28	2,147
	2500	2,35	31,15	91,15	52,63	0,14	0,27	2,190
	5000	2,34	31,75	90,95	54,09	0,14	0,26	2,181

Unbleached, dried pulps

	Beating rev	Fiber lenght mm	Fiber width µm	Shape factor %	Kink angle °	Kink/mm	Kink/fiber	Mean segment
Ref-N	0	2,41	29,85	92,75	52,63	0,08	0,15	2,295
	1000	2,36	30,45	91,75	52,07	0,11	0,22	2,202
	2500	2,34	31,15	91,30	52,75	0,13	0,24	2,184
	5000	2,32	31,60	90,95	54,28	0,14	0,25	2,150
	0	2,45	29,85	92,05	51,32	0,13	0,25	2,240
Ref-S	1000	2,40	30,15	91,70	51,35	0,14	0,27	2,178
	2500	2,36	31,25	91,15	53,81	0,13	0,24	2,191
	5000	2,33	31,70	91,00	54,11	0,13	0,25	2,165
	0	2,37	30,75	93,30	51,76	0,07	0,12	2,281
	1000	2,33	31,25	92,00	51,81	0,10	0,19	2,201
XY-0-N	2500	2,31	31,95	91,55	52,93	0,12	0,22	2,170
	5000	2,30	32,75	91,10	53,21	0,14	0,25	2,145
	0	2,45	30,45	92,50	51,06	0,12	0,24	2,263
	1000	2,37	30,80	91,25	50,69	0,14	0,27	2,160
XY-0-S	2500	2,35	31,65	90,95	52,12	0,14	0,27	2,162
	5000	2,34	32,60	90,60	53,15	0,16	0,30	2,142
	0	2,41	30,45	93,20	52,39	0,06	0,12	2,300
	1000	2,37	31,25	91,70	52,26	0,11	0,21	2,198
XY-late-N	2500	2,33	31,75	91,30	53,68	0,12	0,23	2,158
	5000	2,34	32,45	91,05	53,65	0,13	0,24	2,180
	0	2,40	30,25	92,25	51,17	0,14	0,27	2,130
	1000	2,35	30,45	91,55	51,78	0,14	0,25	2,113
XY-late-S	2500	2,35	31,35	91,30	52,18	0,14	0,25	2,126
	5000	2,34	32,05	91,10	52,83	0,14	0,26	2,117
	0	2,37	29,75	93,00	51,30	0,08	0,14	2,229
	1000	2,33	30,45	91,15	51,28	0,14	0,26	2,084
PR-N	2500	2,28	31,05	91,00	52,23	0,15	0,27	2,050
	5000	2,29	31,75	90,70	52,86	0,16	0,28	2,054
	0	2,41	29,45	91,95	51,11	0,15	0,34	2,146
	1000	2,35	29,95	90,40	51,09	0,21	0,34	2,009
PR-S	2500	2,35	30,60	90,95	51,97	0,17	0,26	2,099
	5000	2,32	31,20	90,65	53,30	0,17	0,31	2,073

Bleached pulps

	Beating	Fiber length	Fiber width	Shape factor	Kink angle	Kink/mm	Kink/fiber
	rev	mm	μm	%	°		
Ref -S	0	2,3535	30,95	87,25	53,8035	0,3475	0,6550
	1000	2,3685	30,85	88,80	53,1630	0,2405	0,4530
	2500	2,3740	31,25	88,80	54,7060	0,2250	0,4250
XY-0-S	0	2,3625	30,90	88,45	53,6065	0,2825	0,5360
	1000	2,3560	31,15	89,60	53,3055	0,1910	0,3620
	2500	2,3645	31,85	89,25	55,7425	0,1925	0,3645
XY-late-S	0	2,3215	30,85	88,85	53,2655	0,2810	0,5205
	1000	2,3510	30,85	90,00	53,5120	0,1795	0,3380
	2500	2,3470	31,45	89,75	55,7275	0,1780	0,3335
PR-S	0	2,3370	30,45	87,20	54,2555	0,3590	0,6655
	1000	2,3375	30,40	89,00	54,4070	0,2355	0,4365
	2500	2,3240	30,85	89,10	54,9585	0,2215	0,4085

APPENDIX 1 F. STRENGTH PROPERTIES

Unbleached, never-dried pulps

	Beating	SR	WRV	Density	Tensile I	Stretch	Tens. energy	Stiffness	Burst I	Tear I
	rev		g/g	kg/m3	kNm/kg	%	abs. I, kJ/kg	mNm/kg	kPam2/g	mNm2/g
Ref-N	0	14,2	1,59	507	59,6	1,69	0,68	8,44	3,26	23,21
	1000	15,2	1,62	589	75,1	2,05	1,03	9,14	4,57	18,49
	2000	16,4	1,71	640	94,6	2,51	1,58	10,12	6,11	16,30
	4000	18,8	1,73	677	98,0	2,97	1,95	9,85	6,83	15,07
	5000	20	1,84	680	101,6	2,99	2,03	10,13	7,64	14,54
Ref-S	0	13	1,4	465	42,1	1,41	0,40	6,92	2,09	19,42
	1000	13,3	1,5	572	63,1	2,00	0,86	8,15	3,78	17,34
	2000	15,1	1,52	619	75,0	2,41	1,22	8,77	4,81	14,75
	4000	17,5	1,64	667	91,7	2,61	1,60	9,81	6,18	13,75
	5000	17,8	1,72	666	92,5	2,82	1,77	9,94	6,23	13,09
XY-0-N	0	14,7	1,65	513	68,0	1,51	0,66	9,14	3,6	19,43
	1000	15,5	1,73	612	82,7	1,98	1,08	9,78	5,42	16,52
	2500	18,2	1,85	670	105,3	2,46	1,69	10,63	7,01	14,36
	5000	26,1	2,03	714	115,0	2,92	2,20	11,00	8,14	13,22
XY-0-S	0	13,4	1,45	458	42,4	1,13	0,31	7,03	2,03	16,78
	1000	14,8	1,56	592	72,1	2,00	0,97	9,02	4,17	15,19
	2500	15,9	1,7	647	89,2	2,24	1,32	9,84	5,52	12,06
	5000	23,2	1,88	656	97,9	2,57	1,66	10,09	6,56	11,49
XY-late-	0	14,9	1,67	522	63,5	1,51	0,62	8,61	3,85	18,46
	1000	15,5	1,71	623	85,3	2,08	1,17	9,69	5,49	16,71
	2500	17,8	1,81	670	101,7	2,65	1,78	10,35	6,51	14,60
	5000	24,9	1,95	712	111,3	2,86	2,10	10,74	7,75	13,07
XY-late-	0	13,2	1,4	480	51,8	1,30	0,45	8,24	2,32	18,72
	1000	14,6	1,55	592	76,2	2,21	1,14	9,23	4,57	14,91
	2500	15,9	1,69	643	88,9	2,33	1,38	9,86	5,86	12,25
	5000	22,5	1,81	678	105,0	2,52	1,75	10,86	7,3	11,56
PR-N	0	15,1	1,66	516	65,6	1,55	0,66	8,92	3,54	21,87
	1000	15,2	1,68	616	79,3	2,09	1,11	9,36	5,18	17,83
	2500	17,3	1,8	651	88,1	2,61	1,55	9,48	6,06	17,68
	5000	20,4	1,85	696	98,3	2,75	1,79	10,00	7,2	14,39
PR-S	0	13,9	1,45	465	41,1	1,26	0,34	6,74	2,04	20,76
	1000	14	1,5	574	65,9	1,92	0,86	8,62	3,52	17,72
	2500	15,5	1,64	634	83,4	2,18	1,22	9,65	5,15	14,11
	5000	19,9	1,77	680	88,2	2,35	1,41	9,89	6,24	12,95

Zero-span, kNm/kg	**Ref**		**XY-0**		**XY-late**		**PR**	
Beating rev.	**N**	**S**	**N**	**S**	**N**	**S**	**N**	**S**
0	197.7	183.0	198.9	175.9	201.9	180.0	194.6	175.9
1000	198.2	179.0	195.0	194.2	204.9	183.9	194.6	180.3
2500	194.5*	180.9*	203.6	172.0	197.2	183.1	195.2	182.8
4000	193.9	180.5	-	-	-	-	-	-
5000	190.5	179.2	189.4	171.9	194.7	181.2	188.7	177.0
Average	195.0	180.0	197.0	173.0	200.0	182.0	193.0	179.0
Change N-S, abs**		15.0		24.0		18.0		14.0
Change N-S, %**		7.4		12.1		8.8		7.4

**2000 beating revolutions*

***Average*

Unbleached, dried pulps

	Revs	SR	WRV	Density	Tensile I	Stretch	Tens. energy	Stiffness	Burst I	Tear I
			g/g	kg/m3	kNm/kg	%	abs. I, kJ/kg	mNm/kg	kPam2/g	mNm2/g
Ref-N	0	11,8	1,2	441	39.21	1.22	0.319	6.725	1,87	16,82
	1000	13,5	1,38	565	61.67	1.98	0.821	7.918	3,87	18,29
	2500	14,9	1,51	614	77.21	2.55	1.324	8.508	5,35	18,46
	5000	17,4	1,63	658	97.25	2.83	1.792	9.576	6,81	16,14
	0	11,8	1,12	440	30.77	0.89	0.178	6.307	1,37	12,35
Ref-S	1000	12,9	1,32	528	52.83	1.83	0.660	7.415	3,06	20,46
	2500	14,1	1,44	604	70.40	2.38	1.140	8.493	4,72	15,30
	5000	18,5	1,58	634	92.71	2.72	1.663	9.509	5,87	13,38
	0	12,1	1,21	501	48.17	1.07	0.328	8.357	2,26	16,40
	1000	13,5	1,41	567	67.97	1.84	0.831	8.693	4,15	17,68
XY-0-N	2500	14,0	1,59	623	84.28	2.65	1.475	8.832	5,66	16,02
	5000	18,6	1,74	670	103.10	2.81	1.890	9.918	7,28	13,57
	0	11,4	1,28	464	32.54	0.89	0.188	6.636	1,43	11,90
	1000	14,0	1,36	567	59.94	1.93	0.785	8.127	3,65	15,91
XY-0-S	2500	14,2	1,54	622	79.16	2.19	1.148	9.137	5,18	13,86
	5000	16,9	1,85	666	97.25	2.64	1.677	9.939	6,54	11,89
	0	12,1	1,26	471	42.75	1.03	0.284	7.623	1,95	16,88
	1000	12,8	1,41	558	61.26	2.02	0.841	7.972	3,96	18,70
XY-late-N	2500	14,5	1,59	618	80.51	2.65	1.422	8.654	5,63	15,93
	5000	16,8	1,93	659	99.16	2.98	1.939	9.587	7,17	14,27
	0	12,2	1,17	459	32.76	0.93	0.202	6.695	1,56	13,90
	1000	13,1	1,38	555	63.69	1.64	0.694	8.817	3,65	17,42
XY-late-S	2500	13,9	1,48	568	79.79	2.19	1.152	9.231	5,24	13,40
	5000	17,4	1,64	668	94.45	2.71	1.669	9.439	6,64	11,76
	0	12,1	1,2	476	34.65	1.01	0.230	6.590	1,5	14,08
	1000	13	1,4	579	61.50	1.92	0.798	8.042	3,4	21,34
PR-N	2500	14,1	1,52	641	76.54	2.75	1.424	8.395	5,14	17,51
	5000	16,1	1,64	664	91.58	3.03	1.840	9.153	6,71	14,66
	0	11,6	1,11	438	25.34	0.79	0.131	5.692	1,06	10,83
	1000	12,9	1,34	533	44.67	1.77	0.542	6.471	2,61	20,54
PR-S	2500	13,8	1,45	605	68.30	2.17	0.997	8.309	4,3	16,42
	5000	15,7	1,58	653	83.50	2.99	1.672	8.535	6,26	13,64

Zero-span, kNm/kg	**Ref**		**XY-0**		**XY-late**		**PR**	
Beating rev.	**N**	**S**	**N**	**S**	**N**	**S**	**N**	**S**
0	194.9	184.2	196.3	170.3	200.2	177.5	194.9	181.4
1000	197.7	180.4	201.5	171.6	196.0	180.5	192.0	175.8
2500	203.6	181.2	188.2	178.2	191.9	177.9	189.1	181.1
5000	191.9	181.5	187.6	177.0	196.8	179.1	192.8	175.3
Average	197	181.8	193.4	174.3	196.4	178.8	192.2	178.4
Change N-S, abs**		15.2		19.1		17.6		13.8
Change N-S, %**		7.7		9.8		8.9		7.2

***Average*

Bleached pulps

	Revs	SR	WRV	Density	Tensile I	Stretch	Tens. energy	Stiffness	Burst I	Tear I
			g/g	kg/m^3	kNm/kg	%	abs. I, kJ/kg	kNm/g	kPam2/g	mNm2/g
Ref-S	0	12,7	1,46	504	41,7	2,07	0,59	5,80	2,51	17,9
	1000	15,3	1,58	640	83,9	2,50	1,37	9,12	5,50	14,1
	2500	17,4	1,71	702	99,7	3,03	1,93	9,35	6,98	11,5
XY-0-S	0	13,1	1,48	534	53,3	1,82	0,65	7,59	2,72	21,3
	1000	16,4	1,65	664	95,1	2,48	1,49	9,96	5,74	11,5
	2500	21,5	1,80	726	115,2	2,63	1,92	10,93	7,11	9,1
XY-late-S	0	14,2	1,49	503	50,0	1,81	0,62	7,31	2,50	20,2
	1000	15,9	1,62	636	91,5	2,32	1,37	9,94	5,41	12,3
	2500	21,1	1,76	715	113,2	2,82	2,04	10,72	7,19	10,4
PR-S	0	135	1,46	497	43,1	1,69	0,50	6,46	1,82	19,1
	1000	15,8	1,57	639	87,5	2,50	1,42	9,32	5,40	13,3
	2500	18,1	1,71	698	104,9	2,70	1,80	10,23	6,99	10,9

	Ref-S			XY-0-S			XY-late-S			PR-S		
Beating rev	0	1000	2500	0	1000	2500	0	1000	2500	0	1000	2500
ZS, kNm/kg	159,8	174,5	174,9	161,7	172	172,1	168,6	178,7	179,4	165,4	170,9	175,8

APPENDIX 2. QUANTIFICATION OF XYLAN ON XYLAN TREATED KRAFT SPRUCE PULP FIBRES USING ELISA

Geoffrey Daniel, Andrea Kaňuchová, SLU

Aims: The aim was to develope an *Enzyme-Linked Immunosorbent Assay* (ELISA) to indirectly quantify the presence of xylan (i.e. bound birch xylan (*BX*)/native xylan available for detection) on the spruce pulp fibres.

Background: Studies with immunofluorescence labeling of xylan on whole fibres and fibre cross-sections (*Appendix 3*) from the experimental pulps showed positive indications for the presence of xylan on the fibre surfaces. Results suggested that pulps treated with birch xylan (BX) gave the most intense immunofluorescence response in both experimental approaches. However, considerable variability was noted with the intensity of labeling with some fibres labeling very strongly, others poorly (if at all) while others showed positive labeling of only certain morphological structures on the fibres. This variability is most probably related to a number of causes including true differences in actual treatment (presence of birch xylan) as well as availability of the xylan for labeling. *Thus there is an important need for a suitable method for the quantification of a much larger fibre population where such differences can be reduced.*

The method chosen involves a similar immuno-approach as for the previous immunofluorescence work on whole fibres and fibre sections but utilizes an enzyme-conjugated secondary probe that affords colour development *in-situ* that can be measured using spectrophotometry. The ELISA approach had two moments: *i)* Development of a suitable xylan standard (over time/concentration) using purified birch xylan; and *ii*) from *i)* using spectrophotometry readings of unknown samples (i.e. our birch xylan treated pulps) to determine the amounts of xylan available for labeling on the surfaces of the fibres.

Material and Methods

Anti-xylan: The same LM 10 monoclonal antibody as described in *Appendix 3* was used for the

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

Table 1. Fibre materials from the different pulp treatments

	Without shearing	With shearing
Kraft pulp treatment	**Description**	
Reference pulp	Reference-N	Reference-S
Extra birch xylan added early during cooking	XY-0-N (early normal)	XY-0-N (early sheared)
Extra birch xylan added late during cooking	XY-late N (normal)	XY-late-S (sheared)
Xylan removed early in cook and added later. Redistribution of softwood xylan	PR-N (normal)	PR-S (sheared)

Enzyme Linked Immunosorbent Assay (ELISA): This involved the development of a standard using birch xylan and subsequent use of this standard for quantifying xylan in unknown pulp samples.

i) Enzyme Linked Immunosorbent Assay- *Standard development*

The birch xylan standard was made in the following way. 1g/L Sigma birch xylan in Na_2HCO_3 buffer (pH 10.9) was autoclaved for 1 hr. After cooling a dilution series (0.1, 0.01, 0.001, 0.0001, 0.00001, 0.000001 g/L) was prepare from this stock solution and 200 µl added to wells of ELISA plates (3 replicates per dilution) and left overnight at 4° C. Next day wells were washed with PBS (pH 7.2) followed by air-drying for 1 hr at room temperature. Wells were then blocked with 3 % w/v BSA in PBS (200 µl/well) for 4 hr at RT and washed again in PBS (3 x 10 mins). Thereafter a 1:100 solution of LM 10 (rat anti-xylan) in PBS (pH 7.2) (100 µl/well) was added and left overnight at 4° C. Next day wells were washed 3 times with PBS (1×5 mins, 1×10 mins, 1×15 mins). Subsequently, an alkaline phosphate conjugated secondary antibody –goat anti-rat- in PBS, 1:1000, 100 µl/well was added and the samples left overnight at 4° C. Next day wells were washed 3 times (1×5 mins, 1×10 mins, 1×15 mins) and the alkaline phosphate liquid substrate (p-nitrophenyl phosphate, pNPP) added (150 µl/well). Absorbance was measured at 405 nm after 5, 10, 15, 30, 45 and 60 mins using a Bio-Tek EL 311 SL microplate recorder (Bio-Tek Instruments, Inc., Burlington, VT) without termination. Readings were corrected for background controls (absorbance of empty wells) and absorbance of samples used to form regression curves for each time measurement. The standard for quantifying the xylan was repeated 5 times and results reflect the mean. From the work, 30 mins incubation time was selected as the most appropriate standard time for colour development. For more details of the method see Daniel and Nilsson (1991); Hafrén and Daniel (2003).

ii) Enzyme Linked Immunosorbent Assay- *Experimental kraft pulps*

Presence of xylan on the experimental pulps was determined using the following developed method. 1.7 mg of never-dried pulp fibres weighed from a filtered pulp fibre suspension suspended overnight in buffer were suspended in eppendorf tubes containing 200 µl 3% w/v BSA in phosphate buffer saline (pH 7.2) and left for 1 hr at room temperature. Thereafter, tubes were centrifuged and 100 µl of 1:100 dilution (previously determined as optimal) of LM 10 in PBS containing 3% BSA was added and samples incubated overnight at 4° C under gentle shaking. Next day, samples were washed in PBS (3×10 mins, 400 µl PBS); each time gently centrifuging the fibres down for exchange of the solution. After each centrifugation the fibres are washed by first manual shaking and then in a shaker. Thereafter, a secondary goat anti-rat secondary antibody IgG conjugated to alkaline phosphatase in PBS at a 1:1000 dilution of (100 µl per sample) was added. Samples were left for 2 hr at RT in a shaker (or overnight in a cold room). Pulps were then washed with PBS (2×10 mins; 400 µl) and with distilled water (2×5 mins, 400 µl); each time gently centrifuging down the fibres for exchange of the solution. Thereafter, the alkaline phosphatase substrate p-nitrophenyl phosphate (150 µl pNPP in 0.1M glycine buffer, pH 10.4) was added and samples incubated for 30 mins. Colour development was terminated with 10 µl 15 M NaOH, fibres centrifuged down and 100 µl transferred to ELISA plates for absorbance determination at 405 nm as described above. Xylan concentration was then determined from the 30 min. standard equation. The assay was repeated 10 times and results shown reflect mean absorbance values.

Both method and specificity controls were included. Method controls included omission of the primary and secondary antibodies in the labeling procedure; all of which were negative. Additional control tests included using the antibody previously preabsorbed with either birch xylan or spruce fibres. As a further positive control, bleached spruce fibres were treated with 1 mg/L birch xylan for two hrs under autoclaving.

Results and Discussion

The ELISA method developed allowed indirect quantification of xylan (principally birch xylan) present and available for anti-xylan labeling on fibre surfaces of the different pulps (*Figures 1, 2*). Since a large number of fibres are included in the assay, the reaction is based on a relatively large and mixed fibre population (EW, LW) of fibres (*ca* 800) increasing its randomness and representativeness of the entire fibre population.

Results showed that the pulps could be divided into three groups: (Reference N and S; PR-normal and PR-sheared); (XY-early normal and XY-late normal); (XY-early sheared and XY-late sheared) of which the most strongly reactive were the pulps with added BX that were sheared (*Figure 2*). The strongest reaction was with kraft pulps treated with birch xylan early- or late in the kraft process and sheared compared with reference spruce pulps (i.e. N and S) and where the softwood xylan was removed early in cook had been added later in the kraft process (i.e. PR normal and sheared) (*Figure 2*). The weaker reactions of the reference and PR kraft pulps no-doubt reflect the absence of birch xylan and weak reaction of the antibody with the softwood xylan as well as probably the amount of xylan present. Results are consistent with the immunofluorescence observations (whole fibres/cross sections; *Appendix 3*) and other work on the specificity of the xylan probe. Shearing gave a stronger reaction suggesting that the process may open the fibre wall allowing for improved access of the primary antibody for xylan.

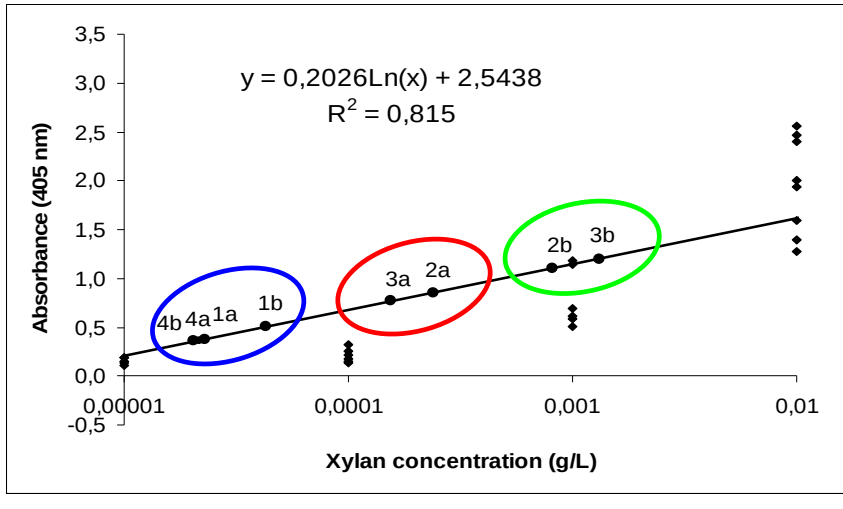

Figure 1. Standard for determining the amount of birch xylan in unknown samples.

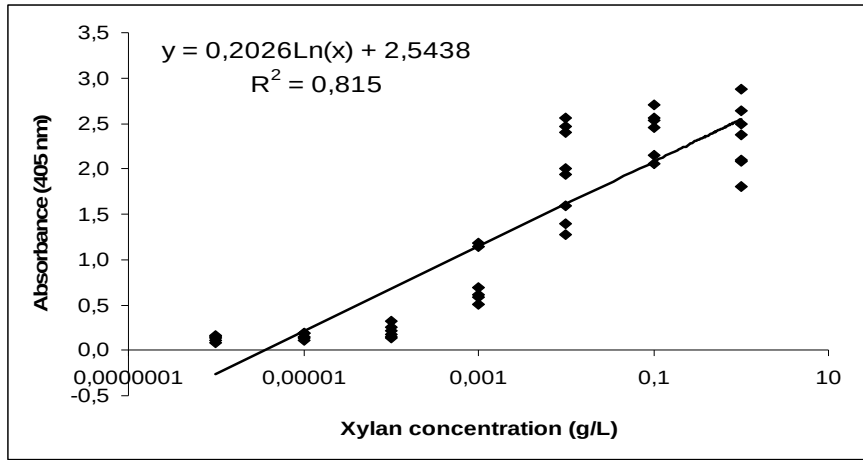

Figure 2. Estimation of xylan (g/L) binding to pulp samples (10 replicates). The kraft pulp samples were separated into 3 groups. Highest xylan concentrations were found for kraft samples subjected to shearing (green ellipse) followed by early- and late application of birch xylan to the kraft process (red ellipse). Samples untreated with birch xylan -both sheared and un-sheared (blue ellipse) gave the weakest reaction. Abbreviations: Group 1: 1a, Reference N, 1b, Reference S; 4a, PR-normal, 4b, PR-sheared; Group 2: 3a, XY-Late normal, 2a, XY-early normal; Group 3: 2b, XY-early sheared, 3b, XY-late sheared.

References

Daniel, G. and T. Nilsson. 1991. Antiserum to the fungus *Phialophora mutabilis* and its use in Enzyme-linked Immunosorbent assays for detection of soft rot in preservative-treated and untreated wood. Phytopathology 81, (10) 1319-1325.

Hafrén, J. and G. Daniel 2003. A bioassay for methylated galacturonan on pulp-fiber surfaces. Biotechnology Lett., 25, 859-862.

APPENDIX 3. VISUALIZATION OF THE SPATIAL MICRODISTRIBUTION OF XYLAN ON BIRCH XYLAN TREATED SPRUCE PULPS USING IMMUNO-FLUORESCENCE MICROSCOPY OF WHOLE FIBRES AND FIBRE CROSS-SECTIONS

Geoffrey Daniel, Andrea Kaňuchová, Lada Filonova, SLU

Aims: The purpose of the study was to: *i)* Visualize birch xylan on the surfaces of treated whole fibres assessing two indirect labeling approaches: xylan specific antibodies and synthetic xylan carbohydrate modules; *ii)* Determine whether there are any apparent differences in the spatial distribution of the birch xylan present on the laboratory treated kraft pulps; *and iii)* Visualize the spatial microdistribution of the xylan in cross-sections of the treated pulps to determine whether the xylan was penetrating the fibre cell walls.

Background: In order to understand the importance of xylan in promoting fibre strength it is imperative to have a high precision technique that will allow visualization of the xylan both intracellularly and extracellularly in/on the fibre wall. Therefore in order to *truly* visualize the birch xylan and thereby have knowledge on its spatial distribution on the pulp fibres during the cook process, a method of analysis coupling both morphology and high specificity is needed. Briefly, two methods that fulfil these requirements are the use of antibodies (proteins) produced against xylan (i.e. anti-xylan) and carbohydrate binding modules (CBM) that can specifically localize xylans on pulp fibres. The xylan antibodies in the present study have been derived from animals immunized against xylan while the xylan CBM have been derived by synthetic evolution of a binding module against xylan (Cicortas-Gunnarsson et al., 2004).

Materials and Methods

1. Monoclonal antibody and carbohydrate binding module.

Visualization of xylan was achieved using a rat monoclonal antibody generated against a low–substituted (1-4)-β-D-xylan (LM10) (McCartney et al., 2005). The antibody was a generous gift from Prof. P. Knox (Leeds Univ., UK). The X-2 carbohydrate binding module (CBM) was as described previously (Cicortas-Gunnarsson et al., 2004; Filonova et al., 2007a, b).

2. Immunolabeling of whole kraft fibres and fibre cross-sections

i) Labeling with LM10 monoclonal antibody

Briefly, whole fibres were treated with 10% H_2O_2 (3 mins), washed in water (2 x 5mins) and labeled with LM10 (1:100) in PBS containing 3 % BSA

overnight at 4° C. Next day fibres were washed with water (4×15mins) and labeled with FITC-conjugated anti-rat antibody (Sigma Aldrich) in PBS at 1:500 dilution for 1 hr at rt and washed with water (3×10mins).

ii) Labeling with X-2 Carbohydrate Binding module (CBM)

For the labeling of fibres, a synthetic carbohydrate binding module (X-2) previously shown to have a high affinity for xylan (Filonova et al., 2007a, b) was utilized (*Tables 1, 2*). Detection of the CBM binding was achieved using the indirect approach based upon antibody-mediated labeling of the CBM bound to the fibre substrate (Daniel et al., 2006, Filonova et al., 2007a, b). Fibres were initially blocked in PBS (pH 7.4) containing 5% w/v ovalbumin to prevent non-specific binding of the CBM. Thereafter fibres were incubated in PBS containing 10 μM X-2 at rt for 6 h. Samples were then washed twice with PBS (5 minutes each step) and incubated with mouse Anti-His antibody (Amersham) at 4° C overnight. After washing three times with PBS (10 mins each step), samples were treated with FITC-conjugated anti-mouse IgG (Sigma) diluted 1:500 in PBS for 1 h at rt. As a method control, the X-2 CBM was omitted in the labeling procedure of fibres. In addition, fibres were incubated in 500 μl of PBS containing 2 μM FITC-conjugated G-4 for 4 h at room temperature followed by washing in PBS. G-4 is a CBM that has been selected from the same library as the xylan binding CBMs and has been shown unable to bind xylan and other hemicelluloses (Cicortas-Gunnarsson et al., 2004) and its FITC-labeled derivative served as a negative control in these experiments.

All experiments with both LM10 and CBM were carried out in 500 μl of incubating/washing solutions in triplicate using Eppendorf tubes with centrifugation between washing labeling steps.

iii) Immunolabeling of fibre cross-sections

In principle, fibre cross-sections were labeled with LM10 as described above except that drops of the primary and secondary antibodies and washing solutions were added directly to sections on object glasses.

iv) Positive and negative and substrate controls

As for the SEM study (*Appendix 4*) positive controls were used as a means of checking fibre specificity (i.e. best case situation for detecting birch xylan on pulp fibres). As a positive control observations were also made on reference fibres in which birch xylan (BX) was sorbed onto the spruce fibres under known conditions. Spruce reference N and S pulp fibres and a fully bleached spruce pulp (from Södra) were treated in two ways according to Table 2 *i)* with 10g/L birch xylan (BX) in Na_2CO_3 (pH 10.9) buffer at 121° C for 1 hr. Conditions may

not exactly reflect the kraft cook process but represent a situation at high and prolonged temperature; *and ii)* with 10g/L BX in Na_2CO_3 (pH 10.9) at RT under 1 bar pressure to determine the likely penetration of BX into the kraft fibres. As a method control, spruce, LM10 or the CBM was omitted from the labeling procedure.

3. Embedding of kraft pulp fibres in resin

Never dried fibres were dehydrated in an ethanol series (20 mins steps of 20, 40, 60, 70, 80, 90, 95, and 99.5% ethanol and embedded resin (Daniel et al., 2004a). Semi-thin sections (*ca* 2.0 µm) were cut using a Reichert E4 Ultramicrotome and mounted on object glasses for labeling.

4. Fluoresence 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. All 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).

*Table 1. Overview of the labeling of treated whole spruce kraft pulp fibres. A similar scheme was also used for the labeling of fibre cross-sections using only the xylan antibody and not CBM. *Xylan monoclonal antibody with high specificity for birch xylan; ** CBM X-2; Carbohydrate binding module with specificity for birch xylan; *** BX; birch xylan*

Kraft Pulp Treatment	Comments	Xylan Ab*/CBM**
Laboratory pilot plant pulps		
Reference-N	**Control sample-orig. SWX[1)]**	**Xylan AB & CBM**
Reference-S	**Control sample + shearing – orig. SWX**	**Xylan AB & CBM**
Extra xylan added early in cook XY-0-N		**Xylan AB & CBM**
Extra xylan added early in cook XY-0-N	**+ Shearing**	**Xylan AB & CBM**
Extra xylan added late in cook XY-late-N		**Xylan AB & CBM**
Extra xylan added late in cook XY-late-N	**+ Shearing**	**Xylan AB & CBM**
Xylan removed early in the cook and added back late in cook	**PR-N**	**Xylan AB & CBM**
Xylan removed early in the cook and added back late in cook	**PR-S**	**Xylan AB & CBM**

Table 2. Overview of additional treatments of the spruce fibres with birch xylan and the detection method used

Positive Controls	**Autoclaving with 10g/L BX*****	
Reference-N	**Autoclaved at 121 C, 1 hr, Na_2CO_3 pH 10.8 + 10g/L BX**	**Xylan Ab & CBM**
Reference-S	**“ “ “**	**Xylan Ab & CBM**
Fully bleached kraft pulp (Södra)	**“ “ “**	**Xylan Ab & CBM**
Positive Controls	**Vacuum pressures with 10g/L BX**	
Reference-N	**Vacuum at 1 bar at RT, in Na_2CO_3 buffer pH 10.8 + 10g/L**	**Xylan Ab & CBM**
Reference-S	**“ “ “**	**Xylan Ab & CBM**
Fully bleached kraft pulp (Södra)	**“ “ “**	**Xylan Ab & CBM**

Results and Discussion

1. Whole fibres

i) Laboratory kraft pulps:

Without quantification it is not so easy to compare the labeling results, however strong fluorescence was noted with XY-early normal, XY-early sheared and XY-late normal and XY-late sheared i.e. all pulps to which birch xylan had been added either early- or late in the cook process (compare *Figures 3, 6* with *Figures 4, 5*). As seen in the images, there is considerable variability in intensity of labeling (i.e. presence of green (FITC) colour) for xylan in both the XY-early and XY-late treated pulps, both with and without shearing (*Figures 4, 5*). This is observed both between fibre types (EW/LW) and even morphological structures (e.g. pits, fibrils) on the fibre surfaces. Latewood fibres were frequently very auto-fluorescent and often appeared less labeled. From the images it is suggested that shearing does not apparently affect the presence of xylan; and once present seems to remain bound. Both the xylan antibody and CBM appeared to react fairly similarly with very strong labeling of the bleached pulps (*Figures 1, 2.*) and variable reaction with the experimental pulps (*Figures 4, 5*).

ii) Positive controls:

The strongest labeled pulp fibres were the positive controls where the birch xylan was added with autoclaving and vacuum impregnation of the pulp fibres (*Figures 1, 2*). Most intensive labeling for xylan as expected was with fully bleached pulps, both with the antibody and the CBM) presumably reflecting the greater porosity and availability of the

cellulose for xylan sorption. The reference laboratory pulps N, S and PR-normal and PR-sheared labeled much more weakly reflecting the poorer specificity of the probes for softwood xylan.

2. Fibre cross sections with xylan antibody

i) Laboratory kraft pulps:

Like immunofluorescence microscopy of whole fibres, labeling of sections also showed some variability but gave a better idea of the ability for the xylan to penetrate the pulp fibres (*Figures 7-11*). In particular, strongest reactions were shown by the XY early normal and sheared and XY-late normal and sheared (*Figures 8, 9*). Indications were given for binding of birch xylan to both the outer fibre walls (presumably S1/primary wall) as well as the wall of the fibre cell lumens wall for both earlywood and latewood fibres in the XY-early and late treated pulps (*Figures 8, 9*). Observations suggested that the more open the fibre wall the greater the staining and thereby green fluorescence. The poor reactions with the reference N, S and PR-normal and sheared cross-sections is further consistent with weak specificity of the xylan antibody for with softwood xylan (*Figures 7, 10*).

ii) Positive controls:

The positive controls like the whole fibres gave strong fluorescence (*Figure 11 A-D*) but suggest that even under best conditions, it may be difficult to sorb xylan into the secondary walls of thick fibres and best penetration will be with earlywood fibres (*Figure 11*).

Overall conclusions:

- Anti-xylan probes (antibodies/CBMs) can be used as means for visualizing the spatial microdistribution of birch xylan when added to the cook process;
- The labeling of whole fibres and fibre cross-sections give complimentary information with whole fibres giving a broader understanding of a larger population of fibres and reaction with different morphological structures on the fibre wall while cross-sections can give information of the penetration of xylan into the fibre wall both from the outer and inner lumen walls;
- However, in order to prove differences a method of quantification is required using xylan standards;
- The present anti-xylan probe has a strong reaction with birch xylan and weak reaction with softwood xylan. It would be advantageous to be able to distinguish between the both.

References

Cicortas Gunnarsson L, E. Nordberg-Karlsson, A. S. Albrekt, M. Andersson, O. Holst and M. Ohlin. 2004. A carbohydrate binding module as a diversity-carrying scaffold. *Protein Eng Des Sel* 2004, 17:213-221.

Daniel, G., J. Volc and M-L. Niku-Paavola. 2004a. Cryo-FE-SEM and TEM Immuno-Techniques Reveal New Details for Understanding White Rot Decay of Lignocellulose. Comptes Rendus, Biologies 327/9-10, Oct. pp 861-871.

Daniel, G., I. Duchesne, C. Tokoh and S. Bardage. 2004b. The surface and intracellular nanostructure of wood fibres: Electron microscope methods and observations. In Proceedings of COST Action: Wood Fibre Cell Walls: Methods to study their formation, structure and properties; 87-104. Ed. U. Schmitt, P. Ander, J.C Barnett, A.M.C. Emmons, G. Jeronimidis, P. Saranpää, S. Tschegg. (http://www-wurc.slu.se).

Daniel, G., L. Filonova, Å. M. Kallas and T. Teeri. 2006. Use of the cellulose-binding module $CBM1_{HjCel7A}$ for morphological and chemical characterization of the gelatinous cell wall layer in tension wood fibres of *Populus tremula* and *Betula verrucosa* using fluoresence and FE-SEM microscopy. Holzforschung, 60, 618-624.

Filonova, L., L.-C. Gunnarsson, G. Daniel and M. Ohlin. 2007a. Synthetic xylan-binding carbohydrate modules (CBM) for the mapping pulp fibre and wood sections. BCC Plant Biology, 7, 54-64.

Filonova, L., A.M. Kallas, L. Greffe, T.T. Teeri, and G. Daniel. 2007b. Analysis of the surfaces of wood tissues and pulp fibres using carbohydrate-binding modules specific for crystalline cellulose and mannan. Biomacromolecules 8, 91-97.

Hafrén, J. and G. Daniel 2003. A bioassay for methylated galacturonan on pulp-fiber surfaces. Biotechnology Lett., 25, 859-862.

McCartney, L., S.E. Marcus and J. P. Knox. 2005. Monoclonal antibodies to plant wall xylans and arabinoxylans. J. Histochem. Cytochem. 53, 543-546.

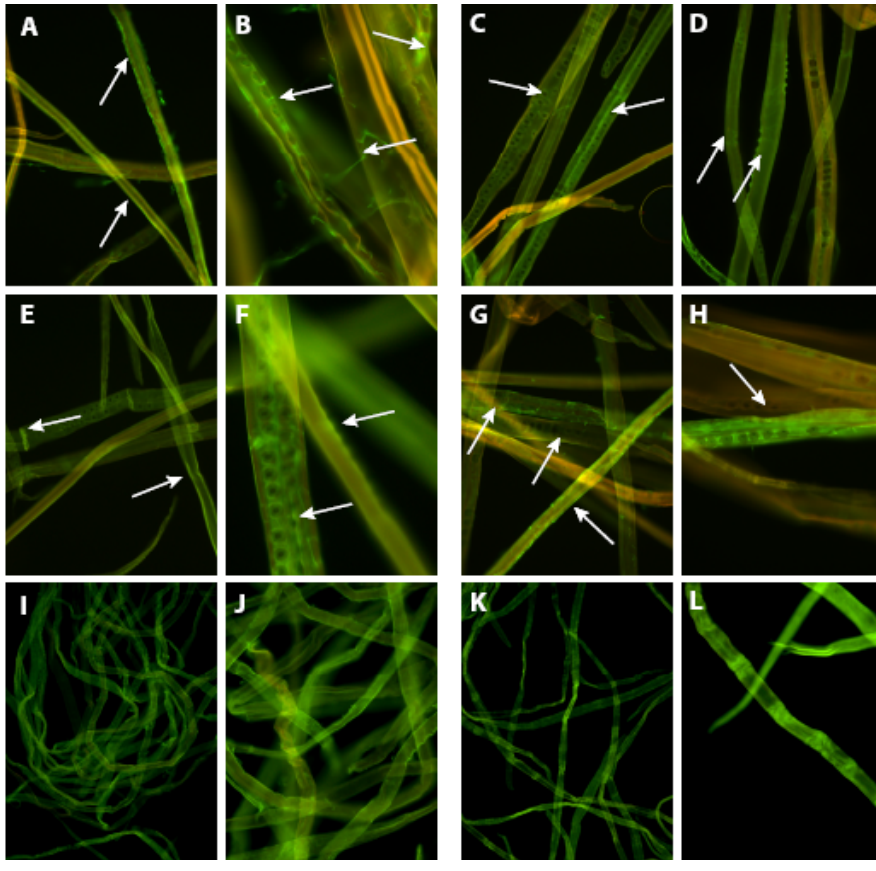

Figure 1. Positive controls; pulps autoclaved with 10 g/L birch xylan. Green fluorescence indicates positive indication for presence of xylan.

Images A-J on left show fibres treated with anti-xylan Ab; Images C-L on right show fibres treated with X-2 CBM.

Images A-D are fibres from Reference-N pulp; Images E-H are fibres from Reference-S pulp; Images I-L are fibres from fully bleached pulps.

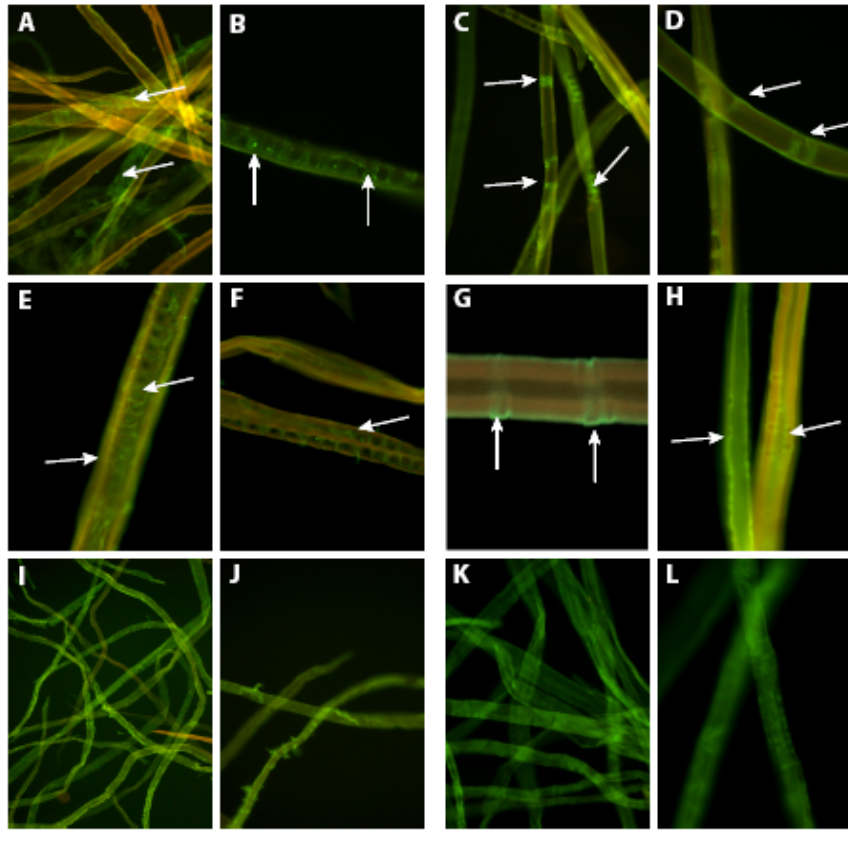

Figure 2. Positive controls; Vaccum impregnated pulps with 10 g/L birch xylan. Green fluorescence indicates positive indication for presence of xylan.

Images A-J on left show fibres treated with anti-xylan Ab; Images C-L on right show fibres treated with X-2 CBM.

Images A-D are fibres from Reference-N pulp; Images E-H are fibres from Reference-S pulp Images I-L are fibres from fully bleached pulps.

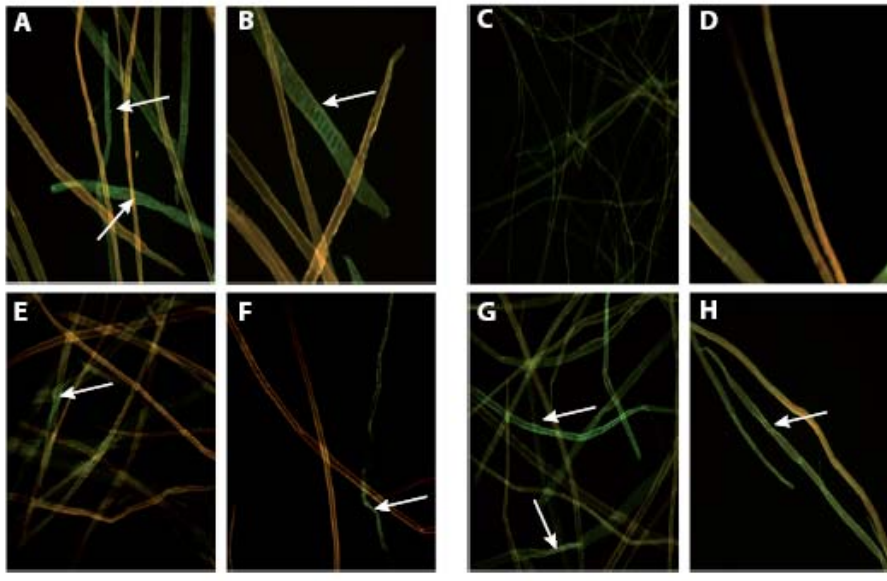

Figure 3. Reference N and S pulps untreated with birch xylan; i.e. only softwood xylan present. Green fluorescence indicates positive indication for presence of xylan.

Images A-F on left show fibres treated with anti-xylan Ab; Images C-H on right show fibres treated with X-2 CBM.

Images A-D are fibres from Reference-N pulp and images E-H are fibres from Reference-S pulp.

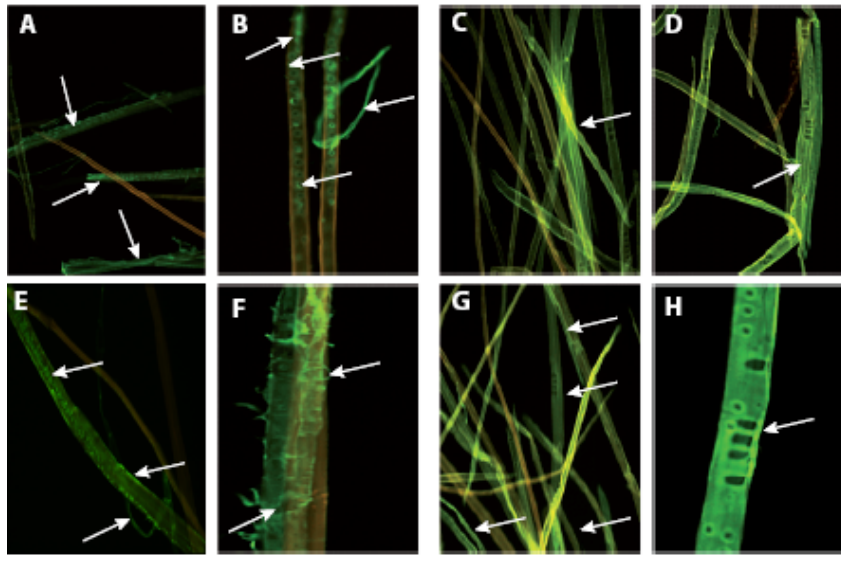

Figure 4. XY-0-N and XY-0-N sheared pulps treated early with 10 g/L birch xylan.

Green fluorescence indicates positive indication for presence of xylan.

Images A-F on left show fibres treated with anti-xylan Ab; Images C-H on right show fibres treated with X-2 CBM.

Images A-D show fibres from XY-0-N to which xylan was added early in cook and images E-H are fibres from XY-0-N plus shearing.

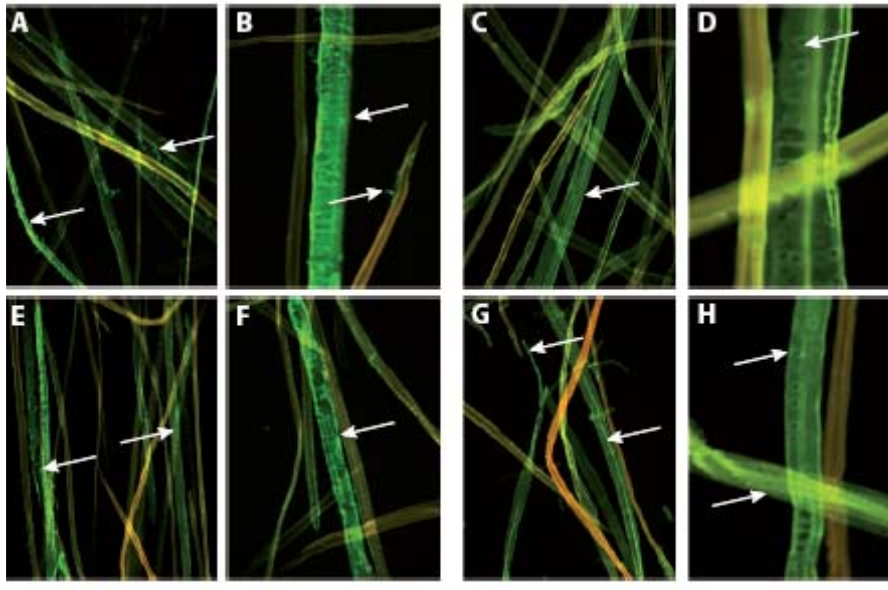

Figure 5. XY-late normal and XY-late sheared treated with 10 g/L birch xylan. Green fluorescence indicates positive indication for presence of xylan.

Images A-F on left show fibres treated with anti-xylan Ab; Images C-H on right show fibres treated with X-2 CBM.

Images A-D are fibres from XY-late-N to which xylan was added late in the cook and images E-H are fibres from XY-late-N plus shearing.

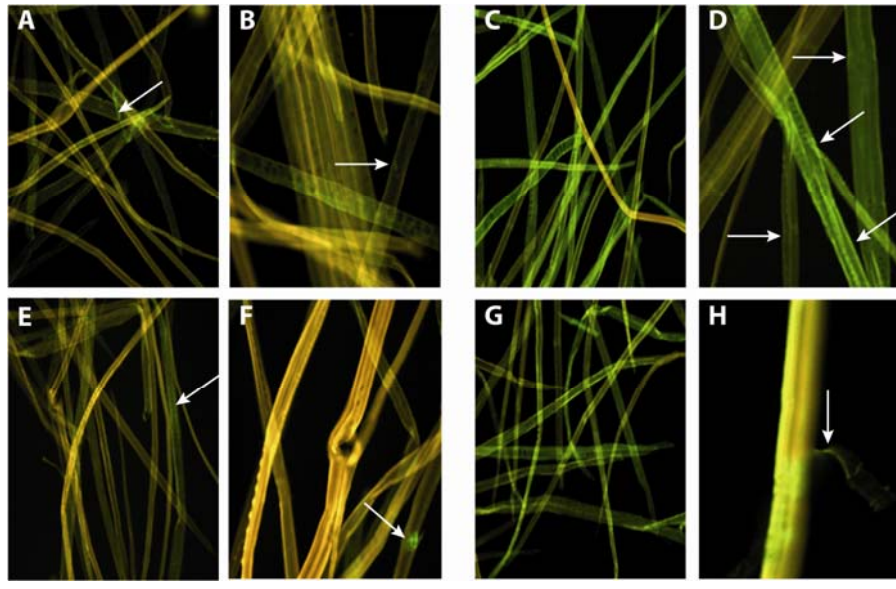

Figure 6. PR-Normal (PR-N) and PR-sheared (PR-S) pulps in which xylan was removed early in the cook and added back late in the cook process. Green fluorescence indicates positive indication for presence of xylan.

Images A-F on left show fibres treated with anti-xylan Ab; Images C-H on right show fibres treated with X-2 CBM.

Images A-D are fibres from PR-N and images E-H are fibres from PR-S.

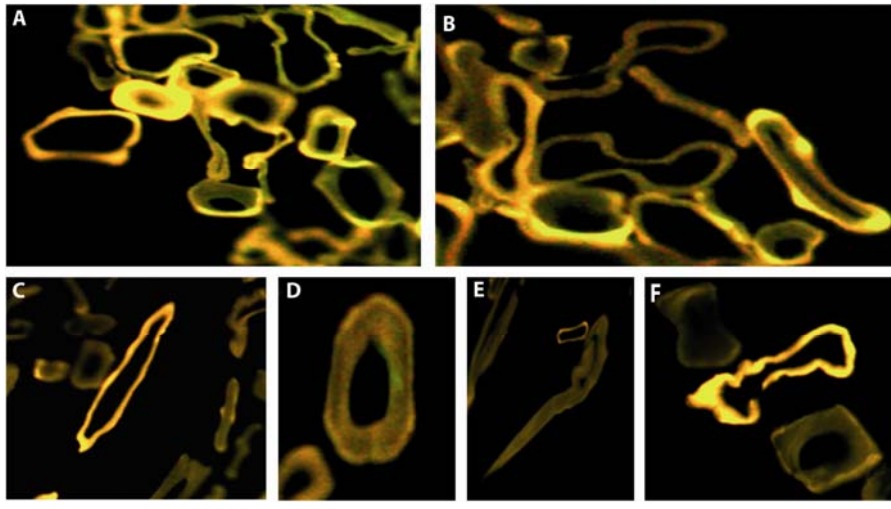

Figure 7. Fibre cross-sections after labeling with anti-xylan Ab.

Images A, B, fibres from Reference-N pulp; Images C-F fibres from Reference-S pulps.

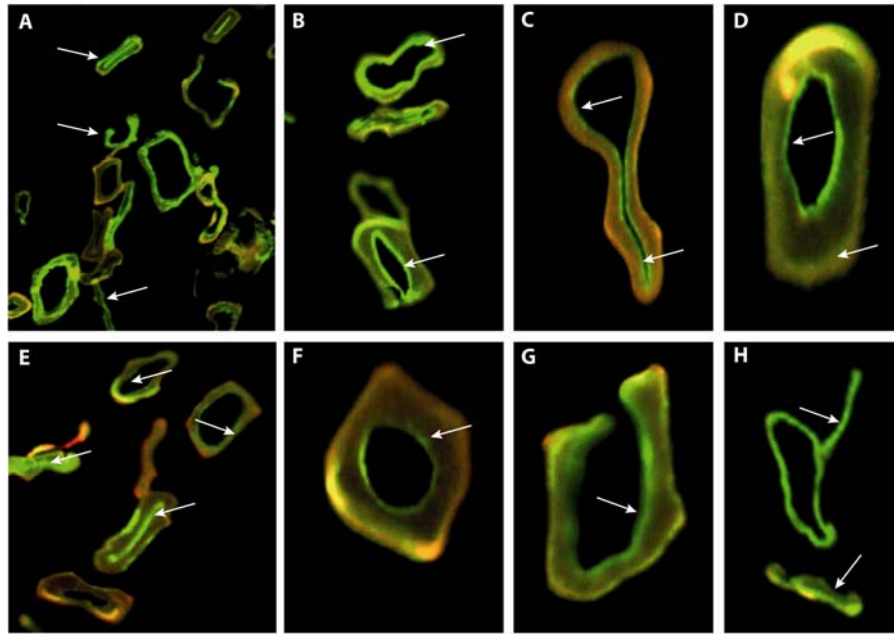

Figure 8. XY-0-N and XY-0-N (plus shearing) fibre cross-sections after labeling with anti-xylan Ab. Green fluorescence indicates positive indication for presence of xylan.

Images A-D; birch xylan added early in cook process.

Images E-H; birch xylan added early in cook process plus shearing.

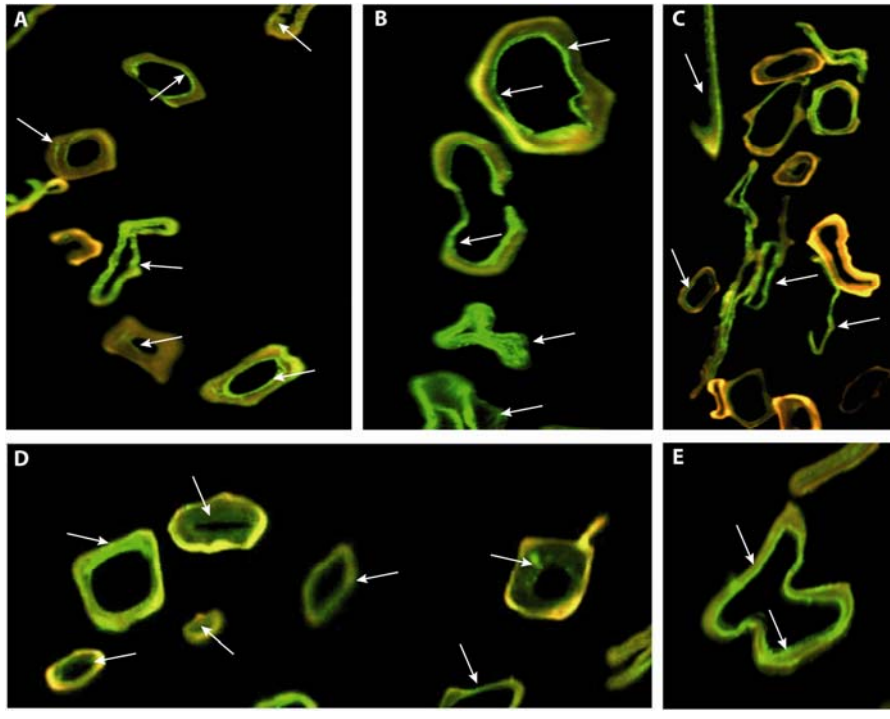

Figure 9. XY-late-N and XY-late-N (plus shearing) fibre cross-sections after labeling with anti-xylan Ab. Green fluorescence indicates positive indication for presence of xylan.

Images A-C; birch xylan added late in the cook process.

Images D-E; birch xylan added late in cook process plus shearing.

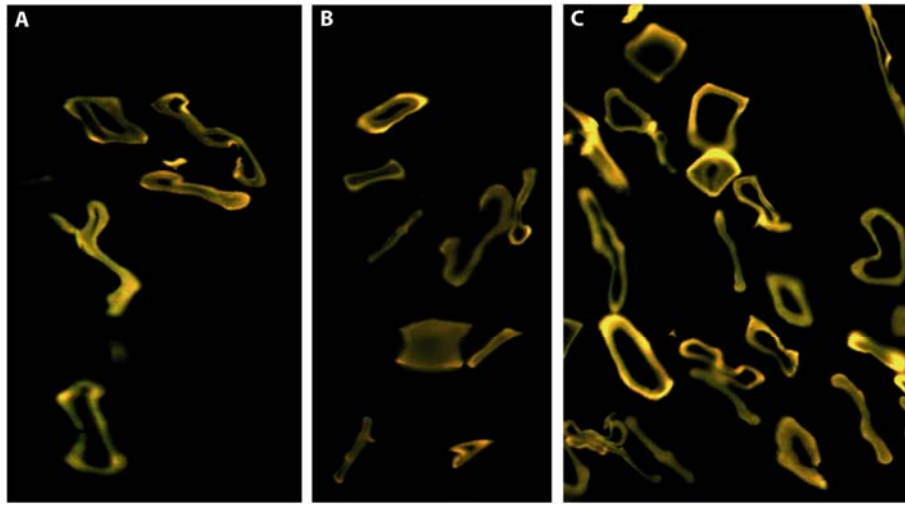

Figure 10. Fibre cross-sections of PR-Normal (PR-N) and PR-sheared (PR-S) pulps in which xylan was removed early in the cook and added back late in the cook process.

Images A-B on left show PR-Normal.

Image C-H on right show PR-sheared.

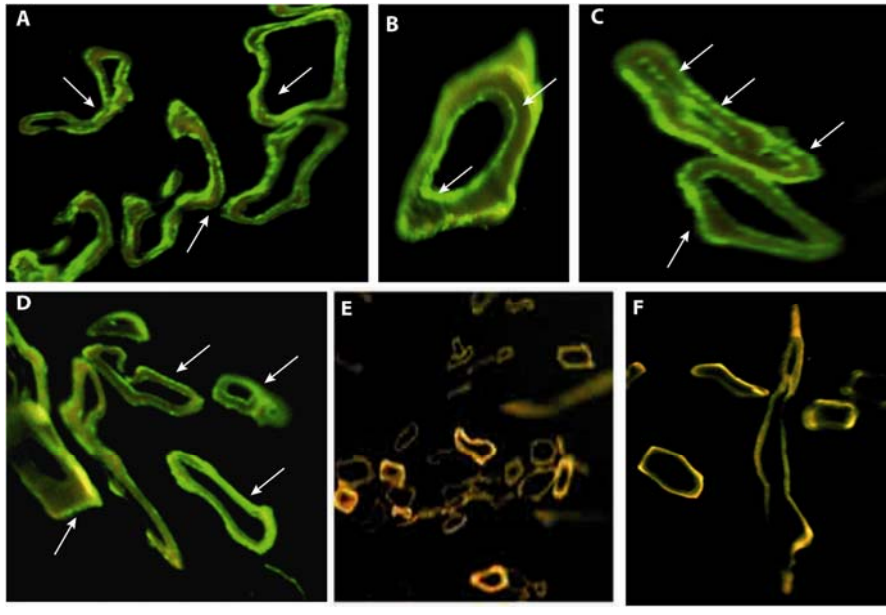

Figure 11. Fibre cross-sections of Reference-N and Reference-S pulp fibres from positive and technical controls. Green fluoresence indicates positive indication for presence of xylan.

Images A-C; Reference-N; Positive control; 10g/L birch xylan added to spruce pulp and autoclaved for one 1 hr;

Image D; Reference-S; Positive control; 10g/L birch xylan added to spruce pulp and autoclaved for one 1hr;

Images E, F; Reference PR-Late; No LM10 anti-xylan AB added but with secondary antibody included during the labeling procedure.

APPENDIX 4. SEM OBSERVATIONS ON SPRUCE KRAFT PULPS TREATED WITH BIRCH XYLAN AND TEM EXAMINATION OF BIRCH XYLAN FILMS

Geoffrey Daniel, SLU

Aims: The purpose of the work was answer the following questions: *i)* Can exogenously added birch xylan be observed morphologically on the surfaces of birch treated kraft pulp fibres using SEM ?; *ii)* Does sorbed xylan precipitate and are there different morphological complexities (e.g. precipitates, aggregates etc) with fibres treated at early- and late stages of the kraft process ?; *iii)* Does xylan show any concentration towards the different fibre morphological features ? i.e. native and processed induced changes (latewood/earlywood. pits, dislocations, kinks etc.); *iv)* How does precipitated birch xylan appear morphologically ?; *v)* Can the affects of shearing be visualized and their affects interpreted with birch xylan addition ?. Naturally *ii)* and *iii)* were dependant on the outcome of *i)*.

Background: Xylans have been previously reported to precipitate on the surfaces of fibres when applied exogenously or directly from the kraft cooking liquor. This can be assessed conveniently by the use of scanning electron microscopy (SEM) which can provide a 3-dimensional overview of the morphological structure of fibres.

Materials and Methods

Fibre materials and importance of control samples:
Analysis of any biological material using microscopy necessitates positive and negative controls. Thus in the present study the positive control was controlled birch xylan sorption onto spruce fibres under known conditions. Spruce reference-N (i.e. normal) and S (i.e. sheared) pulp fibres and a fully bleached spruce pulp (from Södra) were treated in two ways:

i) With 10 g/L birch xylan (BX) in Na_2CO_3 (pH 10.9) buffer at 121° C for 1 hr. Conditions may not exactly reflect the kraft cook process but they nevertheless represent a situation at high and prolonged temperature whereby knowledge on what xylan is likely to appear morphologically when present on fibre surfaces using the SEM may be revealed; *and*
ii) With 10 g/L BX in Na_2CO_3 (pH 10.9) at RT under 1 bar pressure to determine whether BX is likely to penetrate the kraft fibres.

The above approach was taken to represent a best possible case for success. If no difference in morphological appearance of the fibres was apparent with

fibres treated in this way, then it is assumed that it may be difficult to see differences with the test kraft pulps. Reference spruce kraft fibres from N and S represent a negative untreated control (i.e. no birch xylan added). This control is needed in order to visualize fibres that bear remaining softwood xylan.

Scanning Electron Microscopy approach for fibre analysis: For Scanning Electron Microscopy (SEM), the kraft fibre 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 operated at variable kV. Images were digitalized using the embedded software.

Table 1. Fibre materials from the different pulp treatments

	Without shearing	**With shearing**
Kraft pulp treatment	**Description**	
Reference pulp	Reference-N	Reference-S
Extra birch xylan added early during cooking	XY-0-N (early normal)	XY-0-N (early sheared)
Extra birch xylan added late during cooking	XY-late N (normal)	XY-late-S (sheared)
Xylan removed early in cook and added later. Redistribution of softwood xylan	PR-N (normal)	PR-S (sheared)

Transmission Electron Microscopy (TEM) of isolated birch xylans: 10 µl of 1 g/L birch xylan in sodium carbonate buffer pH (10.9) was pipetted onto TEM grids. After blotting, 10 µl of 4% w/v uranyl acetate added. After drying grids were examined using a CM/12 Philips TEM operated at 100 kV. Images were recorded using Kodak film.

Results and Discussion

Positive controls: Positive controls – *autoclaved or vaccum impregnated pulps*- with 10 g/L birch xylan (BX) -with reference N and S and bleached pulps showed that xylan added at 10 g/L could be visualized as aggregates on the fibre surfaces (*Figures 1-5*) although there was considerable variability in microdistribution. Greatest quantities of aggregates appeared on the fully bleached pulp (*Figures 1-3*). Shearing probably caused removal of some of the aggregates from the reference pulp fibres and bleached control pulps as lesser quantities were noted on the surfaces. However, at the

magnification level the SEM was used, shearing did not appear to cause any major changes in fibre surface morphological ultrastructure (*Figures 5, 7, 9, 11*).

Laboratory kraft pulps: SEM observations did not reveal aggregated BX on fibres –neither when applied early- or late in the kraft process (XY-early normal, XY- late normal) nor with sheared (XY-early sheared, XY late sheared) (*Figures 6-11*). Fibres from the PR-normal and PR-sheared pulps were also similar in morphological appearance to the other pulps (not shown). Indeed very little difference in morphological appearance could be observed between the negative reference controls and the test samples. The fibre surfaces of all test laboratory pulps at the magnifications used showed a fairly smooth structure with the outer fibre surface composed of the secondary S1 cell wall layer. Any morphological effects of pulp shearing on fibre structure were not truly apparent at this magnification.

TEM observations showed the xylan as a strongly staining amorphous film containing small rod-like structures (*Figure 12*). The morphological appearance of the xylan was similar to that observed using SEM with spruce fibres treated with 10 g/L birch xylan (*Figure 4*).

Final conclusions from the SEM observations

Evidence for BX was found as aggregates present on/in outer fibre cell walls of positive control fibres and bleached fibres from pulps treated exogenously with BX (10 g/L). However, to date no evidence for aggregates/precipitates of birch xylan was found for the test pulp samples analyzed. This may suggest that any xylan present will principally be found bound in the pores of the outer fibre cell wall. High resolution SEM would probably give verification of this and indicate whether the xylan is also aggregated in the wall structure, but there is still a need to chemically prove that the aggregates represent xylan BX.

References

Daniel, G. and I. Duchesne. 1998. Revealing the surface ultrastructure of spruce pulp fibres using field emission-SEM. In: Proceedings of the 7th Int. Conf. on Biotechnology in the Pulp and Paper Industry, pp. 81-85, Vancouver, Canada.

Daniel, G., J. Volc, M-L. Niku-Paavola. 2004a. Cryo-FE-SEM and TEM immuno-techniques reveal new details for understanding white rot decay of lignocellulose. Comptes Rendus, Biologies 327/9-10, Oct. pp 861-871.

Daniel, G., I. Duchesne, C. Tokoh and S. Bardage. 2004b. The surface and intracellular nanostructure of wood fibres: Electron microscope methods and observations. In Proceedings of COST Action: Wood Fibre Cell Walls:

Methods to study their formation, structure and properties; 87-104. Ed. U. Schmitt, P. Ander, J. C Barnett, A.M.C. Emmons, G. Jeronimidis, P. Saranpää, S. Tschegg. (http://www-wurc.slu.se).

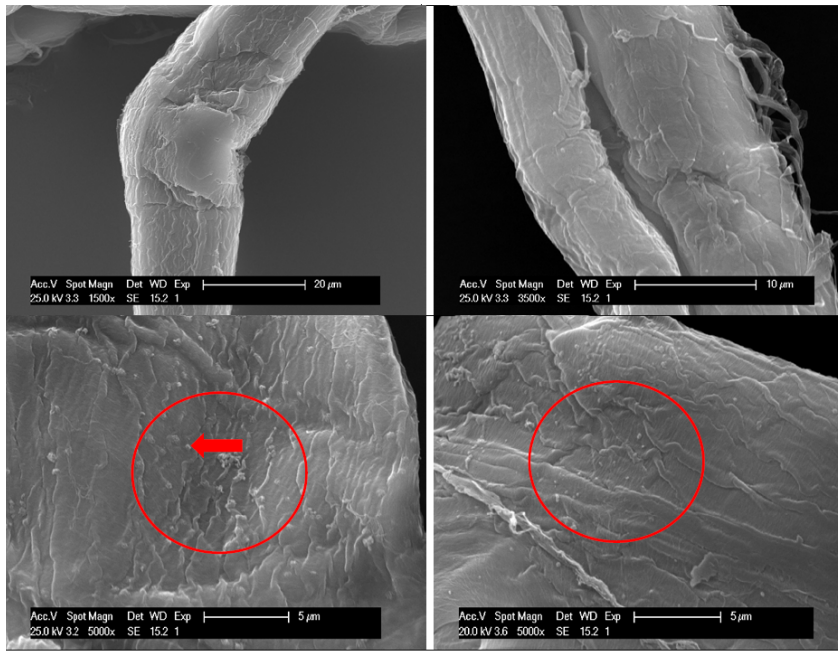

Figure 1a-d. Positive control; fully bleached fibres autoclaved with 10 g/L BX. Aggregates are present (circles) on the outer fibre surface of some fibres, otherwise the surface fibre morphology appeared fairly uniform.

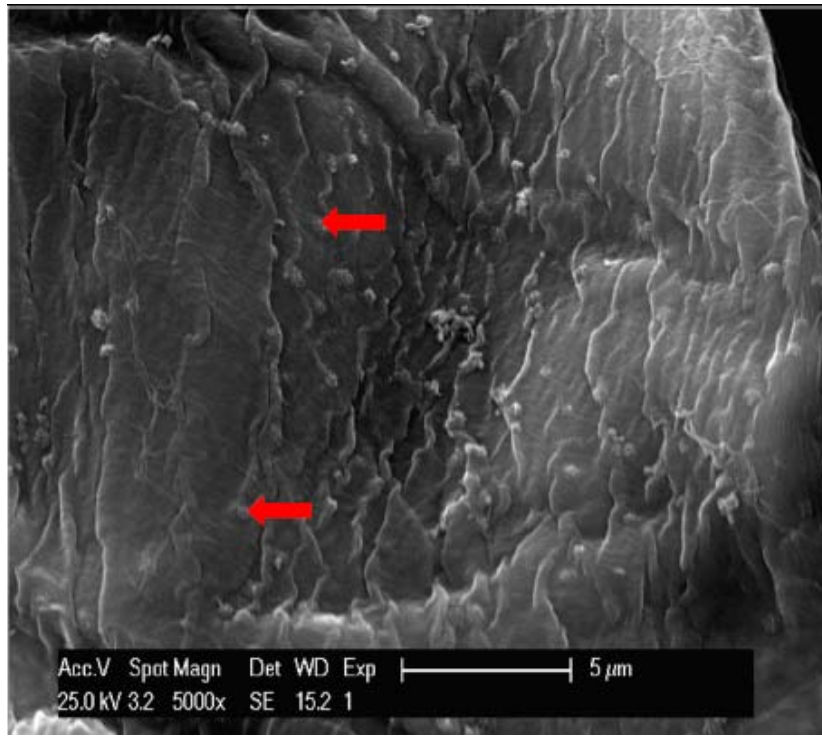

Figure 2. Positive control; fully bleached pulp fibre autoclaved with 10 g/L BX. Arrows indicate xylan aggregates.

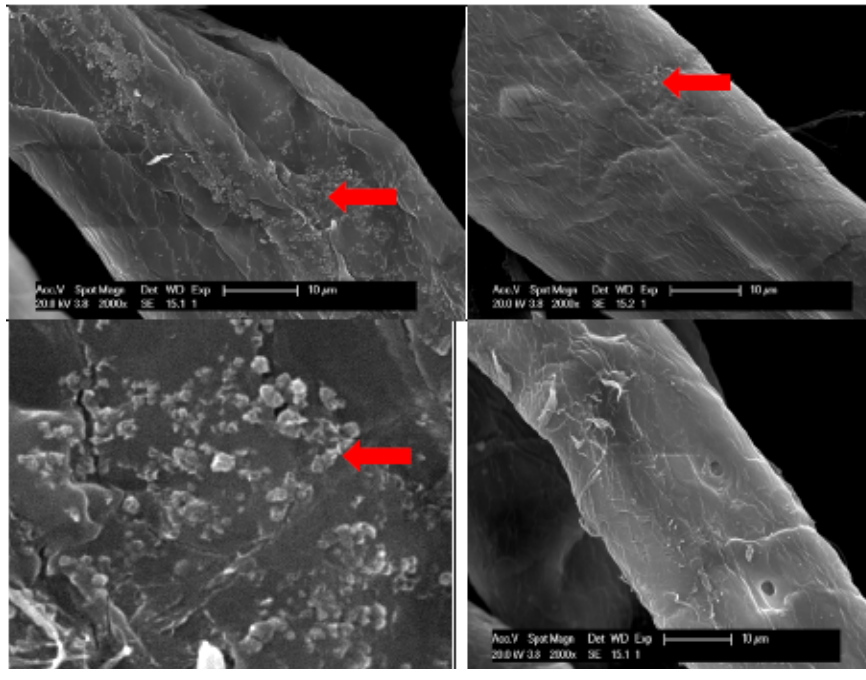

Figure 3a-d. Positive control; fully bleached pulp autoclaved with 10 g/L BX. Aggregates of birch xylan (arrows) present on areas of the outer fibre walls.

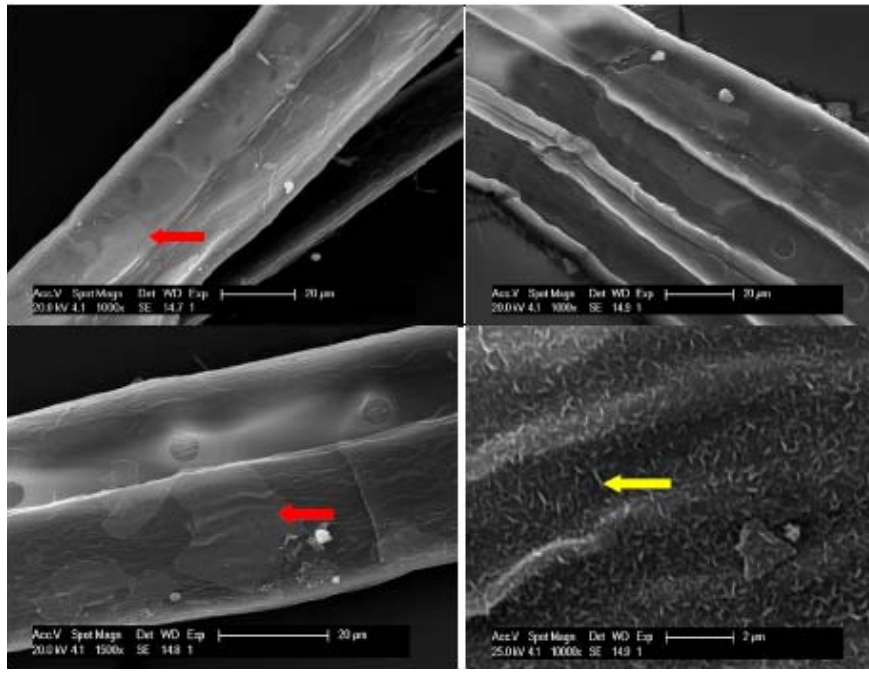

Figure 4a-b. Positive control; Reference-N; autoclaved with 10 g/L BX. Under these conditions the birch xylan can be observed forming isolated films on the fibre surfaces (arrows). Higher magnifications (bottom right) show these islands composed of fine flake-like structures (0.1-0.2 µm long) that presumably represent concentrated xylan. Comparison of the images with Figure 12 shows indicates that the flake-like structures are embedded in a xylan film and may represent crystals.

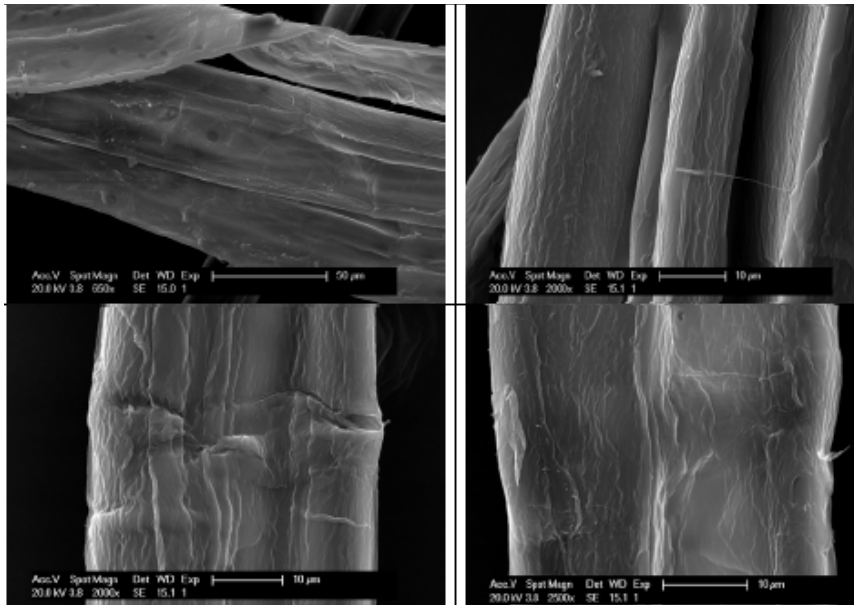

Figure 5. Positive control; Reference S autoclaved with 10 g/L BX.

Laboratory kraft pulps

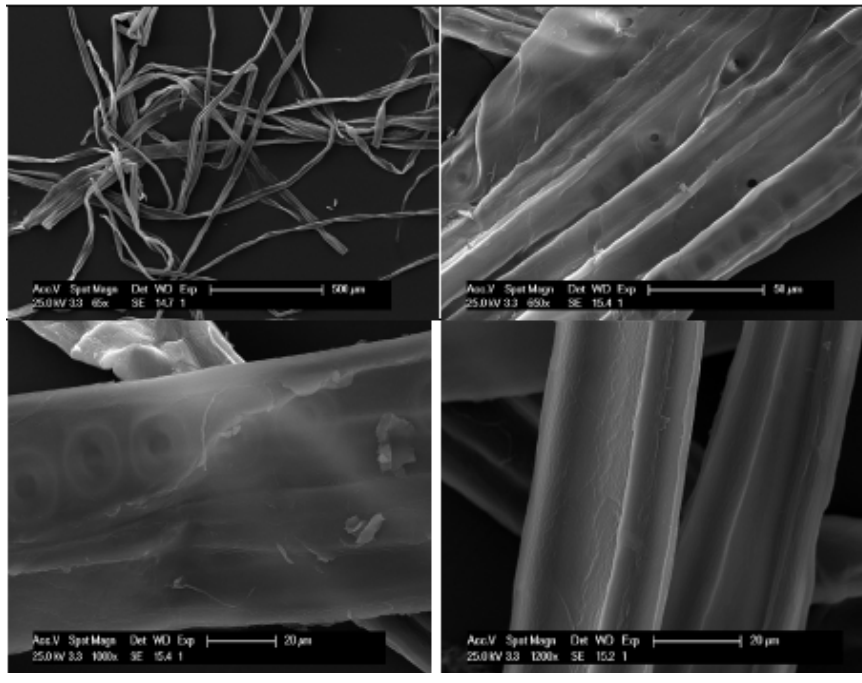

Figure 6a-d. Reference-N control. No birch xylan added.

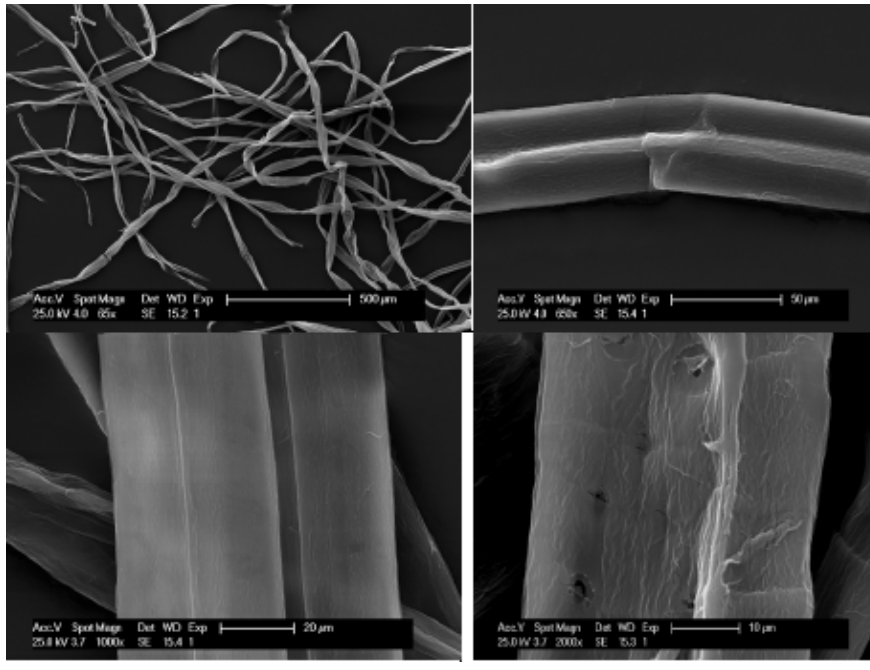

Figure 7a-d. Reference-S control; No birch xylan added.

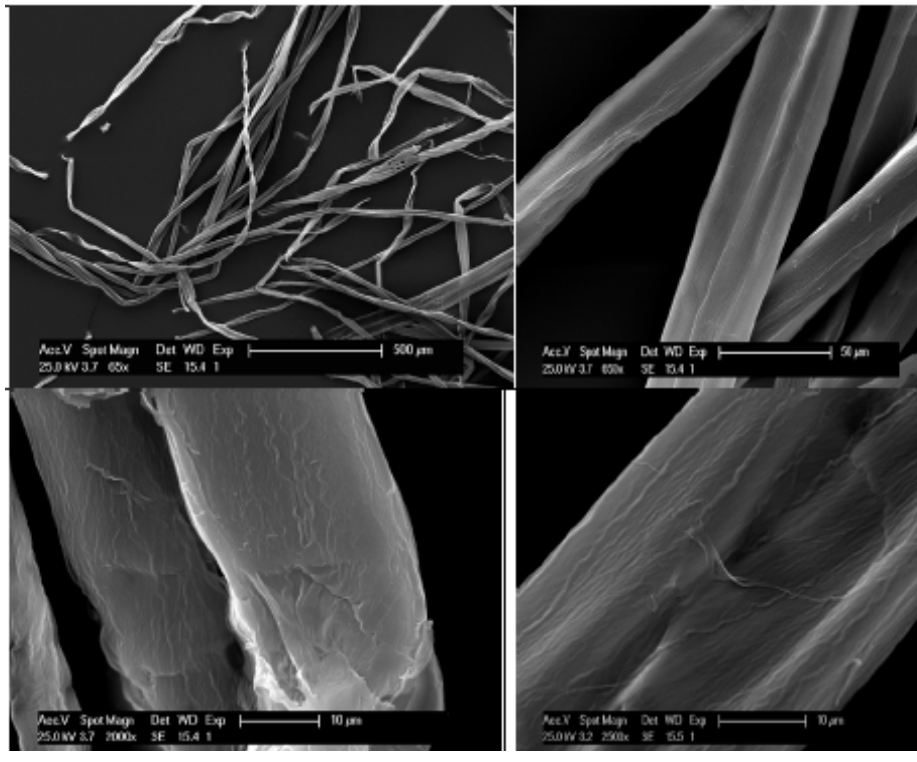

Figure 8a-d. XY-0-N (early normal).

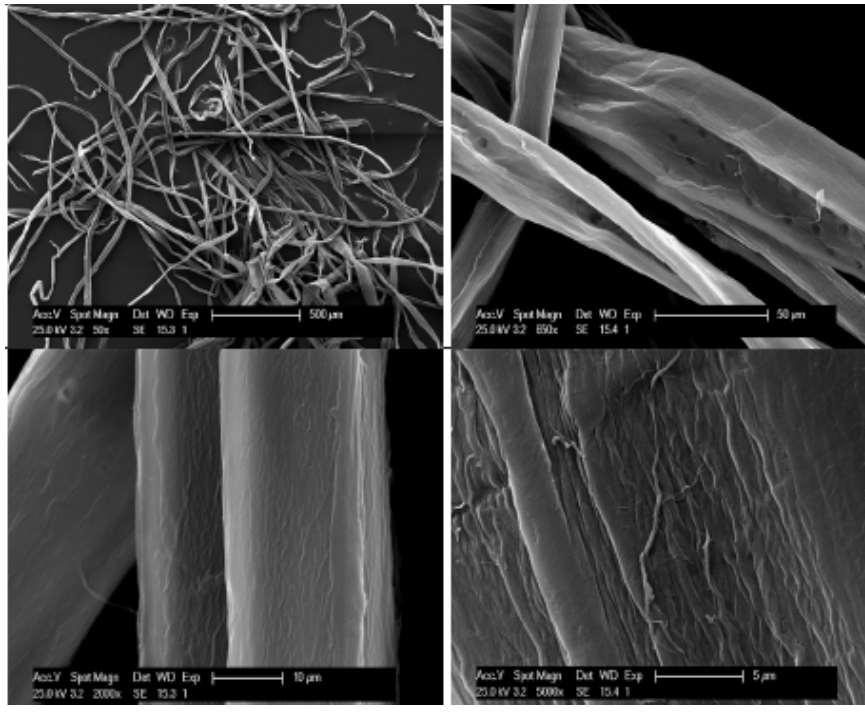

Figure 9a-d. XY-0-S (early sheared).

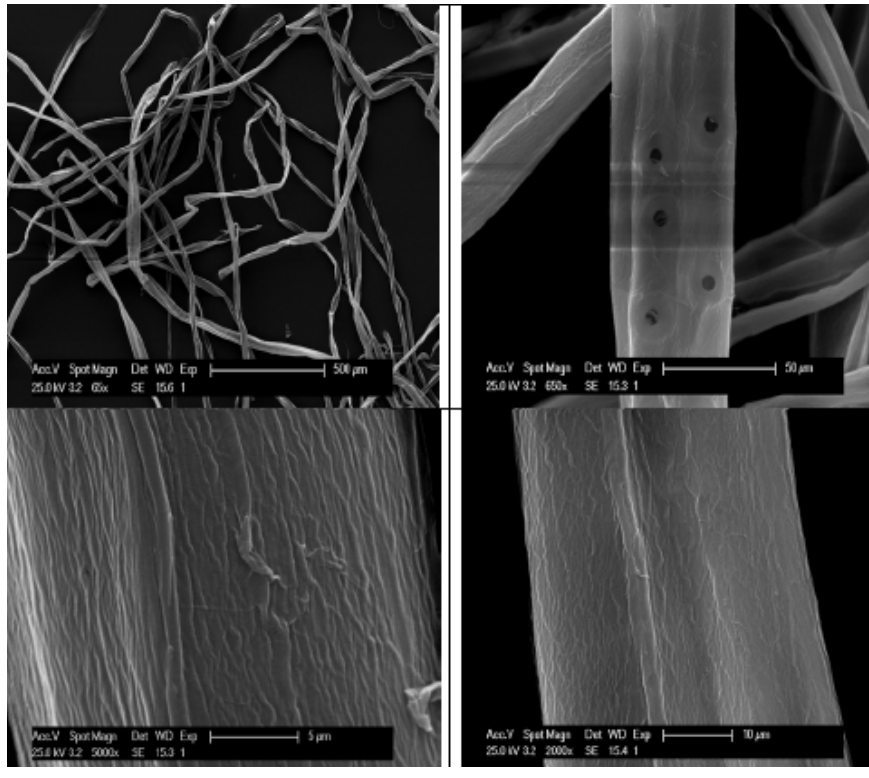

Figure 10a-d. XY-late-N (normal).

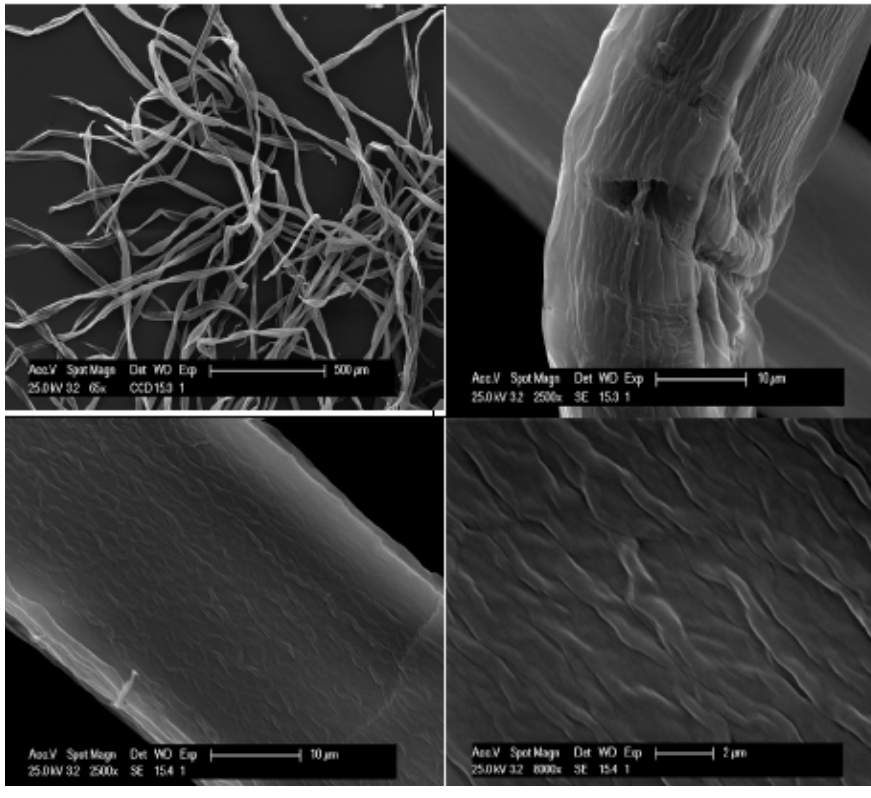

Figure 11a-d. XY- late-S (sheared).

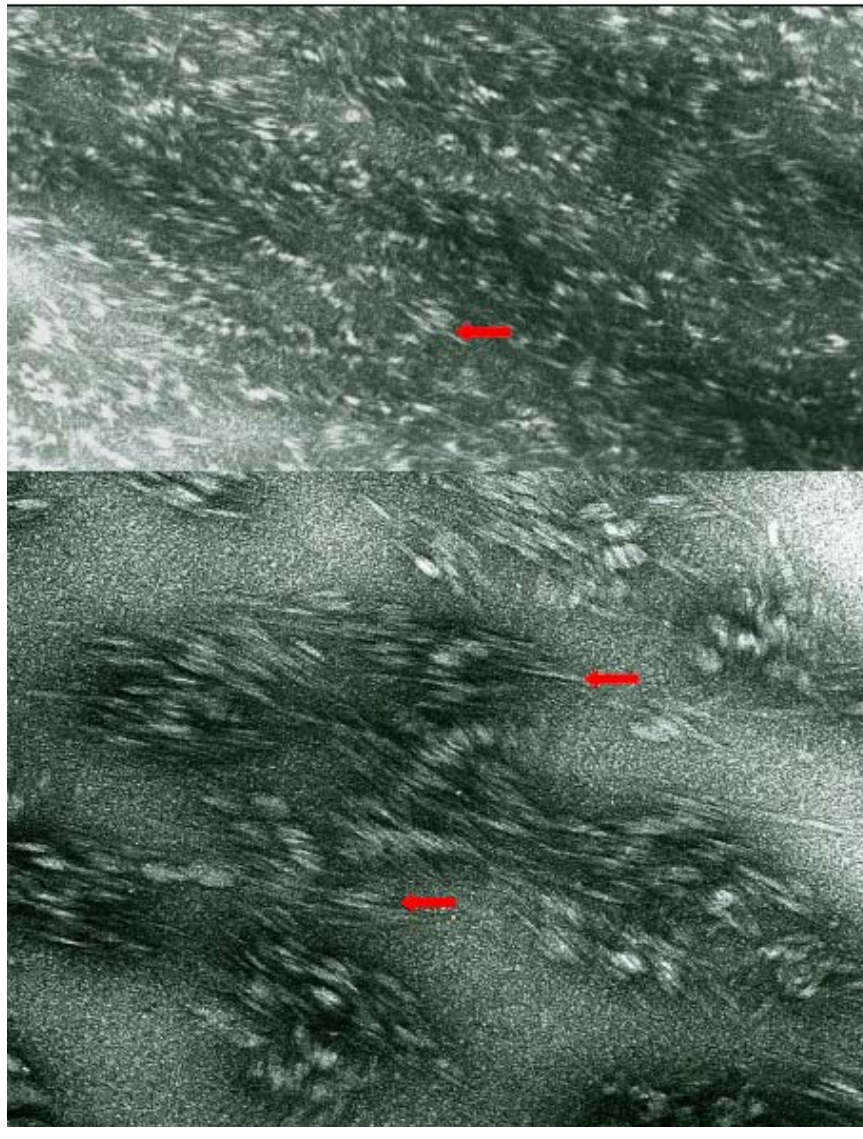

Figure 12. TEM observations of birch xylan film showing mixture of amorphous and rod-like (arrows) forms.

APPENDIX 5. EVALUATION OF XYLAN DISTRIBUTION BY FTIR MICROSCOPY

Anne-Mari Olsson, Lennart Salmén, Innventia

Aim: The purpose of this work was to investigate how added xylan was distributed over the surface of the fibres as well as see if any differences in the localisation of xylan with respect to damaged areas could be noted between mechanically sheared fibres and fibres not subjected to such forces.

Background: It is well known that xylan added in the kraft cooking is deposited on the surfaces of the fibres. However, so far little is known with regard to the distribution of this added xylan as well as to the form of the added xylan depositions. Imaging FTIR-microscopy offers here a possibility for investigating the spatial localisation of xylan both on the surfaces of fibres as well as for an overall amount across fibres based on the specific absorption bands of the xylan polymer. Previous studies using imaging FTIR-microscopy have shown that orientation distributions of xylan using these specific absorption bands may be easily followed (Stevanic, Salmén 2009).

Material

Single fibres from the chemical pulps with different amounts of xylan additions were dried and glued onto glass slides. Both earlywood and latewood fibres were prepared from the different pulps; Ref, XY-0, XY-late and PR.

Method

Surface measurements

The xylan distribution on 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 detector. A CCD camera was used to display the area of interest before it was irradiated with IR light.

Overall composition

Over a length of 1 mm of the whole fibre width, measurements in transmission were taken in order to evaluate the distribution of xylan throughout the entire fibre thickness. The same FTIR system as for the surface measurements was

used with a MCT detector. In transmission mode the resolution is 6.25 by 6.25 μm.

Evaluation

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. In *Figure 1* an example of a typical spectrum from the surface of a fibre is shown together with a spectrum from the birch xylan used as additive in the cook. Generally a typical peak for xylan should be 1740 cm^{-1} which corresponds to the carboxylic acid. Evidently this peak does not occur in the xylan tested. At 1455 cm^{-1} vibrations in the orthogonal CH_2 mainly assigned to xylan occur. In these spectra this peak is seen as a shoulder overlapped by other peaks. As a reference peak the cellulose vibration from the crystalline part at 1370 cm^{-1} was used.

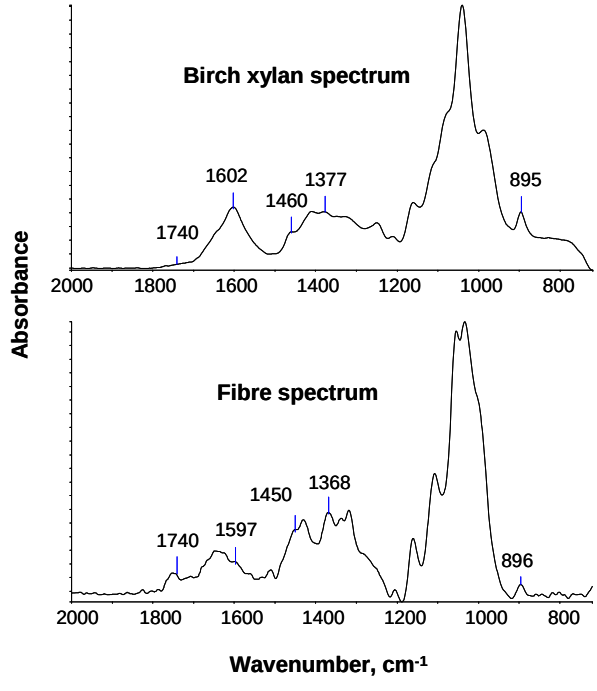

Figure 1. Example of spectra from the surface of a fibre and from the birch xylan added during the cook.

In order to handle the overlapping peaks interfering with the 1455 cm^{-1} peak of the spectra, the second derivative curve was used which results in a peak around 0 for the shoulder. For mapping the distribution of xylan, the band ratio of the area from 1450-1460 cm^{-1} divided by the cellulose reference peak area 1360-1380 cm^{-1} was used. These two peak areas are indicated in *Figure 2*.

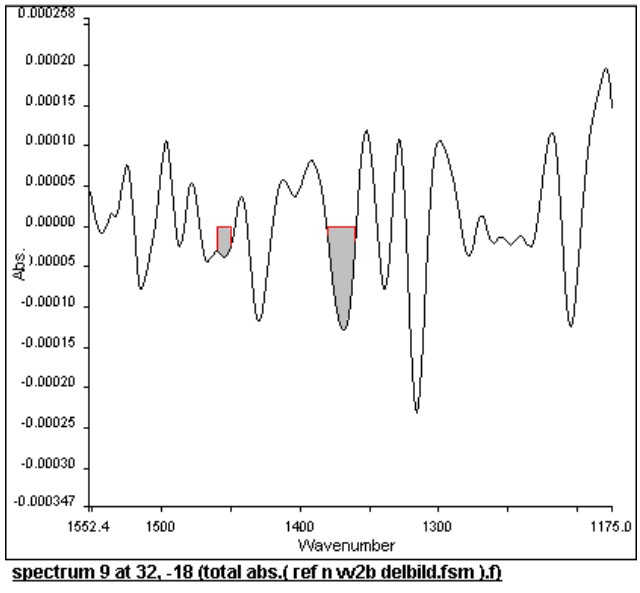

Figure 2. The second derivative curve for a spectrum. The shoulder at 1455 and the peak at 1370 cm^{-1} are indicated in grey.

From this band ratio, xylan intensity maps were obtained. In the images shown, the border of the width of the fibre is not included in the maps. For each pulp, at least 5 different fibres were evaluated. Also an average spectrum for each sample was calculated and the same xylan/cellulose band ratio was determined for each fibre surface area in order to obtain mean values of xylan coverage. For each pulp type, an average of the band ratio for earlywood and latewood fibres respectively was calculated and the values and standard deviations are showed in *Table 1*.

Results

Examples of intensity maps for the different types of fibre are shown in *Figure 3*.

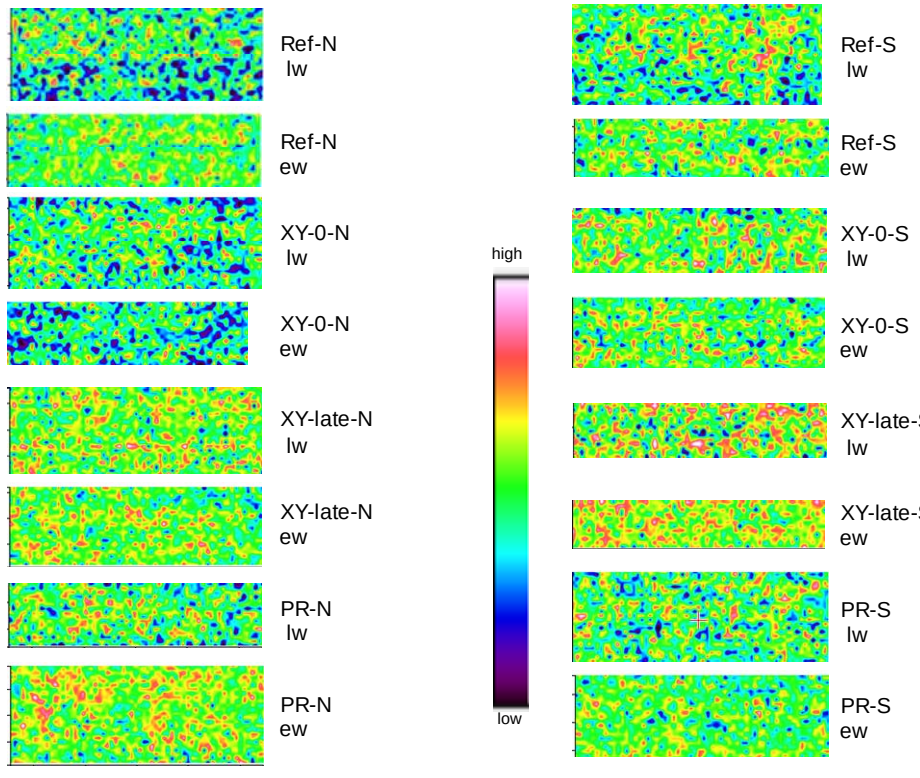

Figure 3. Intensity maps displaying the distribution of xylan on the fibre surface using the same scale for the different pulps. Red colour represents high amounts of xylan and black low amounts (lw=latewood, ew=earlywood).

The xylan seems to cover the entire surface of the fibres and to be distributed as small aggregates. All of the fibres showed similar patterns. However the average amount of xylan was different in the different pulps, i.e. the overall colour differs. In *Table 1*, the average band ratio of (1450-1460 cm^{-1})/(1360-1380 cm^{-1}) which corresponds to the xylan content is presented together with the standard deviation.

Table 1. The average band ratio of (1450-1460 cm^{-1})/(1360-1380 cm^{-1}) for the fibre surfaces of the different pulps (lw=latewood, ew=earlywood)

Fibre	**N – ew**	**N – lw**	**S – ew**	**S – lw**
Ref	0.24 +/-0.04	0.19 +/-0.04	0.28 +/-0.05	0.23 +/-0.05
XY-0	0.20 +/-0.05	0.23 +/-0.06	0.27 +/-0.03	0.27 +/-0.03
XY- late	0.26 +/-0.02	0.24 +/-0.07	0.28+/-0.03	0.26 +/-0.07
PR	0.28 +/-0.04	0.26 +/-0.02	0.23 +/-0.03	0.23 +/-0.04

In *Figure 4* these average band ratios showing the xylan content on the surfaces are shown as bars for the different treatments. The scatter in the results is large, but as an indication the sheared pulps had more xylan on the surface than the normal unsheared pulp. Also the earlywood fibre surfaces had in general more xylan than the latewood fibre surfaces.

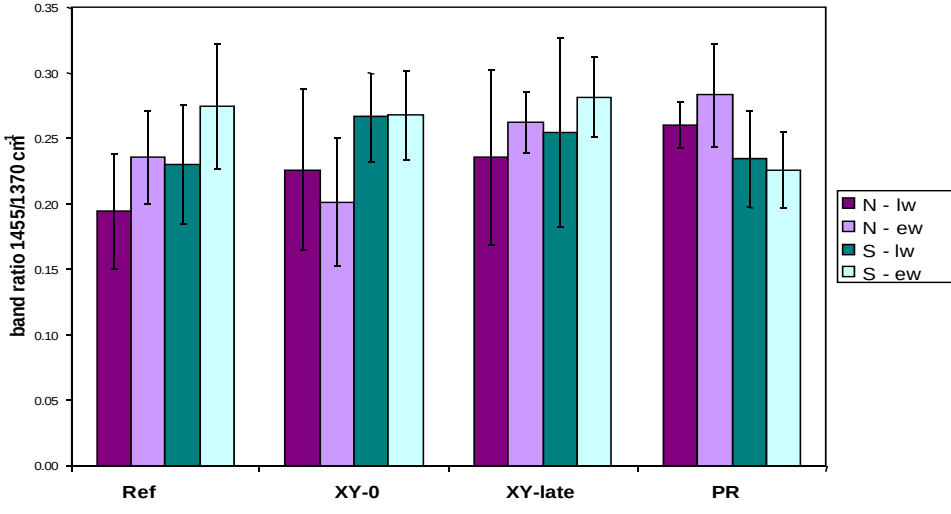

Figure 4. The average amount of xylan on the surface of the different fibres with standard deviation (lw=latewood, ew=earlywood).

A statistical evaluation with Student t-test is presented in *Table 2*. From this analysis it is evident that the differences between sheared and normal pulp and the effect of the treatments are not statistically significant.

Table 2. Result of a Student t-test for the difference between normal and sheared pulp for latewood fibres

Latewood, N/S	**Mean value difference**	**95 % confidence interval**
Ref	0.036	0.052
XY-0	0.040	0.047
XY-late	0.019	0.069
PR	0.026	0.033
Latewood N, Ref/XY-0;XY-late;PR		
XY-0	0.032	0.058
XY-late	0.042	0.063
PR	0.066	0.039

Overall composition

The overall xylan distribution was measured for one fibre of earlywood and latewood respectively on all the 8 types of pulp. Due to the thickness of the latewood fibres these were difficult to evaluate, and therefore only results from the earlywood fibres are shown. In *Figure 5* the eight pulps are represented by three images. The image to the left is the band ratio image (1450-1460 cm^{-1})/(1360-1380 cm^{-1}) showing the xylan amount. In the middle, the microscopy image of the fibre is shown and to the right the total absorbance image showing the amount of material at each spot measured. Evidently the whole width of the fibres were not in all cases covered but a general idea about the evaluation of xylan distribution can still be made. It seems clear that xylan is not evenly distributed along the fibre. Areas with high or low xylan content are however not clearly connected to any structure seen in the microscopy image. To make an accurate evaluation more than one fibre for each pulp would have been needed.

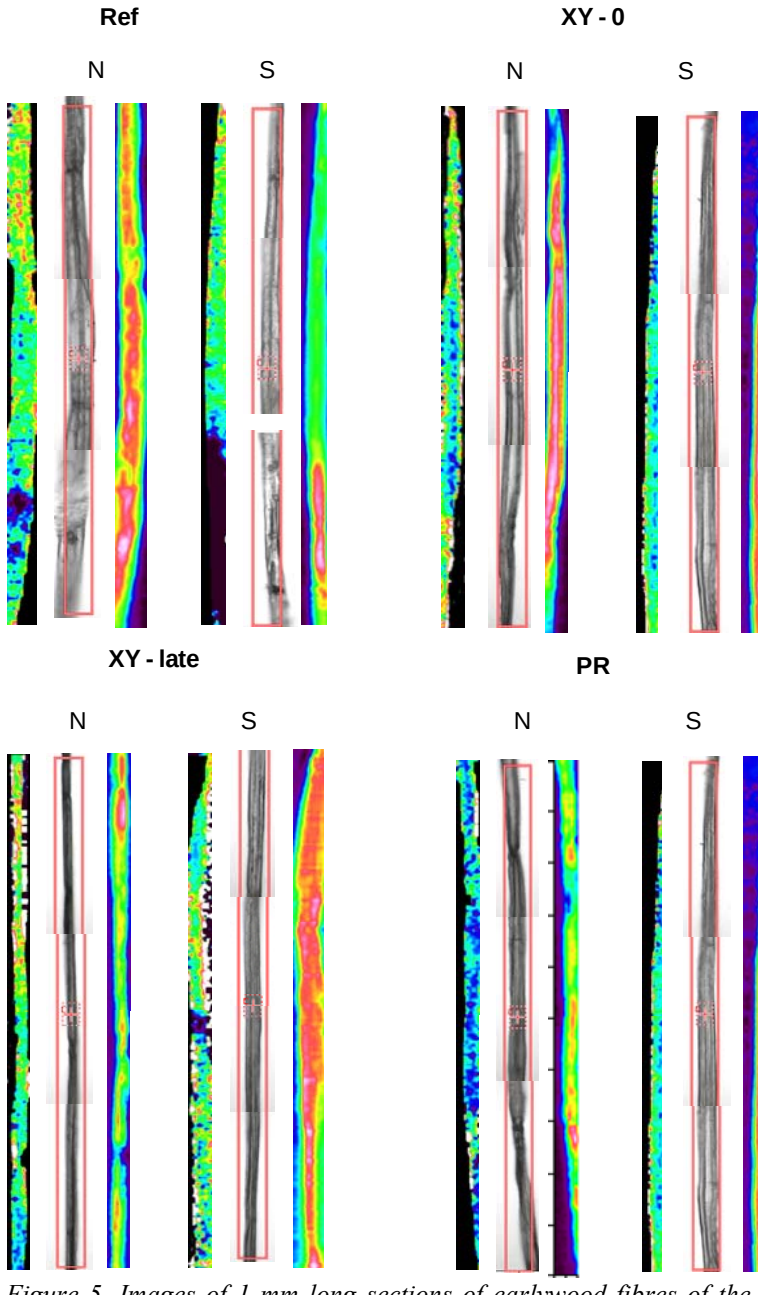

Figure 5. Images of 1 mm long sections of earlywood fibres of the 8 pulps. Each group shows from left to right: the xylan distribution image, i.e. the band ratio (1450-1460 cm^{-1})/(1360-1380 cm^{-1}), the microscopy image, and the total absorbance image.

References

Stevanic S. and Salmén, L. 2009. Orientation of the wood polymers in spruce wood fibres. Holzforschung 63, (5) 497-503.

APPENDIX 6. HCL AND XYLANASE TESTS ON SOFTWOOD KRAFT PULPS IN XYLAN EXPERIMENTS

Paul Ander, SLU

Background

In industrial kraft pulping of softwood fibres a large loss in fibre strength take place as compared with laboratory kraft cook. Studies indicate that reasons for strength loss are fibre damage at both micro- and ultrastructural levels (dislocations and kinks) induced during processing. Research has shown that softwood xylan dissolved into the alkaline cooking liquor may precipitate back onto pulp fibre surfaces giving better pulp strength. An interesting approach is thus to use hardwood or softwood xylan already produced by the pulp and paper industry as a strength enhancing additive. In the present study, unbleached laboratory produced spruce kraft pulps were used to test the influence of adding birch hardwood xylan (BX, 10 g/l) to kraft cook, on pulp and paper properties. In order to simulate industrial conditions shearing compression was done during the laboratory cooking of the used pulps (Salmén and Lundqvist, 2009).

As one part of the project, differences in sensitivity to acid treatment with or without added birch xylan on the kraft pulp fibres, were studied by the HCl method. An attempt was also done to relate release of reducing sugar from spruce fibre xylan and/or from bound birch xylan to possible increased pulp and paper strength. In this connection, unbleached kraft cooked pulp fibres, with or without added birch xylan, were also studied for release of reducing sugars using an endoxylanase that cleaves the xylan backbone.

Experimental

Pulps, birch xylan, xylanase and chemicals

Unbleached spruce kraft pulps were produced by the project industrial members (see *Appendices 1N-1H*).Unbleached birch kraft pulp was obtained from Irina Rauvanto, Fibrea Oy, FI-53850 Lappeenranta, Finland. Birch xylan was purchased from Sigma (Sigma X-0502, birchwood 4-O-methyl glucurono-xylan). This xylan was added to spruce kraft pulp liquors at a concentration of 10 g/l.

Xylanase X2753 from *Thermomyces lanuginosus* was bought from Sigma (Pentopan Mono BG, 37278-89-0) with powder activity ≥ 2500 U/g, a recombinant enzyme expressed in *Aspergillus oryzae*. Originally a product

of Novozyme Corp (EC No. 253-439-7 and thus an endo-1,4-β-xylanase producing xylo-oligosaccharides and xylose).

Disodium 2,2'-bicinchoninate for the BCA reducing sugar analyses was purchased from ICN Biomedicals Inc., Ohio, USA via Chemicon, Malmö and Stockholm. All other chemicals were of proanalysis quality.

HCl method
Normally, 150 mg dry-weight of never-dried pulps were swelled in 20 ml water for 10-15 minutes during stirring, 20 ml 2N HCl added and incubated at 80,5° C ± 0.5 for 4h. The 100 ml Erlenmeyer flasks (duplicates or triplicates) were then allowed to cool during 30 min. final stirring. The acid was washed out on Munktell paper filters 103 using 0.1 M phosphate buffer pH 7.0. The fibres were then transferred to small plastic vials and sent for fibre length analyses on the FibreMaster instrument at Södra Cell Värö. Cleavage per fibre was calculated using fibre lengths obtained in water (L_0 mm) and HCl (L mm) as reported earlier (Ander et al. 2008).

BCA reducing sugar assay
Fibre dry-weights used in this assay were determined in duplicate by drying 200-500 mg fibres at 105° C until no further weight loss was obtained. Usually 150 mg of pulp fibres were used in the treatments. Filtrates after acid or xylanase fibre treatment were diluted 20 times and assayed for reducing sugars as given by Garcia et al. (1993). Absorbance was measured at 560 nm and reducing sugar concentration determined from a glucose standard curve. The assay can detect 0.45 mg/l of glucose.

Xylanase assay
Endoxylanase activity against 150 mg unbleached spruce kraft pulp, 150 mg unbleached birch kraft pulp and 30 mg Sigma birch xylan (BX) were tested in 40 ml acetate buffer pH 6.5 at 45° C for 4h and 18h. From a stock solution of 50 mg / ml water of Sigma xylanase from *T. lanuginosus*, one ml (2.5 units) of xylanase suspension was taken during stirring and pipetted into stirred 100 ml substrate flasks. Flasks were then incubated slowly reciprocal at 45° C. The buffer solutions were filtered off through Munktell filter paper 103 and amount of reducing sugars released measured using the BCA assay at 560 nm (Garcia et al. 1993).

Other fibre treatments
Shearing compression of spruce kraft pulps were done at Innventia as described by Salmén and Lundqvist (2009) in order to simulate industrial cooking.

Mechanical peeling of kraft pulps in a disintegrator was done at a consistency of 3% at EKA Chemicals (Anette Heijnesson-Hultén). Primary and secondary fines were removed from the fibre surface and separated from the pulp by a sieving procedure with a nylon sieve of size 100 µm. The fibres collected on the sieve were air-dried and used in kraft cooking and xylan tests (see pulp production). Before use in HCl and xylanase assays, the dry pulp fibres were swelled and defibrated for 3-4 h during stirring in water or buffer pH 6.5.

Results

HCL AND BCA ASSAYS ON EIGHT SPRUCE KRAFT PULPS IN BIRCH XYLAN TESTS

Eight unbleached spruce kraft pulps 1a-4a and 1b-4b given in *Table 1* were treated with HCl using the HCl method to see if birch xylan (BX) added early or late in the cook gave different fibre lengths and fibre cleavage as compared with no addition of BX as in pulp 1a. Pulp 4a (PR) where spruce xylan had been taken out early and added back late in the cook was also studied. Pulps 1b-4b, with additional shearing was also included in the investigation. *Table 1* and *Figures 1, 2* show that without shearing, BX added to the cook either early (2a) or late (3a) seemed to protect the spruce pulp fibres from HCl attack compared with reference 1a as given by fibre lengths and cleavage per fibre (0.82 0,565 and 0,65). Reference pulp and softwood pulp xylan taken out early and added back late (1a and 4a) to the cook had similar HCl sensitivity

Table 1. Eight spruce kraft pulps and experiments with birch xylan (pulps 2 and 3) and softwood xylan (pulp 4) with or without shearing. HCl method at 81° C with 150 mg fibres in duplicates

Kraft pulps	LWFL (mm) H_2O	LWFL (mm) HCl	Cleavage / fibre
1a. Ref	2.494/2.465 ***2.48***	1.374/1.349 ***1.36***	**0.82**
2a. XY-0	2.451/2.449 ***2.45***	1.542/1.589 ***1.565***	**0.565**
3a. XY-late	2.342/2,413 ***2.38***	1.447/1.426 ***1.44***	**0.65**
4a. PR	2.430/2.380 ***2.41***	1.340/1,346 ***1,343***	0.80
1b. Ref + shearing	2.468/2,508 ***2.49***	0,92/0,95 ***0,94***	1,65 **2,0x**
2b. 2a + shearing	2.412/2.455 ***2.43***	1,028/1,045 ***1,036***	1,35 **2,4x**
3b. 3a + shearing	2,548/2,450 ***2.50***	0,799/0,894 ***0,846***	1,96 **3,0x**
4b. 4a + shearing	2.447/2.484 ***2.465***	0,848/0,846 ***0,847***	1,91 **2,4x**

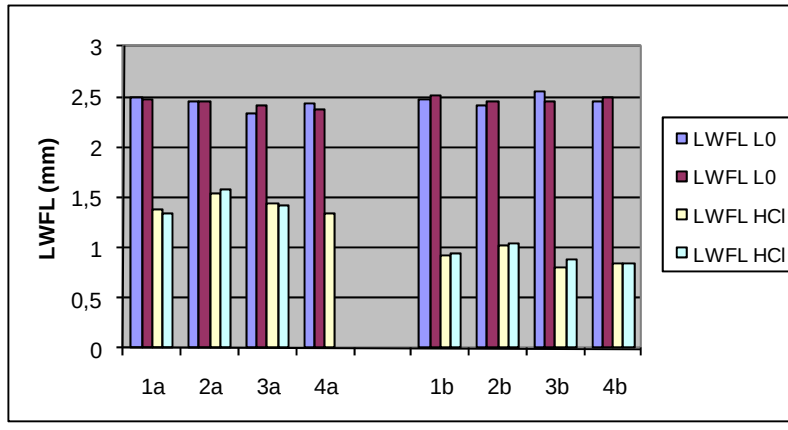

Figure 1. Fibre lengths of softwood pulp fibres before and after HCl treatment. Duplicate values. Normal fibres 1a-4a and sheared fibers 1b-4b as given in Table 1.

Shearing did not change fibre lengths for the control in water. However, after shearing stronger HCl cleavage was obtained which is most easily seen in *Figure 2*. For sheared pulps, the protecting effect of birch xylan was not visible when the xylan was added late (3b) and fibre lengths decreased from 0.94 to 0.85 mm; fibre cleavage increased from 1.65 to 1.96.

Sheared pulp 4b with softwood xylan gave about the same result as for 1b. In general the sheared pulps were very sensitive to the acid giving decreased fibre length and increased cleavage. After HCl normal fibres were 1.34-1.59 mm, while sheared fibres were 0.85-1.05 mm giving increased fibre cleavage in all cases. This effect is not surprising and must be due to formation of more dislocations after shearing and compression of the fibres.

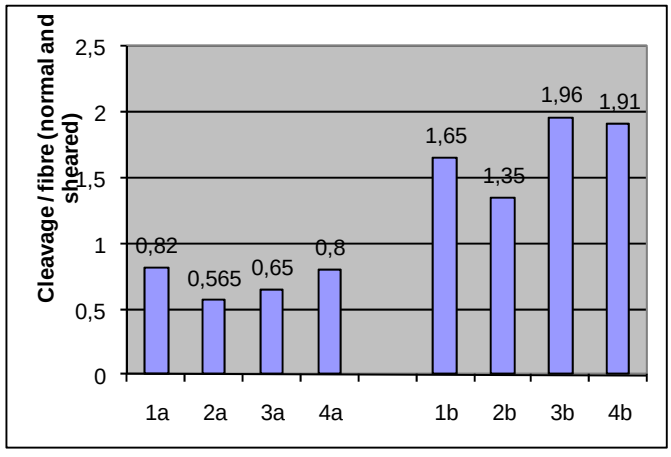

Figure 2. Cleavage per fibre for the eight softwood pulp fibres in Table 1 and Figure 1.

Table 2. Release of reducing sugar from eight spruce kraft pulps and experiments with birch xylan (pulps 2 and 3) and softwood xylan (pulp 4) with or without shearing. BCA method

Kraft pulp treatment	**BCA 560 nm H_2O**	**BCA 560 nm HCl**
1a. Ref	0,533/0.444 ***0,49***	0,568/0,564 ***0,57***
2a. XY-0	0,460/0,450 ***0,455***	0,587/0,665 ***0,63***
3a. XY-late	0,470/0,479 ***0,47***	0,584/0,581 ***0,58***
4a. PR	0,528/0,471 ***0,50***	0,564/0,533 ***0,55***
1b. Ref + shearing	0,592/0,496 ***0,544***	0,499/0,606 ***0,55***
2b. 2a + shearing	0,463/0,521 ***0,49***	0,531/0,530 ***0,53***
3b. 3a + shearing	0,500/0,449 ***0,475***	0,587/0,548 ***0,57***
4b. 4a + shearing	0,481/0,467 ***0,474***	0,597/0,621 ***0,61***

Table 3. Cleavage/fibre for eight spruce kraft pulps as related to % xylose in the fibres and on the fibre surfaces. % xylose is a measurement on the amounts of xylan

Kraft pulps	Cleavage/fibre	% xylose	% xylose on surface
1a. Ref	0.82	7.5	16.9
2a. XY-0	**0.565**	**11.0**	**21.4**
3a. XY-late	**0.65**	**9.7**	**22.2**
4a. PR	0.80	7.6	19.3
1b. Ref + shearing	1,65	7.4	13.6
2b. 2a + shearing	1,35	11.2	20.8
3b. 3a + shearing	1,96	10.3	21.2
4b. 4a + shearing	1,91	7.6	16.1

Table 2 shows that pulps 2a and 3a (XY-0 and XY-late) with added BX in water did not give more sugar from the fibres than pulp 1a. For HCl, 2a gave slightly more sugar (0.63) than 1a (0.57 but this difference is not significant. The reason is probably that too little birch xylan was bound to the pulp fibres in pulps 2a and 3a to be detected (see Discussion and Daniel and Kaňuchová, *Appendix 3*, this report). HCl gave more release of sugar than water.

Table 3 shows that pulps with added xylan (pulps 2 and 3) had most xylan. For the normal pulps 2a XY-0 and 3a XY-late, cleavage per fibre was smaller than for the other pulps as given also in *Table 1*. The trends for pulps 2b and 3b were uncertain.

XYLANASE TESTS ON BIRCH XYLAN, BIRCH KRAFT PULP AND SPRUCE KRAFT PULPS

Positive control on birch xylan and xylanase tests on unbleached birch kraft pulp fibres.

In order to determine if it was possible to measure release of reducing sugars from the Sigma birch xylan (BX), it was treated with *Thermomyces lanuginosus* xylanase at different pHs and times. It was found that the xylanase released sugars from BX and that the substrate BX was saturated at 1.25 xylanase units at pH 6.5 after 4h at 45° C. Colour formation was measured using 2,2'-Bicinchoninate, BCA at 560 nm.

The results for xylanase treatment of unbleached birch kraft pulp and birch xylan are shown in *Table 4*. The xylanase released sugars from both the birch kraft pulp and from the birch xylan.

Table 4. Release of reducing sugars from unbleached birch kraft pulp fibres (BKP 150 mg) and birch xylan (BX 30 mg) using xylanase at pH 6.5 for 4 and 18 h at 45° C

Substrates	Xylanase	Abs 560 nm after 4h	Abs 560 nm after 18h
BKP0	--	0.352	0.378
BKP1	+	0.401	0.419
BKP2	+	0.407	0.422
BX0	--	0.417	0.392
BX1	+	0.445	0.512
BX2	+	0.490	0.512

Release of reducing sugars from unbleached spruce kraft pulps by xylanase The results are shown in *Tables 5a and 5b* below.

Table 5a. Release of reducing sugars from 150 mg unbleached spruce kraft pulp fibres ± xylanase at pH 6.5 for 18 h at 45° C

Pulps exp 1 (150 mg ; 45°C)	Buffer only. Released sugar and fibre weight loss*	Buffer + Xylanase. Released sugar and fibre weight loss*
1a Reference	0.455/0.462 ***0.459 -3 mg***	0.474/0.477/0.467 ***0.473 -5 mg***
2a Xylan added early	0.449/0.456 ***0.453 -17 mg***	0.492/0.480/0.476 ***0.483 -21 mg***
3a Xylan added late	0.434/0.454 ***0.444 -4 mg***	0,514/0.484/0.488 ***0.495 -12 mg***

* Fibre weight loss determined from mean weight of 3 separate filter papers 103 and drying of each filter papers with fibres.

Table 5b. Release of reducing sugars from 150 mg unbleached spruce kraft pulp fibres ± xylanase at pH 6.5 for 18 h at 55° C

Pulps exp 1 (150 mg ; 55°C)	Buffer only. Released sugar and fibre weight**	Buffer + Xylanase. Released sugar and fibre weight**
1a Reference	0.406/0.406 ***0.406 + 10 mg***	0.432/0.439/0.430 ***0.434 + 10 mg***
2a Xylan added early	0.417/0.417 ***0.417 - 10 mg***	0.436/0.434/0.432 ***0.434 - 13 mg***
3a Xylan added late	0.417/0.445 ***0.434 + 9 mg***	0.442/0.460/0.434 ***0.444 + 8 mg***

** Fibre weights (loss or increase) determined using pre-weighed filter papers for each fibre sample

T. lanuginosus xylanase released only small amounts of xylan sugars from the spruce xylan or added BX. The amounts were about 1-2, 5 mg without xylanase and 2.5-3 mg with xylanase according to the BCA-glucose standard curve. Differences between 1a Reference and 2a XY-0/3a XY-late were small, indicating small amounts of BX (see Discussion). Slightly less reduced sugars were released at 55° C as compared with 45° C, although stronger xylanase activity has been reported at 55° C with this enzyme (Alam et al. 1994; Cesar and Mrša 1996; Singh et al. 2000).

Fibre weights after xylanase treatment were measured using filter papers 103 as given in *Tables 5a and 5b*. Pulp 2a (XY-0) gave 10-21 mg weight loss for

both experiments -*with or without*- the presence of xylanase. This weight loss was however not seen as a corresponding amount of reducing sugars, which is surprising. The other pulps (1a and 3a) gave small weight losses or increase in weight. The xylanase used does not contain cellulase (Alam et al. 1994; Singh et al. 2000).

HCL AND XYLANASE TESTS ON PEELED SPRUCE KRAFT PULPS

The unbleached spruce kraft pulps were peeled, which means that fibre surface material such as primary and secondary fines were removed by mechanical peeling in a disintegrator and fractionated. It was of interest to see how this treatment affected HCl sensitivity and release of sugars by acid and xylanase. The results of the HCl method are shown in *Table 6*.

Table 6. Fibre lengths and cleavage fibre for peeled spruce kraft pulps 1a-4a and 1b-4b in the standard HCl method

Peeled kraft pulps	LWFL (mm) H2O	LWFL (mm) HCl	Cleavage/fibre
1a. Ref	2.48	0.687	**2.61**
2a. XY-0	2,45	0.804	**2.05**
3a. XY-late	2.38	0.788	**2.02**
4a. PR	2.41	0.665	**2.62**
1b. Ref + shearing	2.49	0.532	**3.68 (1.41x)****
2b. 2a + shearing	2.43	0.599	**3.06 (1.49x)****
3b. 3a + shearing	2.50	0.571	**3.38 (1.67x)****
4b. 4a + shearing	2.465	0.496	**3.97 (1.52x)****

**As compared with not sheared pulps 1a-4a.

As seen in *Table 6*, the peeled pulps 2a and 3a, with added xylan, had longest fibres and less cleavage and thus were not as HCl sensitive as pulp 1a (cleavage 2.05/2.05 vs 2.61). Pulps which were both peeled and sheared (2b and 3b) were also less sensitive to HCl than pulp 1b. Except for pulp 3b in *Table 1* this result is similar as obtained for unpeeled pulps (*cf. Table 1 and 6*). Again shearing/ compression gave stronger fibre cleavage by the acid treatment (*Table 6*). One can remark that it is not possible to avoid a general fibre destruction caused by fibre shearing and compression by adding birch xylan under the conditions used as seen in *Tables 1 and 6*. However, a certain decrease in fibre destruction by acid treatment is obviously obtained by adding birch xylan. The influence of birch xylan addition on standard pulp and paper strength properties are analysed in other chapters of this report (*see Appendix 1H*).

Table 7. Release of reducing sugars from peeled spruce kraft pulps 1a-4a and 1b-4b with and without T. lanuginosus xylanase at pH 6.5 for 18 h at 45° C

Peeled kraft pulps	BCA Abs 560 nm in Buffer pH 6.5	BCA Abs 560 nm + Xylanase pH 6.5
1a. Ref	0.421	0.431/0.437 ***0.434***
2a. XY-0	0.405	0.465/0.489 ***0.477***
3a. XY-late	0.443	0.458/0.467 ***0.467***
4a. PR	0.432	0.440/0.436 ***0.442***
1b. Ref + shearing	0.460	0.447/0.437 ***0.442***
2b. 2a + shearing	0.427	0.454/0.455 ***0.455***
3b. 3a + shearing	0.436	0.442/0.445 ***0.444***
4b. 4a + shearing	0.422	0.437/0.437 ***0.437***

Table 7 shows that xylanase treatment gave more sugars from pulps 2a and 3a (with added xylan) than pulp 1a (0.477 and 0.467 vs 0.434) corresponding to release of about 2.5 mg bound BX and/or spruce xylan. This result is surprising since it was anticipated that peeling should remove added xylan. Maybe mainly fibrils with little bound xylan were removed by peeling. For the peeled and sheared pulps 1b-4b no clear tendency was obtained.

Discussion

Results indicate that added birch xylan protected the pulp fibres from HCl attack. The presence of small amounts of birch xylan on the pulp fibre surface was indeed proven using FTIR (Olsson and Salmén, *Appendix 5*) and ELISA (Daniel et al. 2010; Daniel and Kaňuchová, *Appendix 2*) and was also found after carbohydrate analyses in the project. Earlier it was shown that bleached spruce kraft pulps (WURC Pulp 2000) with hemicellulose contents ranging from 8.1% to 20.8% had the smallest sensitivity against HCl treatment at the highest amount of hemicellulose 20.8% (Ander and Daniel 2006). Brännvall and Lindström (2007) concluded that fibres with higher xylan content and lower tear vs tensile index, seemed to endure mechanical treatment (high-intensity mixing) better than fibres with lower xylan content.

As the BCA assay lower limit is 0.45 mg/l (0.00045g/l) reducing sugars, only the highest amount of BX (0.001 g/l) detected by Daniel and Kaňuchová (*Appendix 2*) for Group 1 pulps 2b (XY-0 sheared) and 3b (XY-late sheared) can be detected by the BCA assay. No BX sugars can be detected for Group 2 pulps 2a and 3a (XY-0 and XY-late), and certainly not for the Group 1 pulps (1a,b XY-0 N and S; 4a,b PR N and S) in *Figure 2* in *Appendix 3*.

Pulp 2a (XY-0) gave 10-21 mg weight loss with or without xylanase treatment. This weight loss was however not seen as a corresponding amount of reducing sugars, which is surprising and should be further studied. Possibly, bound birch xylan or reprecipitated softwood xylan is rather resistant against hydrolytic degradation with HCl (personal communication, E. Brännvall 2010 in CRUW project) or against xylanase degradation explaining part of the low yield of reducing sugars. Local differences in dry-weights in different parts of the pulps could have affected the results.

The *T. lanuginosus* xylanase should be very suitable for degrading bound BX since xylo-oligosaccharides and xylose are formed in the enzymatic reaction (Lorenzo et al. 2009). Furthermore the enzyme is very active at pH 6.5 and at 45-55° C (Alam et al. 1994; Cesar and Mrša 1996; Singh et al. 2000).

Conclusions

- HCl method: Without shearing and birch xylan added early- or late (2a XY-0 and 3a XY-late) gave longer fibres.
- Sheared pulps were more sensitive to HCl than non-sheared pulps.
- BCA assay: Due to too low sensitivity, fibres with added BX (2a and 3a) did not give more reducing sugars than reference fibres 1a. HCl gave more reducing sugars than H_2O-treated pulps.
- Xylanase released sugars from BX and from unbleached birch and spruce kraft pulp fibres.
- Xylanase released rather little sugar (2-3 mg) from 150 mg unbleached spruce kraft pulp. This amount was not significantly larger for pulps where BX had been added (2a and 3a).
- Pulp 2a (XY-0) lost 10-17 mg in weight in incubation without xylanase and 13-21 mg in weight in the presence of xylanase; this amount was however not detected in the BCA assay.
- Peeled pulps after HCl: Addition of BX gave longer fibres and less fibre cleavage both with and without shearing.
- Peeled pulps + xylanase: Pulps 2a and 3a gave slightly more sugar than pulp 1a.

References

Alam et al. (1994) Production and characterization of thermostable xylanases by *Thermomyces lanuginosus* and *Thermomyces aurantiacus* grown on lignocelluloses. Enzyme Microb. Technol. 16 (1994) 298-302.

Ander, P., Hildén, L., and Daniel, G. (2008). Cleavage of softwood kraft pulp fibres by HCl and Cellulases. Bioresources 3(2), 477-490.

Ander, P. and Daniel, G. (2006). Dislocation counting and comparison of pulp fibre properties after HCl-treatment and fibre length determination. Proc. 5th Plant Biomechanics Conference, Stockholm, Vol. I. August 28 – September 1, 2006. Ed.: L. Salmén. STFI-Packforsk, Stockholm, Sweden, ISBN 91-86018-12-4, pp. 169-174.

Brännvall, E. and Lindström, E. (2007). The hemicellulose composition of pulp fibres and their ability to endure mechanical treatment. Tappi J. 6(10): 19-24.

Cesar, T. and Mrša V. (1996) Purification and properties of the xylanase produced by *Thermomyces lanuginosus*. Enzyme Microb. Technol. 19 (1994) 289-296.

Daniel, G., Kaňuchová, A., Ander, P. and Filonova, L. (2010). Visualisation and quantification of xylan on xylan treated kraft spruce pulp fibres using immunofluoresence microscopy, ELISA, TEM, HCl and Xylanase assays. 11th EWLP Hamburg, August 16 19, 2010.

Garcia, E., Johnston, D., Whitaker, J.R. and Shoemaker, S. (1993): Assessment of endo-1,4-beta-D-glucanase activity by a rapid colorimetric assay using disodium 2,2´-bicinhoninate. J. Food Biochem. 17: 135-143.

Lorenzo, M.L., Nierstrasz, V.A. and Warmoeskerken, M.M.C.G. (2009). Endoxylanase action towards the improvement of recycled fibre properties. Cellulose 16: 103-115.

Salmén, L. and Lundqvist, F. (2009). Effects of mechanical forces for strength delivery in kraft cooking. ISWFPC, Oslo, Norway.

Singh, S. et al. (2000) Relatedness of *Thermomyces lanuginosus* strains producing a thermostable xylanase. J. Biotechnol. 81 (2000) 119-128.

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"*.