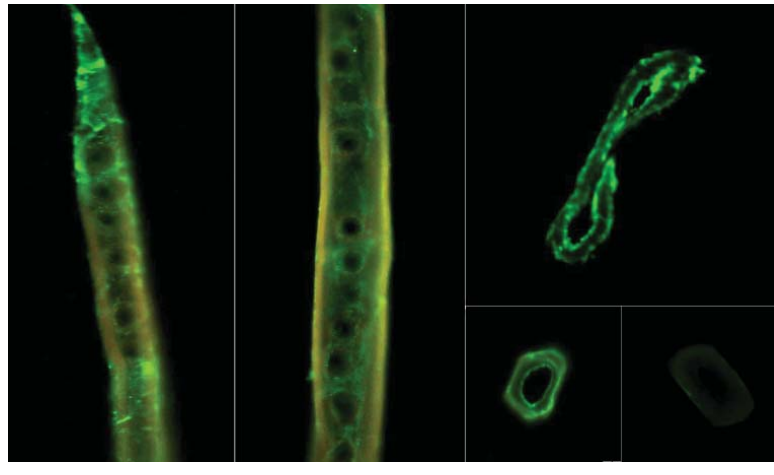
Chemical Pulping

The influence of xylan on the sensitivity towards fiber damage: Xylan added in the oxygen stage

Geoffrey Daniel, *SLU*, Anne-Mari Olsson, *Innventia*, Paul Ander, *SLU*, Andrea Kaňuchová, *SLU*, Lennart Salmén, *Innventia*, Lada Filonova, *SLU*

Karin Sjöström, *Södra*, Leif Boussard, *Stora Enso*, Staffan Berg, *Iggesund Paperboard, Holmen*, Anette Heijnesson-Hultén, *Eka Chemicals*, Curt Lindström, *Smurfit Kappa*



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Summary

The aim of this study was to investigate if extra xylan added during oxygen delignification of kraft spruce fibers could contribute to reduce the effect of fiber damage introduced during the cook.

Two pulps were produced, one with and one without mechanical treatment at the end of the cook. The pulp produced without mechanical treatment was oxygen delignified with xylan added in the oxygen stage. The pulp produced with mechanical treatment was oxygen delignified both with and without xylan added.

Results did not indicate that xylan added in the oxygen stage could repair the already introduced fiber damage.

Added xylan had a fiber straightening effect as seen earlier when xylan was added in the cook. Xylan added in the oxygen stage resulted in improved tensile strength development. However, the negative effect from introduced mechanical treatment still influenced the strength properties more than could be compensated by the added xylan.

SEM-images could not identify any differences between the investigated pulps. It seems likely that the birch xylan added forms a uniform but non-homogeneous coating on the fiber surfaces. Possibly the xylan has penetrated into the fiber wall to a greater extent compared to previous studies when xylan was added in the cook.

Contents

1 Background

Previously the influence of xylan on the sensitivity towards fiber damage during mechanical action in the cook has been investigated (Daniel et al. 2010). Kraft pulps from spruce with different amounts of xylan were produced in the laboratory, either by adding birch xylan at different stages in the cook process or by redistribution of the spruce xylan liquor. At the end of the cook, fiber damages were introduced by subjecting the fibers to shear and compression forces. No direct positive indications were found that the extra xylan added during the cook could lower the amount or affect the introduced fiber damage.

In this study, pulps were produced with or without mechanical treatment in the cook without birch xylan added. Instead, the xylan was added in the subsequent oxygen stage. The aim was to investigate if added xylan could "heal" the damaged fiber areas (introduced in the cook) and by that contribute to improved strength properties. The hypothesis behind the study was that the increased removal of lignin could result in a more open structure that would facilitate xylan penetration into the fiber wall to a greater extent than during the cook.

2 Experimental

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

Pulps were produced at Innventia, using a digester equipped with a device that can introduce mechanical forces, i.e. shear and compressive forces on the pulp. The digester is further described by Salmén and Lundqvist (2011). Two pulps were produced, one with and one without mechanical treatment at the end of the cook. The two pulps were produced using exactly the same conditions during the cook i.e. half of the pulp in the digester was subjected to mechanical forces and half was not. To obtain enough pulp, five cooks were performed and merged. Primary data for all cooks are shown in *Appendix 1a*. The two pulps were cooked to about the same kappa number of around 32. The cooking conditions used are shown in *Table 1.*

Table 1. Cooking conditions

	Compr, %	**EA, %**	**H-factor**	**Cooking time[1] min**	**Residual OH-g/L**	**Kappa no N[2], S[3]**
N-, S-	45	21	2000	197	3,9	30.5, 30.8

[1] *at 165° C*
[2] *N cooks without mechanical treatment*
[3] *S cooks with compression and shearing forces for 2 minutes, 15 minutes before the end of cook*
All data are average values from 5 cooks.

The pulps were further oxygen delignified. The pulp produced with mechanical treatment was oxygen delignified both with and without birch xylan added in the oxygen stage. The pulp produced without mechanical treatment was oxygen delignified with only xylan added. To obtain the same kappa number, a longer time was needed for the pulps with added xylan in the oxygen stage *(Table 2)*. The birch xylan used was obtained from Sigma Aldrich. Data on carbohydrate composition, molecular weight and degree of substitution for the birch xylan used can be found in *Appendix 1a* in Daniel et al. (2010). The birch xylan addition was 7.33% which is equivalent to a concentration of 10 g/L in the liquor used in the oxygen stage. The conditions used in the oxygen stage are showed in *Table 2*.

Table 2. Conditions used during oxygen delignification

	Temperature °C	Conc. %	Time min	$MgSO_4$ %	NaOH %	Final-pH
S-0	100	12	110	2.5	3.5	10.3
S-X	100	12	140	2.5	3.5	10.1
N-X	100	12	140	2.5	3.5	10.1

2.1 ANALYSIS

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

Fiber properties and strength properties were evaluated after PFI-refining. The analyses were performed by Eka, except analysis of rewetted zero-span which was performed by Södra on sheets prepared at Eka. All strength properties are shown in *Appendix 1b*.

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

Visualization of adsorbed xylan on whole fibers and on cross section 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 of 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 pulps were evaluated regarding introduced damage and strength properties after oxygen delignification. When producing the pulps, the variables were with and without mechanical treatment in the digester and birch xylan addition or absence in the oxygen stage. In total, three pulps were included in the study (see *Table 3* for description of the pulps).

The aim was to investigate if added xylan in the oxygen could "heal" the damaged fiber areas introduced in the cook and by that contribute to improved strength properties.

Table 3. Pulp variants included in the study

Mechanical treatment in the cook	Xylan added in the oxygen stage	Description
Yes	No	S-0
Yes	Yes	S-X
No	Yes	N-X

3.1 INVESTIGATED PULPS

The pulps had approximately the same kappa number after the cook (ca 32) and after oxygen delignification (*ca* 16) (*Tables 4, 5*). The pulp produced with mechanical treatment at the end of the cook (S) had a higher dry content compared to the pulp produced without mechanical treatment (N), as analyzed after a standardized centrifugation procedure as previously reported (Daniel et al. 2010). A higher dry content after centrifugation was still observed for the pulps produced with mechanical treatment in the cook after oxygen delignification. No difference was seen between the pulp oxygen delignified with added xylan and that without (*Table 5*). The yield after cooking for the pulps investigated was *ca* 48 % (*Table 4*).

Table 4. Cooking results

	Residual OH^- g/L	Kappa no	Dry content %	Shives %	Tot. yield %
S-0	3.9	31.8	31.2	1.8	48.0
S-X	3.9	31.8	31.2	1.8	48.0
N-X	3.9	31.5	25.5	1.8	48.0

Table 5. Results after oxygen delignification

	Kappa no	Viscosity mL/g	Brightness % ISO	Adsorbed xylan % of charged	Dry content %
S-0	15.9	1110	37.2	-	29.4
S-X	16.1	1135	37.1	46	29.4
N-X	15.8	1145	37.4	61	27.0

3.2 XYLAN DISTRIBUTION WITHIN THE FIBER WALL; VISUALIZATION

SEM-images could not identify any differences between the investigated pulps. It seems likely that the xylan added forms a uniform but non-homogeneous coating on the fiber surfaces.

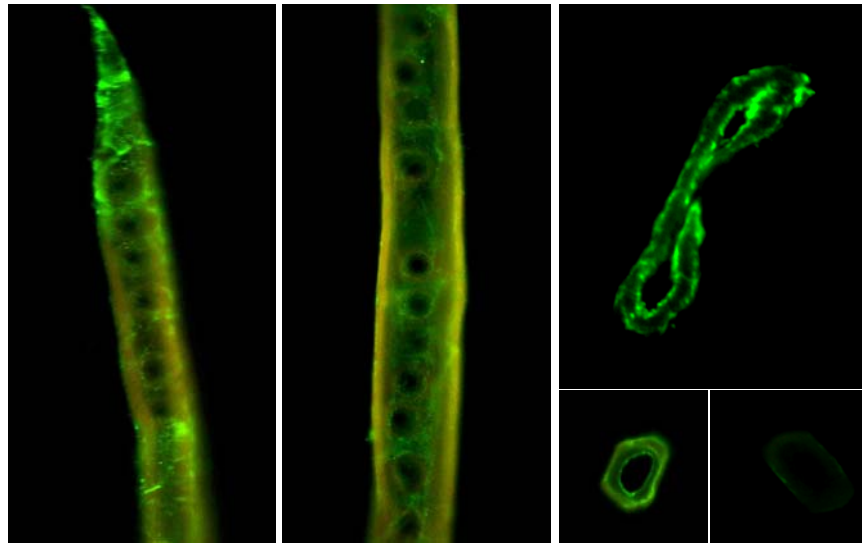

Figure 1. Visualization of adsorbed xylan. S-X (left) and N-X (middle) whole fibers showing non-homogeneous micro-distribution (green colour) of BX on the fiber surfaces. Far right shows cross-sections of S-X fiber with BX present on the fibres outer and lumen walls. Bottem far right shows absence of labeling on S-X fibers without anti-xylan probe. For more details see Appendix 3.

Immunolabeling suggests greatest reactions for the birch xylan on the fiber surfaces (*Figure 1*). Possibly the xylan has penetrated into the fiber wall to a greater extent, compared to previous studies when xylan was added in the cook, due to an more open structure of the fibers (Daniel, et al. 2010).

3.3 CARBOHYDRATE COMPOSITION AND FIBER CHARGES

No difference in xylan content or in surface charge could be seen between the two pulps with added xylan in the oxygen stage (*Table 6*).

Table 6. Carbohydrate content and fiber charges in the oxygen delignified pulps

		S-0	S-X	N-X
Galactose	Rel. %	0.4	0.4	0.3
Glucose	Rel. %	83.9	81.0	80.7
Mannose	Rel. %	7.0	6.8	6.8
Arabinose	Rel. %	0.7	0.7	0.7
Xylose	Rel. %	8.0	11.2	11.5
Galactose anhydro.	%	0.3	0.3	0.3
Glucose anhydro.	%	83.9	81.0	80.7
Mannose	%	6.8	6.3	6.5
Arabinos anhydro.	%	0.7	0.6	0.7
Xylose anhydro.	%	7.6	10.2	10.8
Tot anhydro sugar	%	96.3	92.9	95.8
Hydrorest	%	2.4	2.5	2.5
Tot acid groups	mmole/kg	111	123	129
Surf. Charge (neg)	meqv/kg	10	19	19

The ELISA-analyses show a somewhat higher xylan content in the S-X pulp compared to the N-X-pulp (*Figure 2*). Higher xylan content has also previously been analyzed with ELISA on the mechanical treated pulps compared to an identically cooked pulp but produced without mechanical treatment (Daniel et al. 2010).

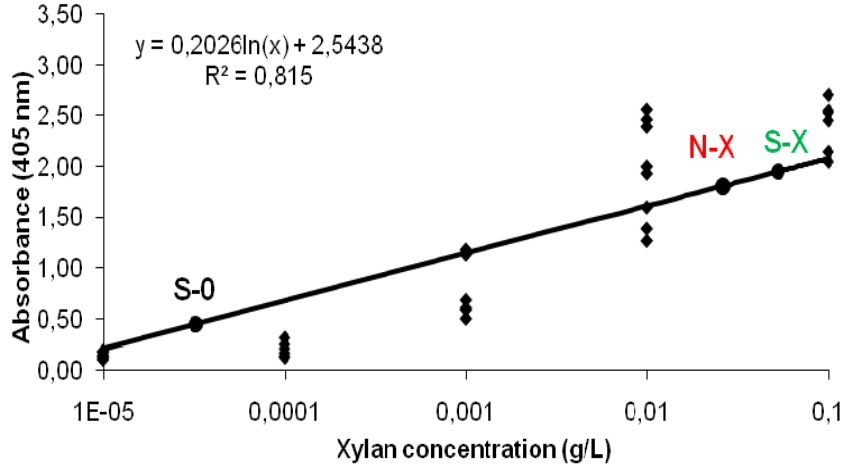

Figure 2. Xylan concentration estimated with ELISA for the investigated pulps.

3.4 Introduction of fiber damage and influence on sheet properties

Added xylan in the S-X and N-X pulps in the oxygen stage resulted in straighter fibers, fibers with higher shape factor/lower amount of kink/mm, compared to the pulp without xylan added in the oxygen stage (*Figures 3, 4*). No significant difference could however be observed between the pulps with added xylan and those produced with or without mechanical treatment introduced in the cook (S-X and N-X). All fiber data for the investigated pulps are shown in *Appendix 1c.*

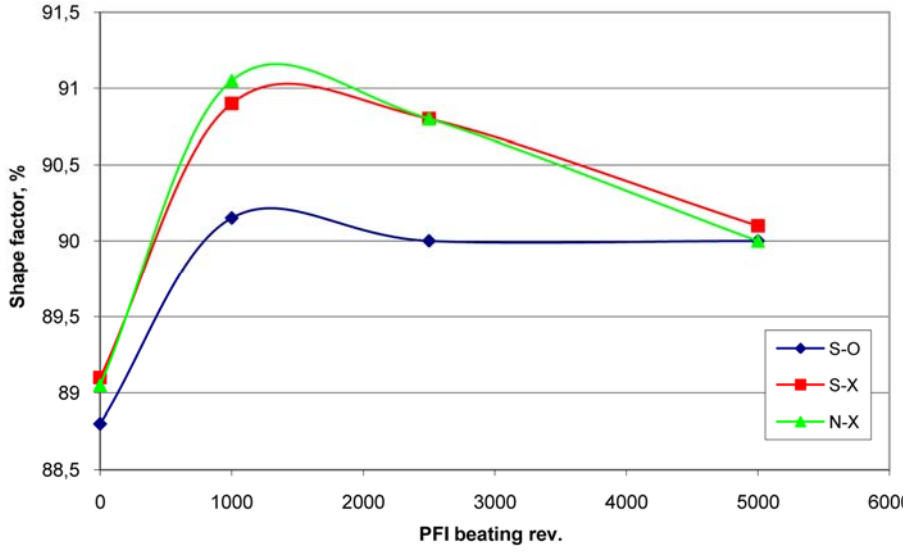

Figure 3. Shape factor vs PFI beating revolutions.

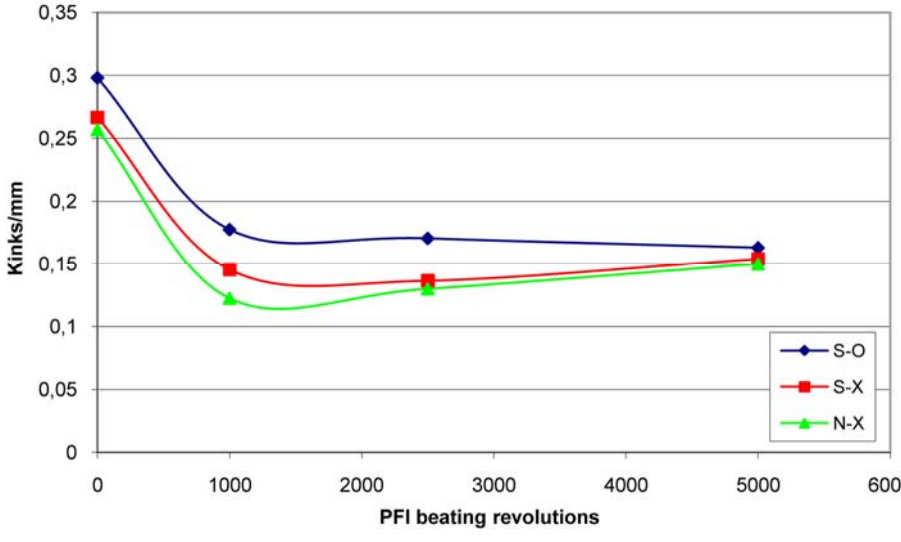

Figure 4. Kinks/mm vs PFI beating revolutions.

Pulps exposed to mechanical damage in the cook and with extra xylan added in the oxygen stage did not contain a lower number of damaged areas compared to the pulp without xylan added in the oxygen stage, analysed as number of cleavage/fiber by the HCl-method (*Table 7)*. Introduced damaged areas were apparently not "healed" by the added xylan. The pulp produced without mechanical treatment in the cook contained a significantly lower

amount of damaged areas compared to pulps produced with mechanical treatment, i.e. showed a significantly lower number of cleavages/fiber.

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

		S-0	S-X	N-X
LWFL H2O	mm	2,21	2,32	2,26
LWFL after HCl	mm	0,746	0,774	1,19
Cleavage/fiber		1,97	1,99	0,897

All strength properties are shown in *Appendix 1b*. The zero-span levels were similar for all pulps (*Figure 5*). The stretch was higher for the pulp produced without mechanical treatment and xylan added in the oxygen stage, although the shape factor were on the same level as for the pulp produced with mechanical treatment and with xylan added in the oxygen stage (*Figure 6*). Also the tear index at a certain tensile index was highest for the pulp produced without mechanical treatment in the cook (*Figure 7*). Between the pulps produced with mechanical treatment in the cook, a slightly lower tear index level was seen for the pulp with xylan added in the oxygen stage. This may be explained by the higher yield for that pulp.

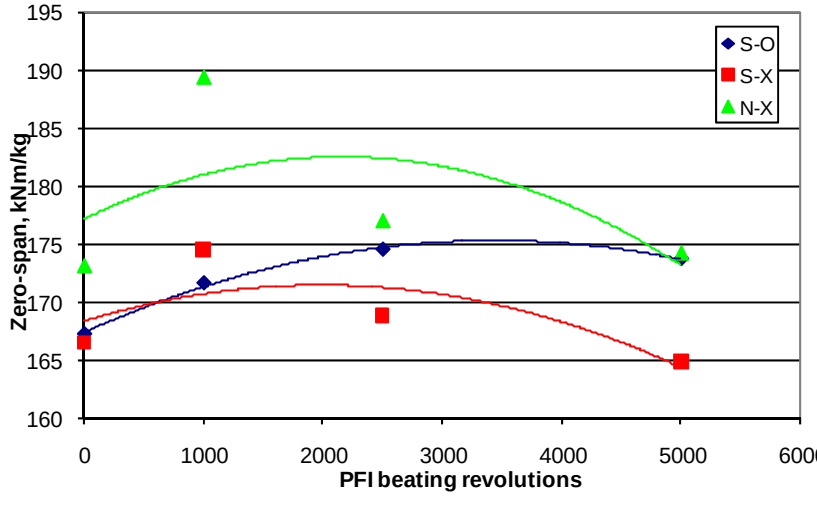

Figure 5. Zero-span vs PFI beating revolutions.

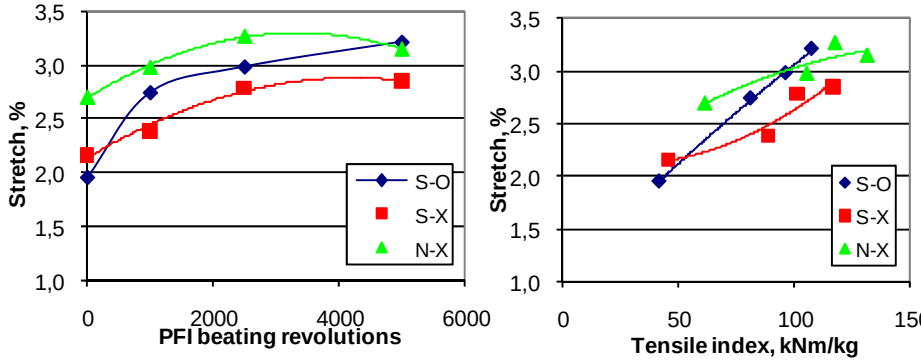

Figure 6. Stretch vs PFI beating revolutions, and stretch vs tensile index.

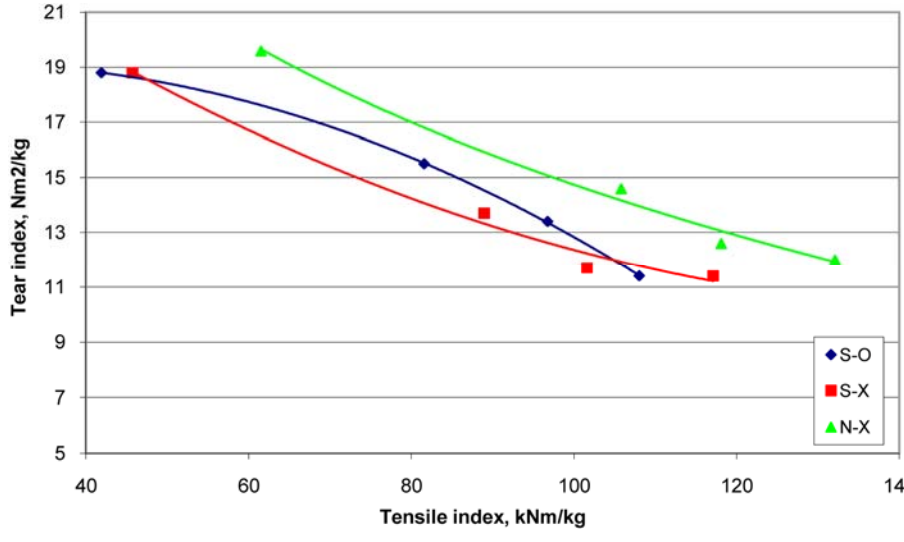

Figure 7. Tear index vs tensile index.

The tensile index development as a function of PFI beating revolution was significantly higher for the N-X pulp compared to the S-X-pulp (*Figure 8*). The negative effect from the introduced mechanical treatment still influences the strength properties more than the added xylan can compensate. However, the xylan addition in the oxygen stage also improved the tensile strength significantly for the pulp produced with mechanical treatment in the cook. Tensile index vs density for the investigated pulps is shown in *Figure 9*.

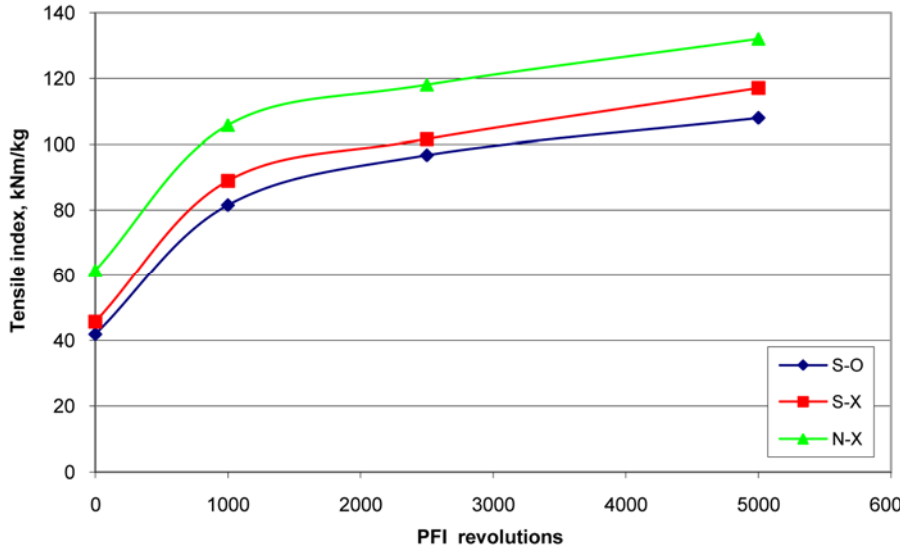

Figure 8. Tensile index vs PFI beating revolutions.

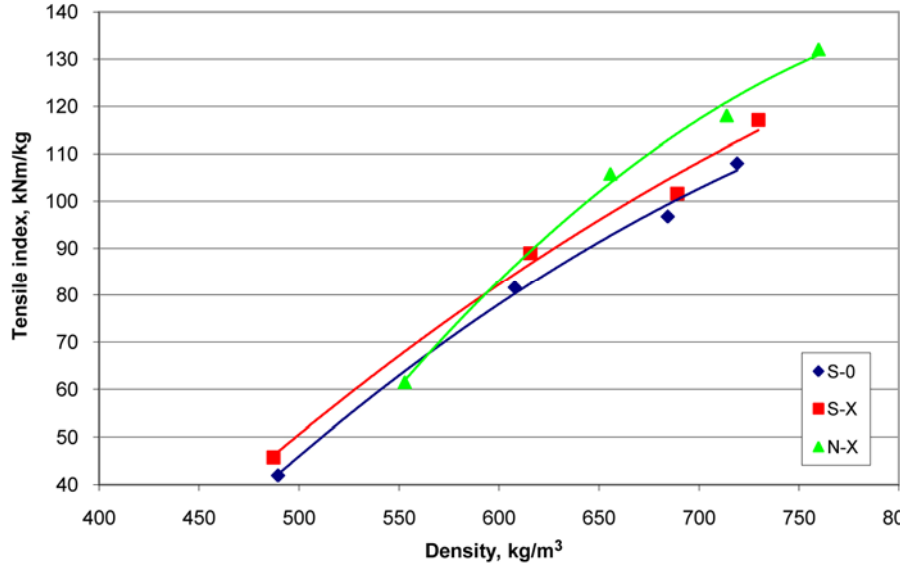

Figure 9. Tensile index vs sheet density.

The dewatering properties are shown in *Figures 10* and *11* as tensile index vs WRV respectively tensile index vs SR. At low refining levels the tensile index–WRV- as well as the tensile index-SR-relationship seems be more advantageous for the mechanically treated pulps. The added xylan only

initially influences the relationship slightly negatively. For more refined pulps the relationship becomes more negative for the pulp produced with mechanical treatment compared to that produced without mechanical treatment when comparing the two pulps with added xylan.

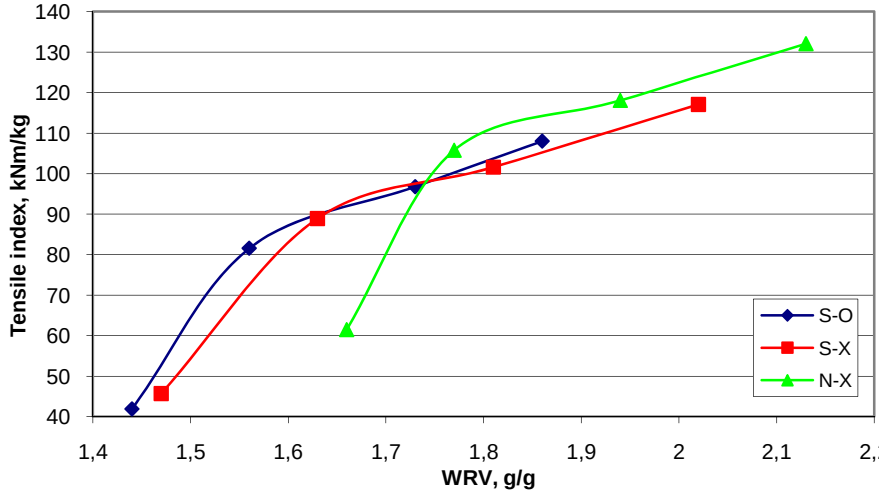

Figure 10. Tensile index vs WRV.

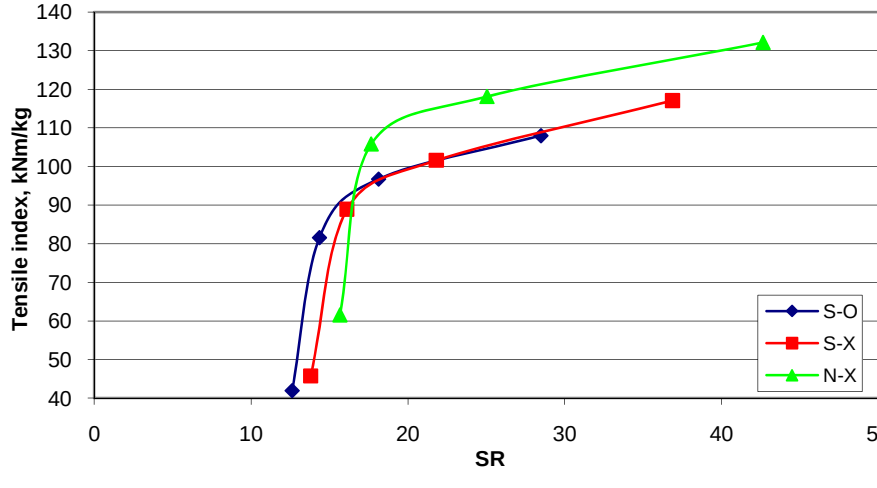

Figure 11. Tensile index vs SR.

4 Conclusions

Results did not indicate that the added xylan in the oxygen stage could "heal" the fiber damages introduced in the cook. Possibly an improvement can be reached if the native xylan in the fiber wall could be preserved to a higher extent.

Added xylan had a fiber straightening effect when added in the oxygen stage as seen earlier when added in the cook (Daniel et al., 2010). The added xylan resulted in improved tensile strength development. However, the negative effect from the introduced mechanical treatment still influences the strength properties more than the added xylan can compensate.

SEM-images could not identify any differences between the investigated pulps. It seems likely that the xylan added forms a uniform but non-homogeneous coating on the fiber surfaces. Possibly the xylan has penetrated into the fiber wall to a greater extent, compared to previous studies when xylan was added in the cook.

5 Recommendation

From the results in Daniel et al. 2010, conclusions could be drawn that replacement of the xylan rich SW black liquor early in the cooking cycle with one xylan free, resulted in inferior resistance against mechanical treatment and also affected the pulp strength negatively. By withdrawal of the xylan rich liquor early in the cook and replacing it with a xylan free, the transportation of xylan out from the cell wall has probably been higher.

Extra xylan added early and late in the cook and that adsorbed on the fiber surfaces has not improved the resistance towards mechanical treatment, nor has added xylan in the oxygen stage been able to "heal" previously introduced damages.

Possibly an improvement, i.e. reduced sensitivity towards mechanical treatment may be reached if the native xylan in the fiber wall could be preserved to a higher extent.

To perform cooks with very high carbohydrate content in the surrounding liquor already in the impregnation stage could be one way to determine if it is possible to prevent the xylan in the fiber wall diffusing out into solution, and what effect that might have on the resistance towards mechanical treatment and strength properties. A recommendation is therefore to perform such cooks and analyze to what extent the native xylan can be preserved in the fiber cell wall. A major challenge will be to determine the changes in xylan in the fiber wall and to distinguish between the native spruce xylan and that added.

6 References

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

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

7 Appendices

APPENDIX 1A. COOKING CONDITIONS

Cook	EA, %	H-factor	Cooking time at 165°C	Residual OH g/l	Total shive %	Total yield %
CR.22	21	2000	197	4,5	1,8	47,51
CR.23	21	2000	197	3,9		
CR.24	21	2000	197	3,7	2,1	48,01
CR.25	21	2000	197	3,6		
CR.26	21	2000	197	3,7	1,5	48,12
Average				3,9	1,8	48

APPENDIX 1B. STRENGTH PROPERTIES

	Beating revs	SR	WRV g/g	Density kg/m^3	Tensile I kNm/kg	Stretch %	Tens. energy abs. I, kJ/kg	Stiffness kNm/kg	Burst I kPam^2/g	Tear I mNm^2/g	Zero-span kNm/kg
S-O	0	12,6	1,44	490	41,9	1,95	0,58	6,4	2,6	18,8	167,2
	1000	14,4	1,56	608	81,6	2,74	1,51	9,0	2,6	15,5	171,6
	2500	18,1	1,73	684	96,8	2,98	1,91	9,7	7,0	13,4	174,5
	5000	28,5	1,86	719	108,0	3,21	2,27	10,1	8,3	11,4	173,7
S-X	0	13,8	1,47	487	45,7	2,16	0,70	6,4	3,0	18,8	166,5
	1000	16,1	1,63	616	89,0	2,39	1,40	9,6	5,7	13,7	174,5
	2500	21,8	1,81	689	101,6	2,79	1,85	10,3	7,3	11,7	168,8
	5000	36,9	2,02	730	117,1	2,85	2,18	11,1	8,1	11,4	164,8
N-X	0	15,7	1,66	553	61,5	2,70	1,16	7,4	4,5	19,6	173,1
	1000	17,7	1,77	656	105,8	2,98	2,05	10,2	7,1	14,6	189,4
	2500	25,0	1,94	714	118,1	3,27	2,47	10,3	8,4	12,6	177
	5000	42,6	2,13	760	132,1	3,15	2,66	11,4	9,5	12,0	174,2

APPENDIX 1C. FIBERMASTER RESULTS

	Beating revs	Fiber length mm	Fiber width µm	Shape factor %	Kink angle °	Kink/mm	Kink/fiber
S-O	0	2,38	31,5	88,8	52,4	0,30	0,57
	1000	2,40	31,4	90,2	53,0	0,18	0,34
	2500	2,39	31,8	90,0	54,6	0,17	0,33
	5000	2,38	32,2	90,0	55,4	0,16	0,31
S-X	0	2,39	31,5	89,1	53,3	0,27	0,52
	1000	2,41	31,4	90,9	53,4	0,15	0,28
	2500	2,37	31,9	90,8	55,5	0,14	0,26
	5000	2,37	32,5	90,1	56,0	0,15	0,29
N-X	0	2,32	32,4	89,1	53,3	0,26	0,48
	1000	2,37	32,2	91,1	54,8	0,12	0,23
	2500	2,34	32,2	90,8	55,9	0,13	0,24
	5000	2,30	32,2	90,0	57,4	0,15	0,27

APPENDIX 2. QUANTIFICATION OF BIRCH XYLAN ADDED TO SPRUCE PULPS DURING THE OXYGEN DELIGNIFICATION STAGE OF KRAFT COOKING USING ELISA

Geoffrey Daniel, Andrea Kaňuchová, SLU, Uppsala

Aims: The aim of the study was to use the *Enzyme-Linked Immunosorbent Assay* (ELISA) to indirectly quantify the presence of birch xylan bound to the surfaces of spruce kraft pulp fibres after adding during the oxygen delignification stage.

Background: ELISA represents an interesting approach that allows for the specific detection and quantification of biological components *in-situ* (e.g. Daniel, G., Nilsson, T., 1991) including characterization of pulp fibre surfaces (Hafrén, J., Daniel, G., 2003). In previous studies (Daniel et al., 2010a, 2010b) the ELISA method was developed to detect and indirectly quantify the absorption of birch xylan (BX) on spruce pulp fibres added during kraft cooking. The ELISA approach developed involves use of specific antibodies to carbohydrates or the use of a carbohydrate binding module (CBM) to detect xylan. Detection involves a three step process: *i)* Detection of the presence of xylan using primary antibodies or CBMs against BX, *ii)* Detection of the primary antibody using a specific enzyme-linked-FITC-conjugated secondary antibody; and *iii)* Determination of the amount of enzyme bound by addition of its substrate and measurement of colour development at 405 nm using spectrophotometry over a fixed period of time. The approach allows for the determination of unknown quantities of xylan binding to pulp fibres by comparison to a standard curve where different concentrations of pure birch xylan absorbed to the surfaces of microtitre plates have been determined in a pre-examination study. In the present context the advantage of the ELISA approach is that it allows determination of the presence of xylan on a relatively large population of fibres (i.e. *ca* 700-1000 fibres) thereby reducing the problems of substrate (fibre) variability from the true reactivity (e.g. differences in morphological structure, chemistry) when only a few fibres or fibre areas are analyzed as is often the case with microscopy approaches (e.g. fluorescence microscopy). The ELISA approach therefore complements microscopy methods. The approach can be adopted for analysis of any unknown carbohydrate or component assuming a specific antibody or CBM is available for detection.

Material and Methods

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

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

Table 1. Fibre materials used from the different pulp treatments

Mechanical treatment in the cook	Xylan added in the oxygen stage	Description
Yes	No	S-0
Yes	Yes	S-X
No	Yes	N-X

Enzyme Linked Immunosorbent Assay (ELISA): This involved the development of a standard using BX and subsequent use of this standard for quantifying xylan on the oxygen delignified pulp samples.

i)* Enzyme Linked Immunosorbent Assay- *Standard development
The xylan standard was developed as described previously (Daniel et al., 2010a, b) and used in the present study.

ii) Enzyme Linked Immunosorbent Assay- *Experimental oxygen delignified pulps*

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

absorbance values. 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.

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 (*Figure 1*). Since a large number of fibres are included in the assay, the reaction is based on a relatively large and mixed fibre population (i.e. early- and latewood) of fibres increasing the assays randomness and representativeness of the entire fibre population.

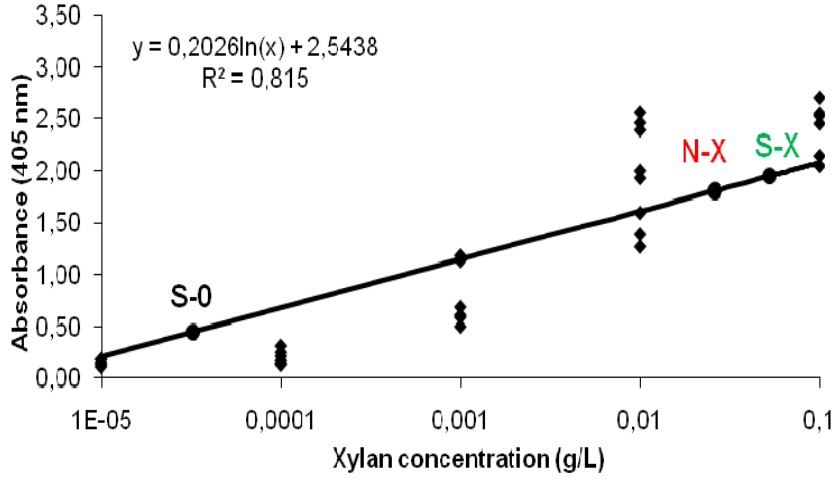

Figure 1. Estimation of xylan (g/L) binding to the oxygen delignified pulps as shown from the standard curve: Absorbance (405 nm) vs. xylan concentration (g/L). Results for the O_2 treated pulp fibres represent mean from 16 individual measurements of pulp samples. Highest xylan concentrations were found for pulps with added xylan and subjected to shearing (i.e. S-X, green oval) treatment followed by xylan treated but non-sheared pulps (N-X, red oval). Sheared but non treated xylan pulps (S-0) reacted very poorly if at all (black oval).

Overall conclusions

- Most xylan was found on birch xylan treated and sheared (S-X) pulps although the difference with xylan treated and non-sheared (N-0) was not distinct as suggested by ELISA (*Figure 1*). Similar observations were observed with the immunolabeling for the presence of birch xylan on whole fibres and fibre cross-sections (*see Appendix 3*);
- Reference pulp (i.e. non xylan treated) plus shearing did not or only very poorly reacted confirming the poor specificity of the antibody for spruce xylan;
- The ELISA method gives an indication of the amount of xylan available for detection on the exposed surfaces of the pulp fibres. It does not however give any indication of the xylan penetrating into the fibre cell walls. This was shown using immunofluorescence staining of cross-sections of the pulp fibres.
- A comparison with ELISA results achieved with kraft pulps treated with BX during the cooking process (Daniel, 2010a) indicated that more xylan was present on the O_2 treated pulps. This suggests that more xylan has been bound or that there is more available for reaction with the antibody. Despite evidence for the presence of xylan see here, an improvement in "healing" of fibre damage was not apparent from the conventional testing results.

References

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

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

APPENDIX 3. VISUALIZATION OF THE SPATIAL MICRODISTRIBUTION OF BIRCH XYLAN ADDED DURING OXYGEN DELIGNIFICATION OF KRAFT SPRUCE PULP USING IMMUNOFLUORESCENCE MICROSCOPY OF WHOLE FIBRES AND FIBRE CROSS SECTIONS

Geoffrey Daniel, Andrea Kaňuchová, Lada Filonova, SLU, Uppsala

Aims: The purpose of the study was to: *i)* Visualize birch xylan (BX) on the surfaces of treated whole fibres treated with xylan during the O_2 delignification stage; *ii)* Determine whether there are any apparent differences in the spatial distribution of the BX present on the oxygen delignified pulps with and without shearing; *and iii)* Visualize the spatial microdistribution of the xylan in cross-sections of the treated pulps to determine whether BX was penetrating the fibre cell walls.

Background: In order to fully understand how xylan and other carbohydrates may be used to improve/retain the strength of kraft pulps, it is important to visualize the association of added xylan with the pulp fibre cell walls. In order to achieve this, it is necessary to have techniques that allow visualization of the spatial microdistribution of both the fibre surface and xylan penetrating into the fibre wall. In the present work, immunofluorescence microscopy in conjunction with specific antibodies against BX was used as a high precision technique to visualize BX associated with spruce fibre cell walls when added during the oxygen delignification stage with and without shearing.

Materials and Methods

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

Mechanical treatment in the cook	Xylan added in the oxygen stage	Description
Yes	No	S-0
Yes	Yes	S-X
No	Yes	N-X

2. Monoclonal antibody

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

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

i) Whole fibres

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

ii) Fibre cross-sections

In principle, the oxygen delignified pulps were processed (i.e. embedded in resin) and fibre cross-sections cut and mounted on object glasses and labeled with the monoclonal antibody for immunofluorescence microscopy as described previously (Daniel et al., 2010a).

iv) Positive and negative and substrate controls

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

4. Fluorescence microscopy

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

Results and Discussion

1. Whole fibres:

Xylan treated whole S-X and N-X fibres showed strong immunofluorescence (i.e. green colour) and evidence for presence of BX (*Figures 1a-c; 2a, b*). However, the immunofluorescence of BX on both S-X and N-X fibres showed variability with some fibres labeling strongly, others weakly and some hardly at all indicating a non-homogeneous distribution of BX in both the fibre population and individual fibres (*Figures 1a, b, 2a, b*). On some areas of the fibre surface, BX was associated with materials surrounding bordered pits (*Figure 1a, 2a*) suggesting presence of certain chemical groups/surface materials on the fibres that react more strongly or are able to bind the xylan. With immunofluorescence it is not possible to visualize quantitatively a difference between the surface labeling of S-X vs N-X fibres although the sheared fibres (S-X) gave an impression of an overall stronger reaction as was suggested in earlier studies (Daniel et al., 2010b). Fibres

untreated with birch xylan (N-0) showed no immunofluorescence (*Figure 2c*), results consistent with the ELISA observations (*Appendix 2*).

2. Fibre cross sections:
Immunofluorescence of fibre cross-sections also showed variability but gave a better idea of the ability for the xylan to penetrate the pulp fibres (*Figures 3-4*). Both the outer fibre and inner lumen wall surfaces showed evidence for the presence of birch xylan in both S-X (*Figure 3a-e*) and N-X treated fibres *(Figure 4a-d)*. Strongest BX immunofluorescence and penetration was found for highly porous and collapsed earlywood fibres in S-X (*Figure 3a, d*) and N-X treated fibres *(Figure 4c, d*). Latewood fibres appeared mostly coated on the fibre cell wall inner lumen surface of both S-X (*Figure 3b, c, e*) and N-X fibres (*Figure 4a, b*) with limited BX penetration. Cross-sections of fibres untreated with BX (N-0) showed no immunofluorescence thereby confirming the high specificity of the antibody for birch xylan (*Figure 5a-d*).

Overall conclusions

- Immunofluorescence using an anti-xylan monoclonal antibody showed strong positive evidence for the presence of birch xylan associated with both early- and latewood fibres when added during the oxygen delignification stage for S-X (with shearing) and N-X fibres (without shearing);
- Immunofluorescence of whole fibres and fibre cross-sections gave complementary results with the latter giving a general understanding of a larger population of fibres and reaction with different surface morphological structures on the fibre wall while the cross-sections gave information of the penetration of BX into the fibre wall from both the outer fibre surface and inner lumen walls. Greatest penetration was found for highly porous and collapsed earlywood fibres;
- Despite the apparent strong immunofluorescence reactions for birch xylan on the S-X and N-X fibres, an improvement in strength was not shown indicating that either insufficient BX was present as a whole on the fibres (i.e. total number of treated fibres) or that the xylan present was not giving the desired reparation of fibre damage (i.e. fibres not sufficiently penetrated by xylan and/or the xylan not retained). This would also suggest that possibly a different xylan type (e.g. greater substitution) should be used.
- The present anti-xylan probe has a strong reaction with birch xylan and only a very weak reaction with softwood xylan. It would be advantageous to be able to distinguish between the both.

References

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

Daniel et al., 2010b. Daniel G., Olsson, A-M., Ander P., Kaňuchová A., Salmén L., Filonova L., Sjöström K., Boussard L., Berg S., Heijnesson-Hultén A., Lindström C. 2010b. CRUW Chemical Pulping 1. The influence of xylan on the sensitivity towards fiber damage. Intern report nr 2, 88p.

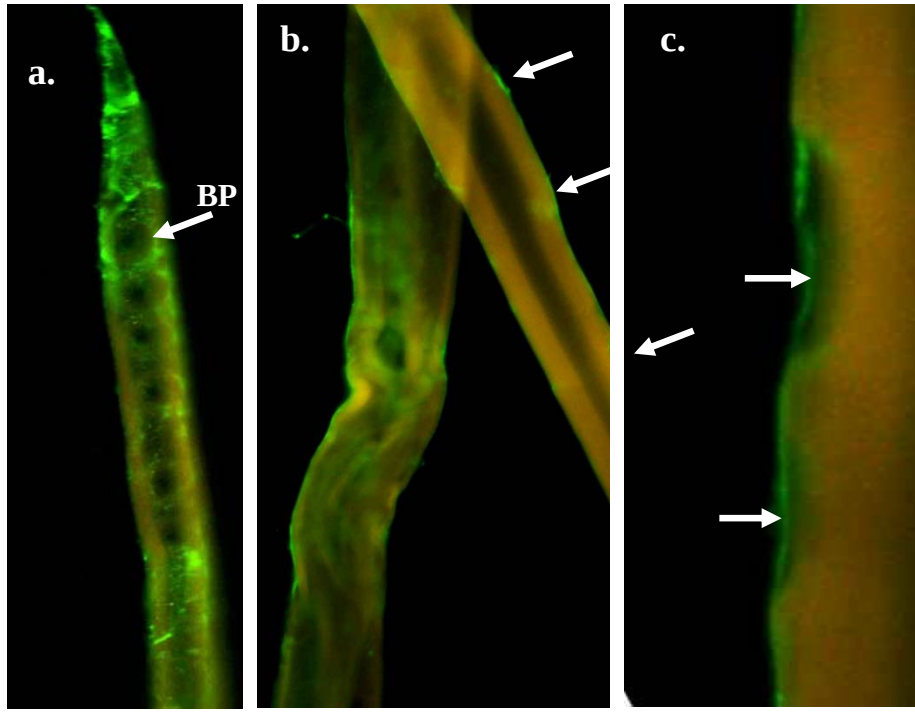

Figure 1a-c. S-X fibres showing positive immunofluorescence for BX (green colour) on the fibre surface. Labeling was typically non-homogeneous with the xylan frequently forming a thin outer layer on the fibre wall as shown with focus through the wall (b, c, arrows). In some areas of the fibre surface, xylan was associated with materials surrounding bordered pits (BP) (a). See also 2a.

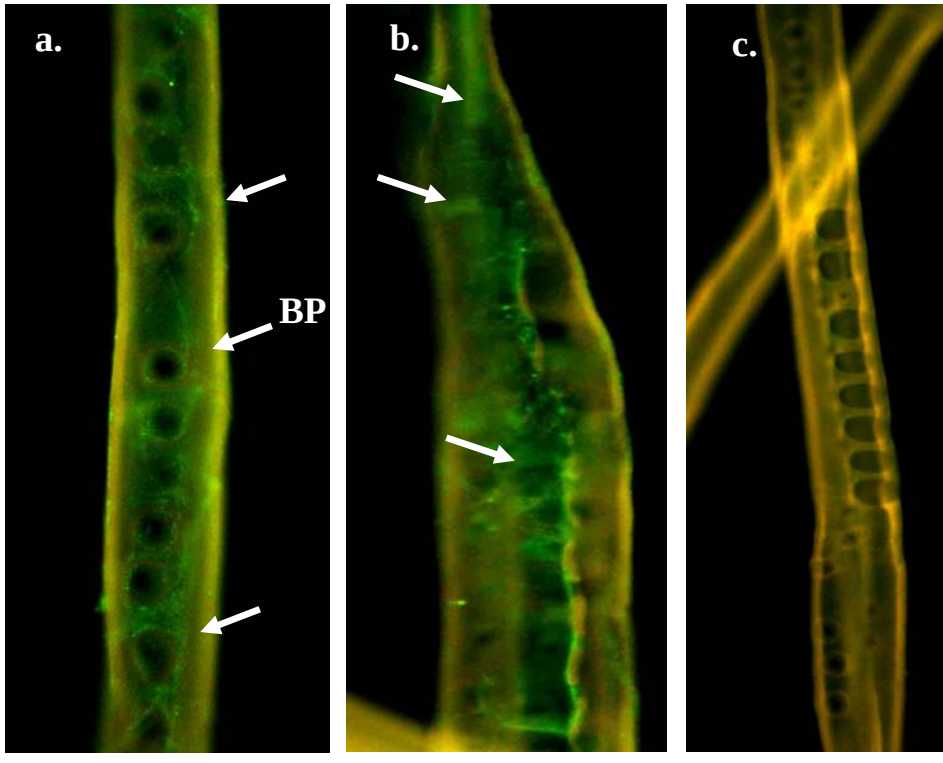

Figure 2. N-X fibres (a, b) and S-0 (c) fibres. N-X fibres showing positive green immunofluorescence (arrows) for BX reminiscent to that observed for S-X. S-0 fibres (c) showed no fluorescence. BP, Bordered pit.

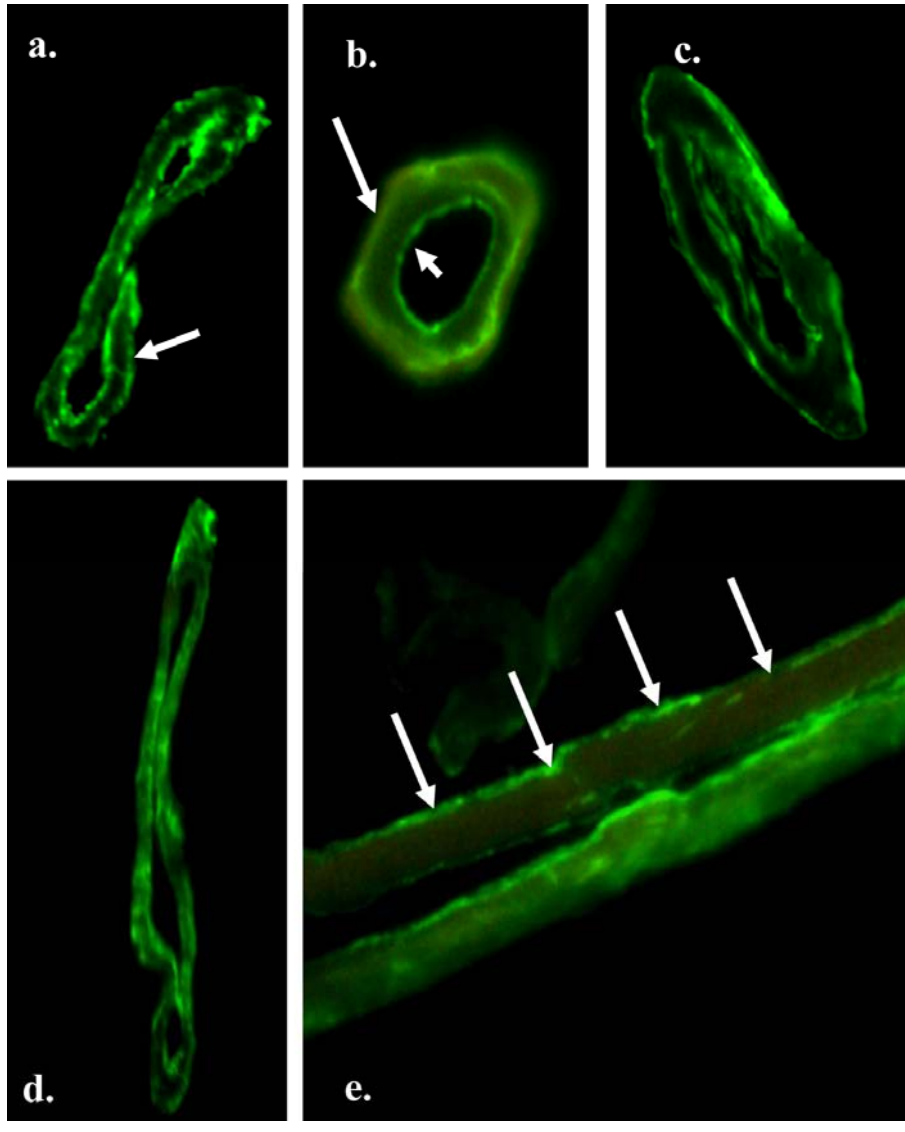

Figure 3a-e. S-X cross-sections of both early- (a, d, e) and latewood (b, c) fibres showing positive immunofluorescence and presence of xylan on the fibre surfaces with some penetration into the wall (e). Both the outer (a-e) and lumen (b) wall surfaces were labeled (arrows) for presence of BX. Ruptured and highly porous earlywood fibres showed evidence for immunofluorescence within the fibre wall (a, d) indicating penetration of BX.

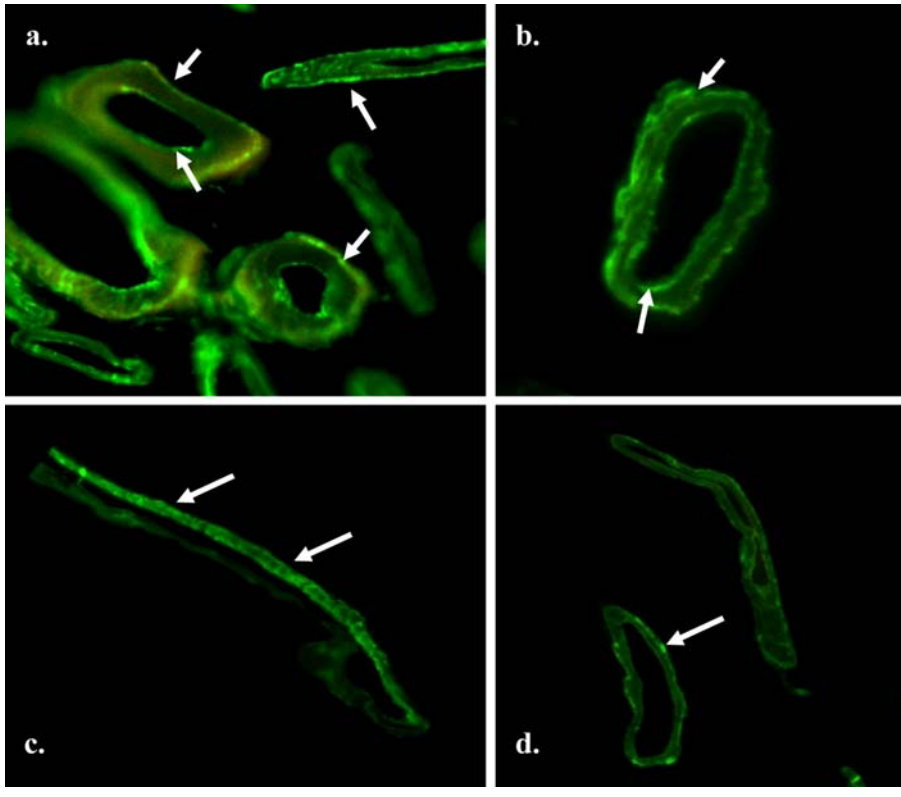

Figure 4a-d. N-X cross-sections of both early- (c, d) and latewood (a, b) fibres showing positive immunofluorescence and presence of BX primarily on the fibre surfaces (arrows).

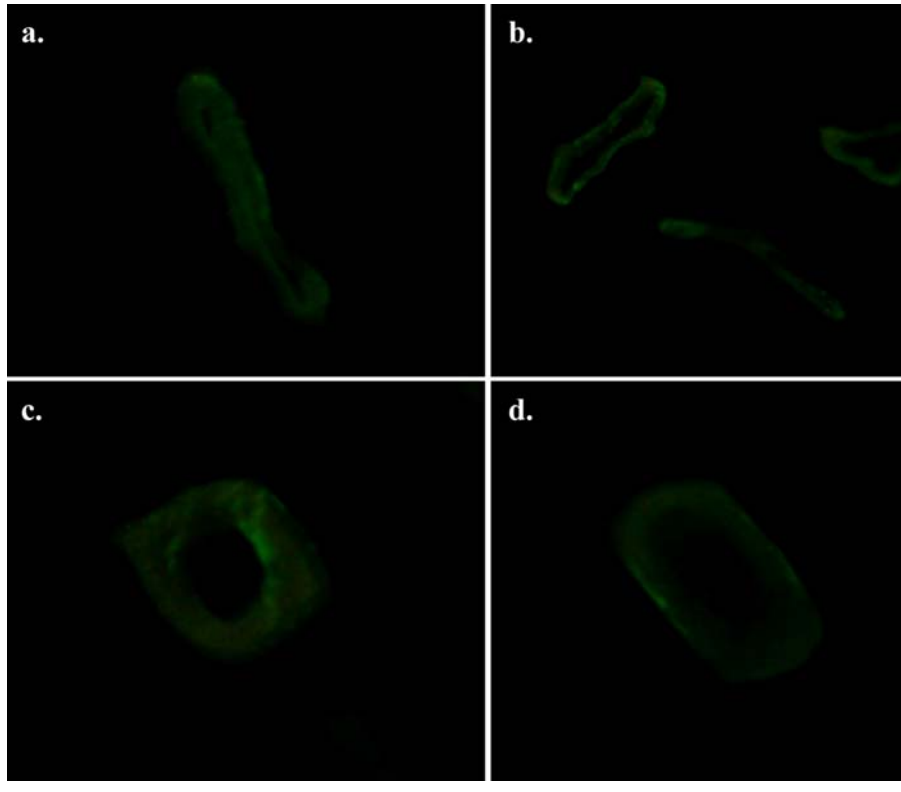

Figure 5. Cross-sections of S-X control early- (a, b) and latewood (c, d) fibres processed with omission of the anti-xylan probe. Fibre cross-sections did not show any evidence for immunofluorescence and presence of BX.

APPENDIX 4. SEM OBSERVATIONS ON OXYGEN DELIGNIFIED SPRUCE PULPS TREATED WITH BIRCH XYLAN

Geoffrey Daniel, SLU, Uppsala

Aims: The purpose of the study was to answer the following questions: *i)* Can exogenously birch xylan (BX) be observed on the surfaces of spruce pulp fibres when added at the oxygen delignified stage using SEM?; *ii)* If present, does the xylan show any concentration towards the different fibre morphological features?; *iii)* Can the affects of shearing be visualized and their affects interpreted with BX addition?

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 provides a 3-dimensional overview of the morphological structure of fibres. In the present project, the immuno-detection systems ELISA and immunofluorescence gave evidence for the presence of BX on the surfaces of both xylan treated S-X and N-X treated fibres. SEM was therefore used in the present study to try and visualize the xylan on the surface of treated fibres to ascertain its morphological state.

Materials and Methods

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

Table 1. Fibre materials used from the different pulp treatments

Mechanical treatment in the cook	Xylan added in the oxygen stage	Description
Yes	No	S-0
Yes	Yes	S-X
No	Yes	N-X

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.

Results and Discussion

At the magnifications used, SEM observations did not reveal any evidence for aggregated birch xylan on the oxygen delignified spruce pulp fibres with (*Figures 1a-d, 2a, b*) and without shearing (*Figure 3a-d*). Indeed, very little difference was observed in the morphology of the fibres after xylan addition compared to reference sheared fibres (*Figure 4a-d*). Some small precipitates were noted on some of the S-X fibres although these were not uniformly distributed (*Figure 2a, b*). The fibre surface morphology 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 and in places the remaining primary wall. Any morphological effects of pulp shearing on fibre structure were not truly apparent at this magnification.

Overall conclusions from SEM observations

- No evidence was found for the presence of xylan aggregates at *the magnifications used* on the S-X and N-X test fibres observed;
- Morphologically there appeared to be little differences in any of the pulp samples S-X, N-X and S-0, whether treated/non-treated with xylan and/or sheared;
- Since the presence of xylan was confirmed *–although with some variability-* on both early and latewood fibres in the both the ELISA assays and immunofluorescence, present observations showing the absence of aggregates in the present SEM studies would indicate that it is likely to be forming a more uniform but non-homogeneous coating on the fibre surfaces;
- Verification of xylan present on the fibre wall using this approach would require high resolution SEM in combination with immuno-electron microscopy.

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.

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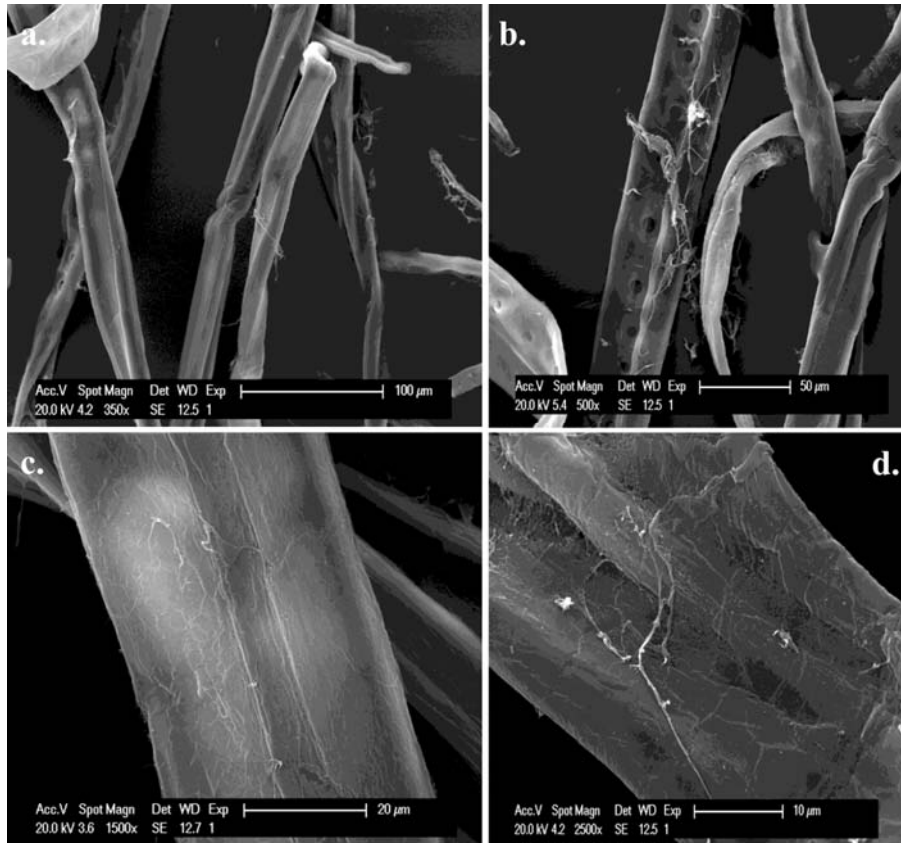


Figure 1a-d. S-X oxygen delignified spruce fibres with added birch xylan and shearing.

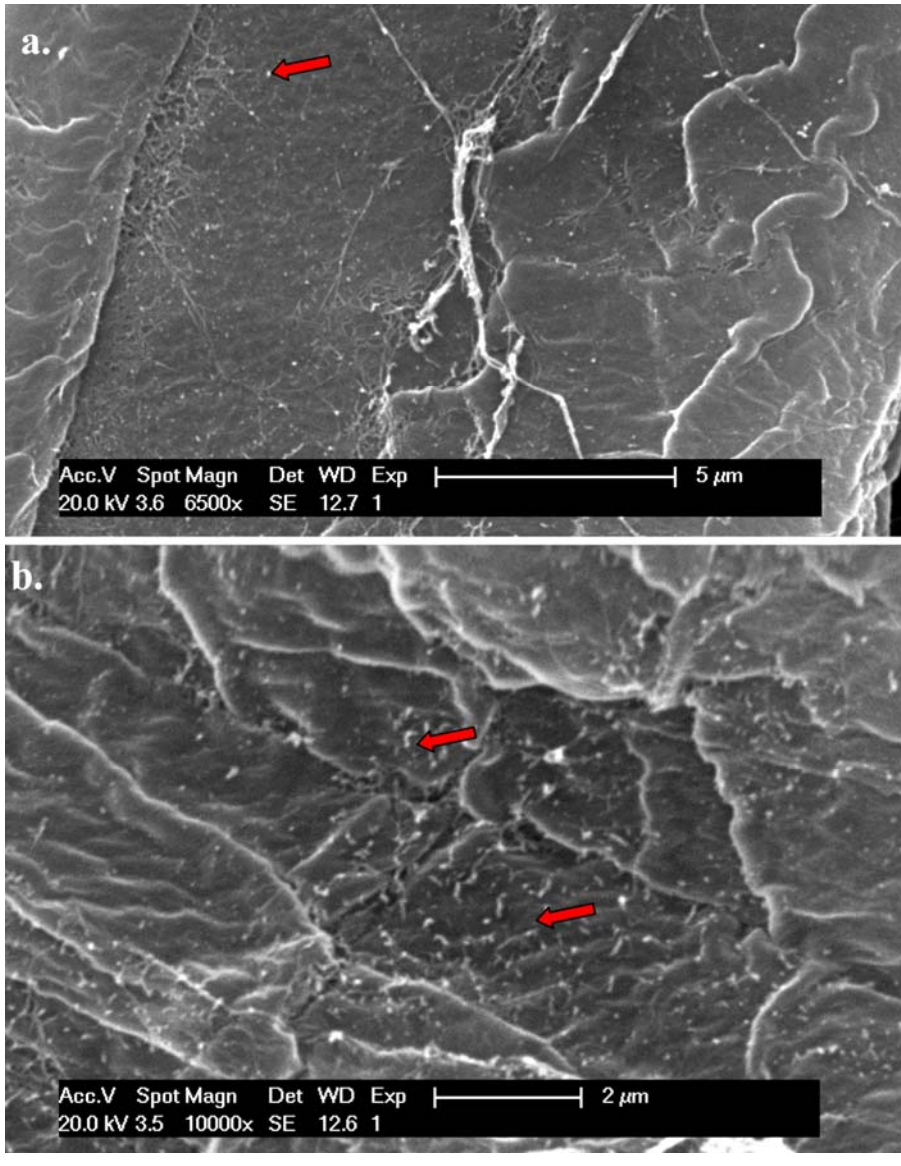


Figure 2a-b. S-X oxygen delignified spruce fibres with added xylan and shearing. Some rather small precipitates were noted in areas of the fibre surfaces (arrows).

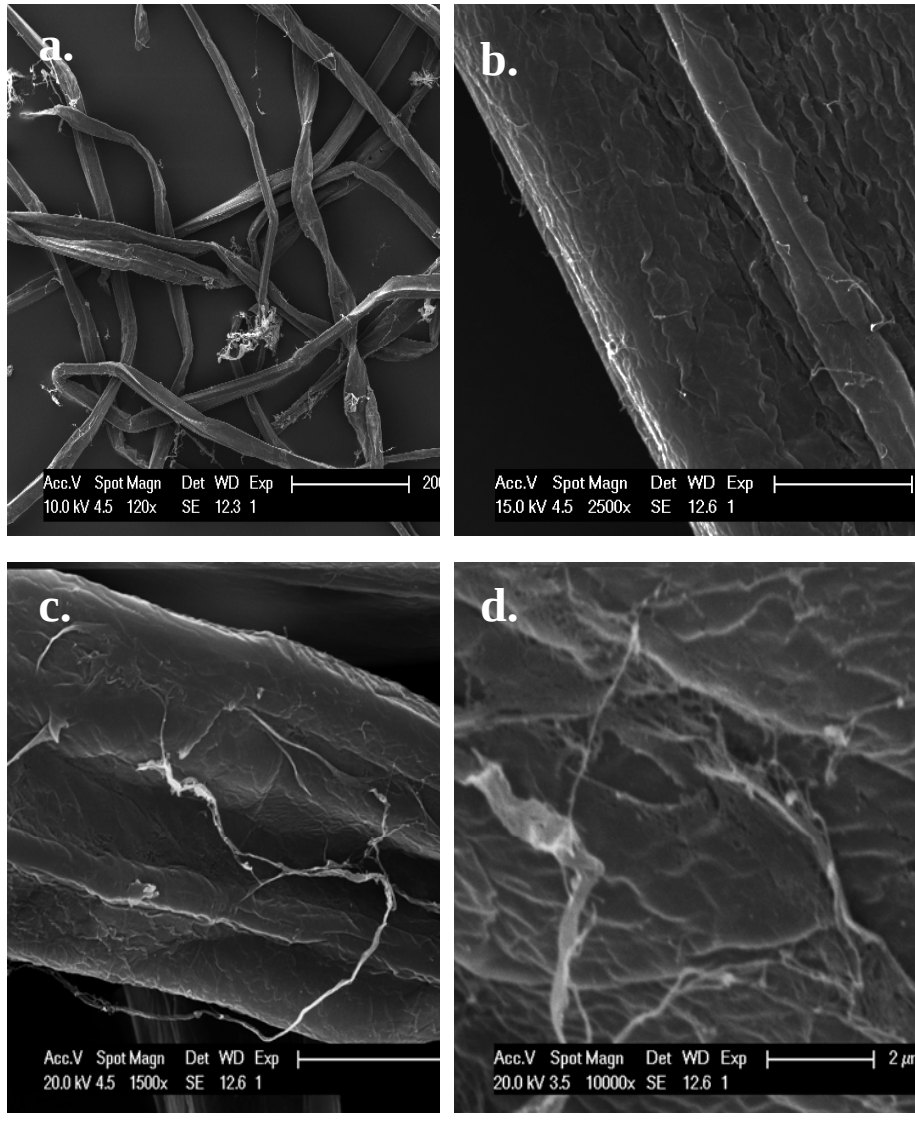


Figure 3a-d. N-X oxygen delignified spruce fibres with added birch xylan.

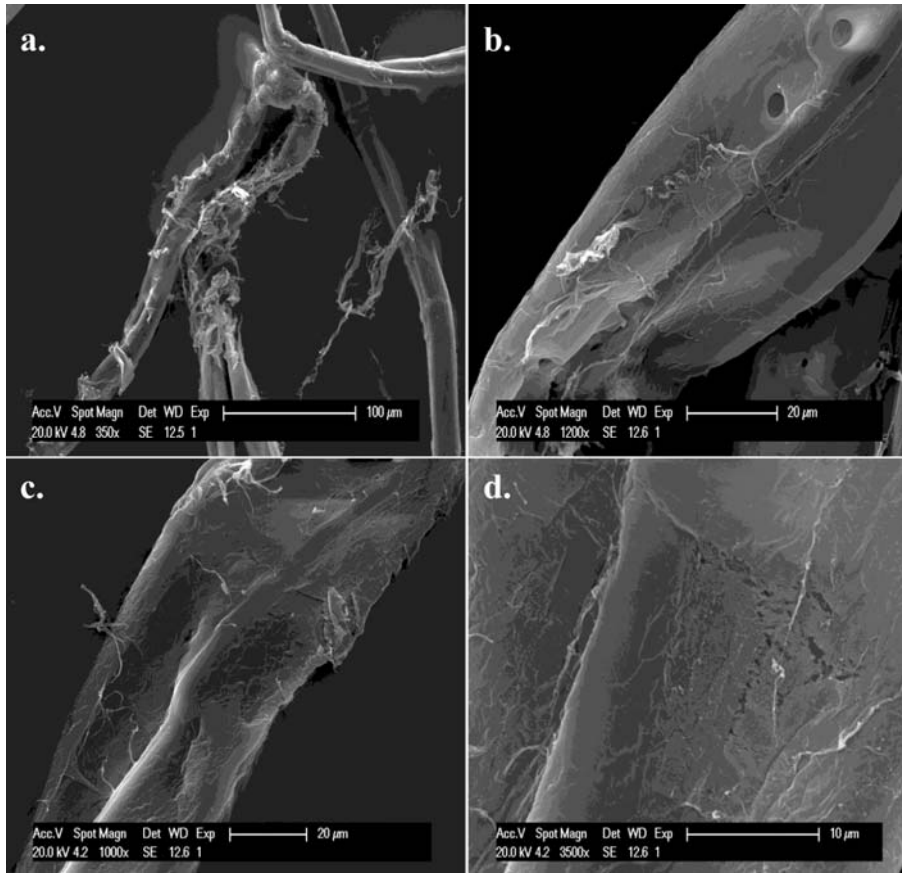


Figure 4a-d. S-0 oxygen delignified sheared spruce reference fibres without xylan.

APPENDIX 5. EVALUATION OF XYLAN DISTRIBUTION BY FTIR MICROSCOPY

Anne-Mari Olsson and Lennart Salmén, Innventia, Stockholm

Aims: The purpose of this work was to investigate how added xylan was distributed over the surface of fibres and to see if there was any difference between the adsorption distribution for fibres subjected to shearing during pulping as opposed to those that had not been sheared.

Background: It is well known that xylan added in kraft cooking is deposited on the surfaces of the fibres. However, so far little is known with regard to the 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 surface imaging FTIR-microscopy have for unbleached pulp fibres indicated a somewhat uneven xylan distribution although that differences between different levels of addition was difficult to see (Daniel et al. 2010).

Material

Single fibres from the three pulps S0, SX, and NX were dried and glued onto glass slides. Both earlywood and latewood fibres were prepared, 10 fibres were analysed for each pulp.

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. In order to evaluate the surface composition, spectra were acquired over a length of 100 μm covering most of the fibre width, from subareas 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.
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 programe. Also average spectra from all the measured subareas were calculated for each fibre.

MULTIVARIATE ANALYSIS

The average spectra were analysed with multivariate analysis using Simca (Umetrics). From a PCA analysis the principal components that separate the different pulps were identified in scoreplots, and in loadingplots significant wavenumbers for this separation is shown.

Results

The scoreplot in Figure 1 shows that in principal component 5 there is a weak separation between pulps where xylan was added and where no addition of xylan was made. The regions for the pulps are not fully separated, but no other component showed any separation between the pulps. No separation due to mechanical treatment could be found, nor any difference between earlywood and latewood.

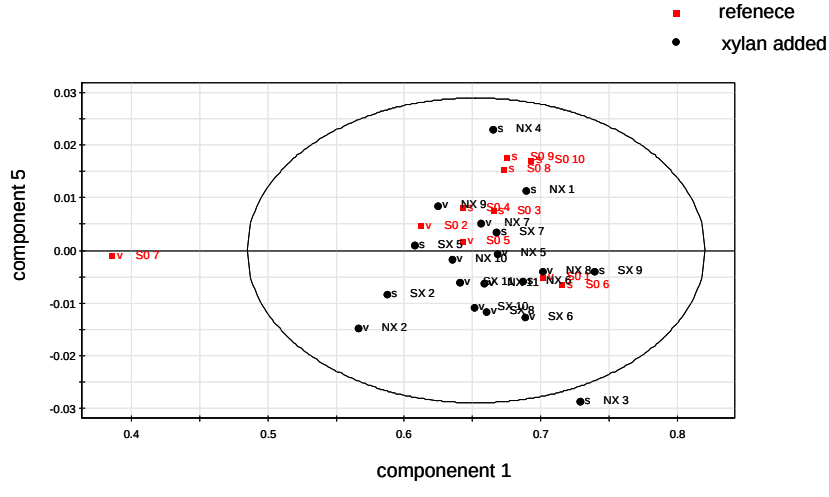


Figure 1. A scoreplot calculated from the average spectra of each fibre measured. Black marks samples where xylan was added (SX, NX) and red marks the S0 fibres.

The loadingplot for principal component 5 shows the wavenumbers that separates fibres with xylan from those without. In *Figure 2* the loadingplot and the spectrum for the added xylan is shown.

The birch xylan added to the fibres contains lignin-like substances absorbing at 1600 cm^{-1} but the 1500 cm^{-1} lignin peak is not seen. Also no peak from the carboxylic group at 1750 cm^{-1} in xylan can be seen. At 1460 cm^{-1} a shoulder is shown from the CH_2 symmetric bending from xylose.

The loadings for principal component 5 in Figure 2 shown in green shows peaks at both 1750 cm^{-1} and 1460 cm^{-1} which are typical for xylan. Negative peaks correspond to the samples with negative scores i.e. those with added xylan.

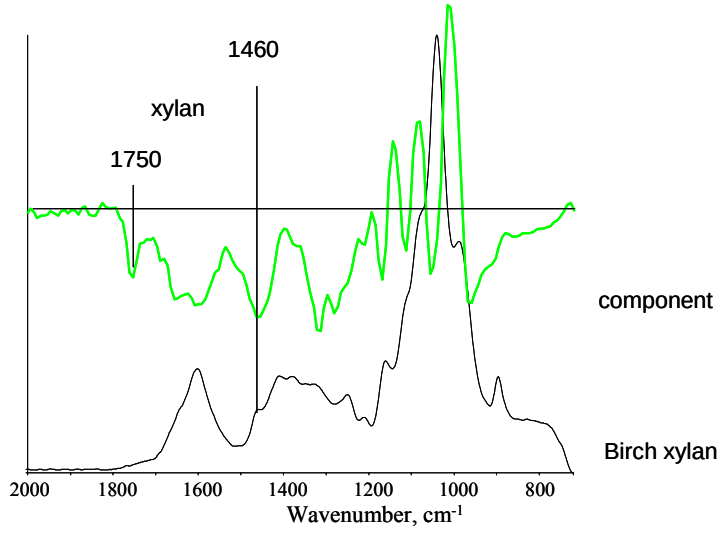


Figure 2. Loadingplot for principal component 5 and the IR spectrum for the birch xylan added. In the spectrum for component 5, negative peaks are significant for the samples with xylan added.

For each measured fibre a distribution image can be made showing wavenumbers corresponding to different components. In the loadingplot in Figure 2 the wavenumber 1750 cm^{-1} corresponding to the carboxylic acid group in xylan, was significant for the samples with xylan added. This wavenumber was chosen to map xylan distribution on the fibre surfaces. In order to eliminate the effect of total absorption the peak height at 1750 cm^{-1} was divided by the total absorbance in the range from 800 to 1800 cm^{-1}. The distribution plots are shown in Figure 3. Dark blue represents a low amount and red and white represents high amounts of xylan.

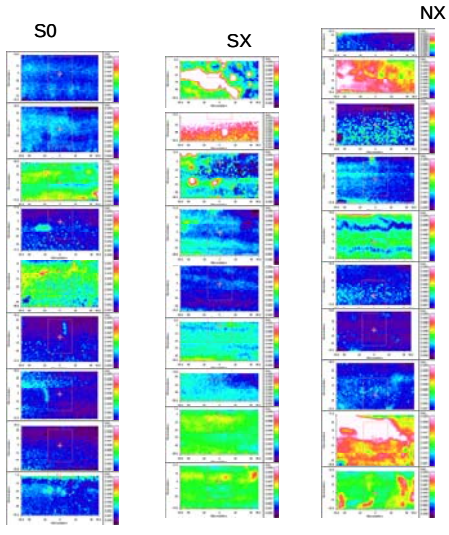


Figure 3. Distribution plots for xylan in comparison to the total absorbance in the spectra. (1740-1760 cm^{-1} / 800-1800 cm^{-1})

There are large variations in the amount of xylan within each fibre type represented by charged carboxylic acid (1750 cm^{-1}). It seems that when xylan is not added, the amount on the surface is lower. The mechanically treated SX was not different from the non-sheared NX sample.

Conclusion

Measurements with FTIR microscopy may distinguish between samples that are very different in surface composition, but small differences are difficult to determine.

References

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

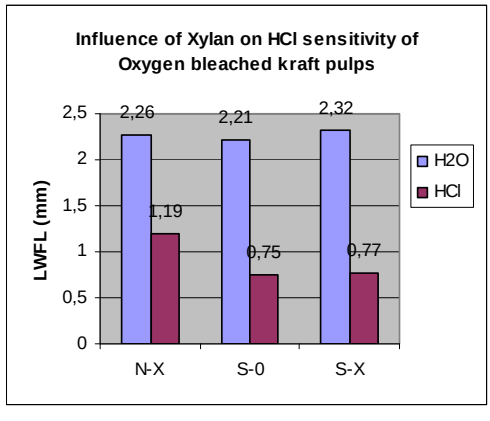
APPENDIX 6. HCL - METHOD USED ON OXYGEN BLEACHED SOFTWOOD KRAFT PULPS IN XYLAN EXPERIMENTS

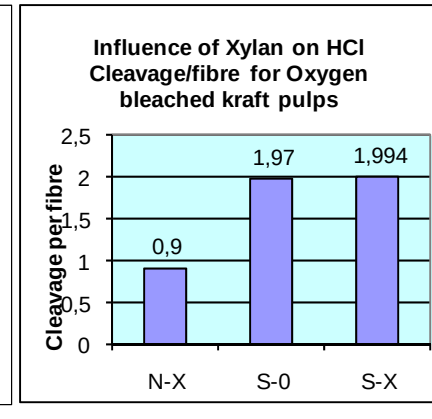
Paul Ander, SLU, Uppsala

Oxygen bleached pulps with or without added birch xylan were tested with the HCl-method (Ander et al., 2008; Daniel et al., 2010; Heinemann and Ander, 2011). The purpose was to determine if xylan addition could protect the fibres against shearing producing deformations and dislocations. The conditions for xylan addition and shearing treatment are outlined in the experimental section of this report (page 3). The results are shown in *Table 1* and in *Figures 1, 2*.

Table 1. HCl-sensitivity of oxygen bleached kraft pulps

O2 bleached pulps	LWFL (mm) L0 H2O	LWFL (mm) L HCl	Cleavage / fibre
N-X (Normal + Xylan)	2.318/2.196 ***2.26***	1.181/1.198 ***1.19***	**0.897**
S-0 (Sheared)	2.337/2,089 ***2.21***	0.816/0.675 ***0.746***	**1.968**
S-X (Sheared + Xylan)	2.342/2.294 ***2.32***	0.829/0.719 ***0.774***	**1.994**





Figures 1, 2. Fibre lengths and cleavage of softwood fibres treated with xylan and tested with the HCl-method. Denotation of the fibres as in Table 1.

All three pulps were 2.2 to 2.3 mm long before HCl treatment and after HCl treatment the N-X pulps were 1.19 mm long, while the sheared pulps were 0.75-0.77 mm long. The shortening is due to the shearing treatment and xylan addition could not decrease this fibre shortening affect. *Table 1* and *Figure 2* also show that fibre cleavage was strongest after shearing and that

birch xylan at 10 g/l could not protect the pulp against cleavage during shearing compression. N-X pulps had cleavage 0.90 while after shearing, S-0 and S-X pulp fibres had fibre cleavage of 1.97-1.99.

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Collaborative Research on the Ultrastructure of Wood Fibres (CRUW)

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