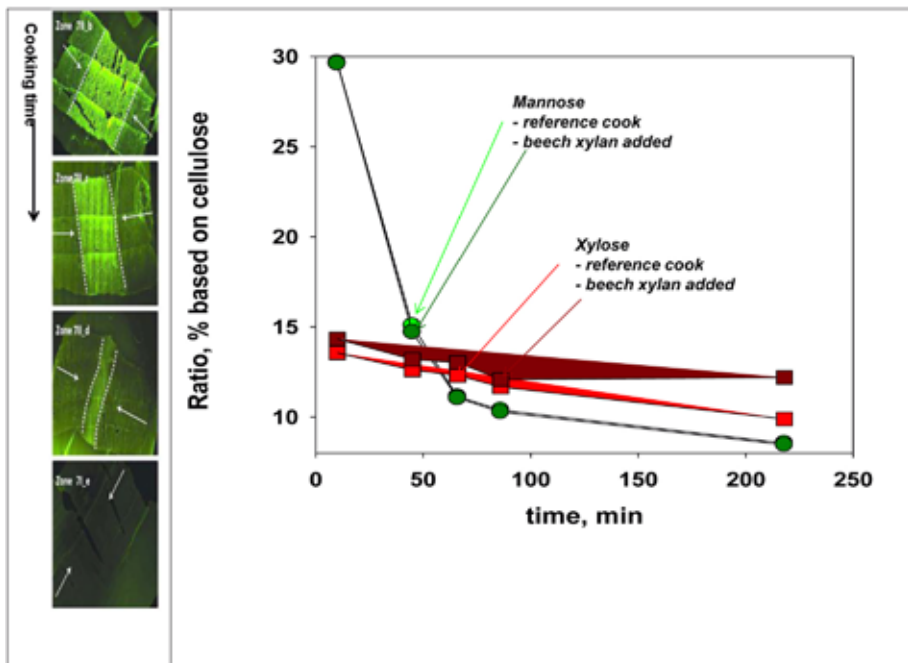# Chemical Pulping

Localization of xylan (native and added) during cooking

Lennart Salmén, *Innventia;* **Lada Filonova,** *SLU*; **Anne-Mari Olsson**, *Innventia;* **Jonas Hafrén,** *SLU;* **Geoffrey Daniel,** *SLU*

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Summary

The aim of this study was to investigate how the xylan content is changed within a wood chip and within the fiber cell walls at different positions of the chip during cooking. It was hoped that such information would give an understanding regarding the critical factors for xylan deposition within the cell wall and possible ways of affecting strength delivery of the pulp.

Two sets of cooking trials were performed; one Ref-pulp and one with beech xylan addition in the impregnation stage (10g/L). The cooks were interrupted at specific times and the chips taken out and analyzed for chemical composition and distribution of wood polymers within the chips as well as within the fiber cell walls. The pulps produced with beech-xylan addition had about 2 % higher xylan content at the end of cooking compared to the Ref-pulp.

Results indicated that the chips already contained an increased amount of xylan after 10 minutes of impregnation when beech xylan was added to the cooking liquor. However, only chips at the end of the cook showed a substantial increase in xylan sorption. During cooking, a large gradient in lignin content was noted between the surface and the inside of the chips which always showed higher lignin content even at the end of the cook. A progressive increase in xylan content was also noted in the chips as cooking proceeded. From the studies made it was not possible however, to determine if the addition of xylan led to any increased xylan content within the fibre walls. In cooks with beech xylan added during early stages, an increase in xylan was noted in the fibre lumen areas. In later stages and with increased delignification, increased xylan content was also noted between fibres in degraded middle lamellae regions.

Contents

APPENDIX 1A. COOKING RESULTS

APPENDIX 1B. ANALYSIS OF BEECH XYLAN

APPENDIX 2. HISTOCHEMICAL AND IMMUNOHISTOCHEMICAL ANALYSIS OF LIGNIN AND NORMAL/LOW MOLECULAR WEIGHT XYLAN DISTRIBUTION WITHIN MODEL WOOD CHIPS
Lada Filonova, Geoffrey Daniel, SLU, Uppsala

APPENDIX 3. LIGNIN AND XYLAN DISTRIBUTION EVALUATED BY FTIR
Anne-Mari Olsson and Lennart Salmén, Innventia, Stockholm

APPENDIX 4. FLUORESCENCE LOSS INDUCTED BY PHOTOBLEACHING (FLIP)
Jonas Hafrén, SLU, Uppsala, Florian Salomons, KI Stockholm

1 Background

Previously the influence of the addition of high xylan concentration in the cooking liquor early in the cook has been studied. Beech- or wheat xylan was added with a concentration of 30 g/L (Daniel et al. 2012). The investigated pulp-variants were compared to a reference pulp produced without xylan addition. All pulps where produced with- and without introduced mechanical damage in the cook.

Cooking with added beech xylan did not lead to less dissolution but less readsorption compared to the reference cook. For the cook with added wheat xylan, possibly more spruce xylan has been readsorbed compared to the reference cook. No significant differences in sensitivity towards mechanical treatment were noted between the different pulps.

In this study the distribution of xylan in softwood chips during cooking was followed by interrupting the cooks at different times both with- and without added xylan. The added xylan was in this case a beech xylan obtained from an external source.

The aim of this study was to investigate how the xylan content is changed within a chip and within the fibre cell walls at different positions of the chip during cooking. It was hoped that such information would give an understanding regarding critical factors for xylan deposition within the cell wall and possible ways of affecting strength delivery of the pulp.

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.

Batch cooks were conducted on 50 g chips each with the addition of two model chips to be used for the detailed analysis of the distribution of wood polymers within the chips as well as within the cell walls at different positions. Impregnation was made at 120 $^{\circ}$C and the cooks were performed at 165 $^{\circ}$C; liquor/wood ratio – 6/1. For the cooks with added beech xylan, the xylan was pre-dissolved in the cooking liquor prior to impregnation. At specific time intervals the cooks were disrupted and the chips collected for analysis. This was made by draining the liquor followed by cooling and washing the chips in cold water

The following steps were studied as indicated in *Figure 1*:
10 minutes after impregnation, temp 120 $^{\circ}$C
45 minutes after impregnation start, impregnation completed.
10 minutes into cooking, 165 $^{\circ}$C
30 minutes into cooking, 165 $^{\circ}$C
2 hours into cooking, cook ends.

The cooking conditions used are shown in *Table 1* and *Appendix 1a*.

The beech xylan was obtained from Sigma (lot BCBG5928V; X4252). Data on carbohydrate composition, molecular weight and degree of substitution for the beech xylan is shown in *Appendix 1b*.
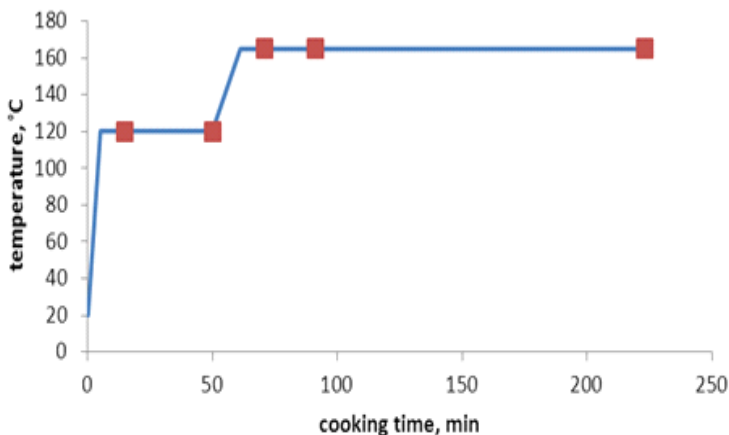

Figure 1. Stages at which cooking was stopped and material collected for analysis.

Table 1. Cooking conditions

	Time (min) at temp. (ºC)		H-factor	Residual OH- g/L	Alkali cons. kg/t	Total yield %
Ref.	10	120 ºC	2	33.6	65	93
	45	120 ºC	7	23.1	127	82
	10 *	165 ºC	164	18.6	153	72
	30 *	165 ºC	384	16.9	165	63
	162 *	165 ºC	1705	12.0	194	51
Beech	10	120 ºC	2	32.1	73	94
	45	120 ºC	7	24.4	120	79
	10 *	165 ºC	162	17.9	158	70
	30 *	165 ºC	374	15.9	171	63
	162 *	165 ºC	1703	10.4	203	49

* at cooking temperature

2.1 ANALYSIS

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

For studies of local composition within chips and cell walls, the model chips added in each of the cooks were used. These model chips after removal from the pulps were frozen in liquid nitrogen and stored at -70°C. For characterizing purpose, one half of a model chip was divided into 3 parts with approximate cross section dimensions of 5*5 mm and 30 mm in the fibre direction. From these sticks, material was taken from 6 positions as indicated in *Figure 2*.

Histochemical and *immunohistochemical* analysis of lignin and xylan distribution were performed using specific labeling techniques. More details are presented in *Appendix 2*.

The distribution of lignin and xylan was also investigated by *FTIR spectroscopy and FTIR-microspectroscopy*. More details can be found in *Appendix 3*.

Diffusion properties within the chips were studied by *Fluorescence loss Induced by photoleaching (FLIP)*. More details can be found in *Appendix 4*.
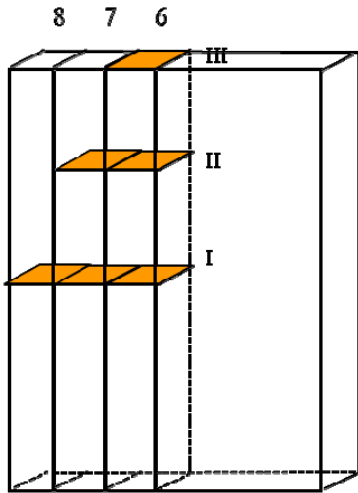
Figure 2. Schematic image of a model wood chip with positions of sampling.

3 Results and discussion

Two sets of cooking trials were performed; one Ref-pulp and one with beech xylan addition in the impregnation stage (10g/L). Cooks were interrupted at specific time intervals and the chips were taken out and analyzed for chemical composition and distribution of wood polymers within the chips as well as within the cell walls of fibers.

The aim was to investigate how the xylan content changed both within a chip and fiber cell walls at different positions of the chips during cooking and what effects the addition of external xylan to the impregnation liquor would have on the behavior. It was hoped that such information would give a better understanding regarding critical factors for xylan deposition within the cell wall and possible ways of affecting strength delivery of the pulp.

3.1 INVESTIGATED CHIPS/PULPS

The cooking trials resulted in 5 different batches of chips/pulp for each of the two cooks; reference and with beech xylan added related to different stages in the process (*Figure 3*.). The chips/pulps produced with- and without addition of beech xylan had a fairly similar yield in the different stages of the process as seen from *Figure 3*.
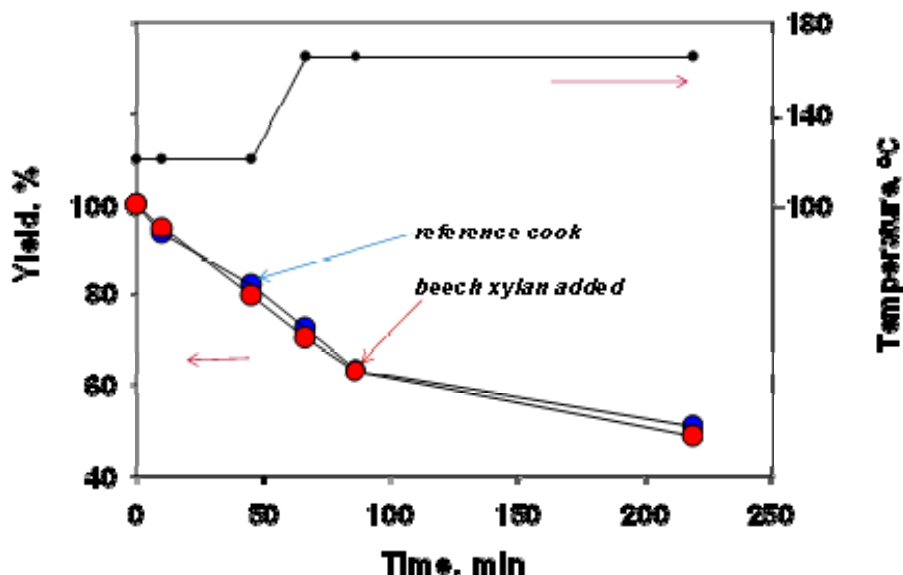

Figure 3. Yield as a function of time in the cooks for reference and with addition of beech xylan to the original cooking liquor at 10g/l; liquor/wood ratio 6:1. The temperature profile during the cook is also indicated (upper line).

3.2 CHEMICAL COMPOSITION

For all the different stages in the cook, the chips/pulps produced with beech-xylan addition contained a much higher xylan content compared to the Ref-chips/pulps (*Table 2*). The lignin content was affected in the same way for the two sets of cooks as seen in *Figure 4;* the increase of relative lignin content during the impregnation step being a reflection of the dissolution of glucomannan. As seen in *Figure 5*, the amount of glucomannan (indicated as mannose) decreased in a similar manner for both sets of cooks with the largest change during the impregnation phase. The amount of xylan was higher in the cooks with xylan added already after 10 minutes of impregnation (*Figure 5*). The increased xylan content was maintained through the cook until the final pulp stage. Only at this stage and at the end of the cook was there a substantially higher increase in xylan content for the cooking set with beech xylan added to the cooking liquor.
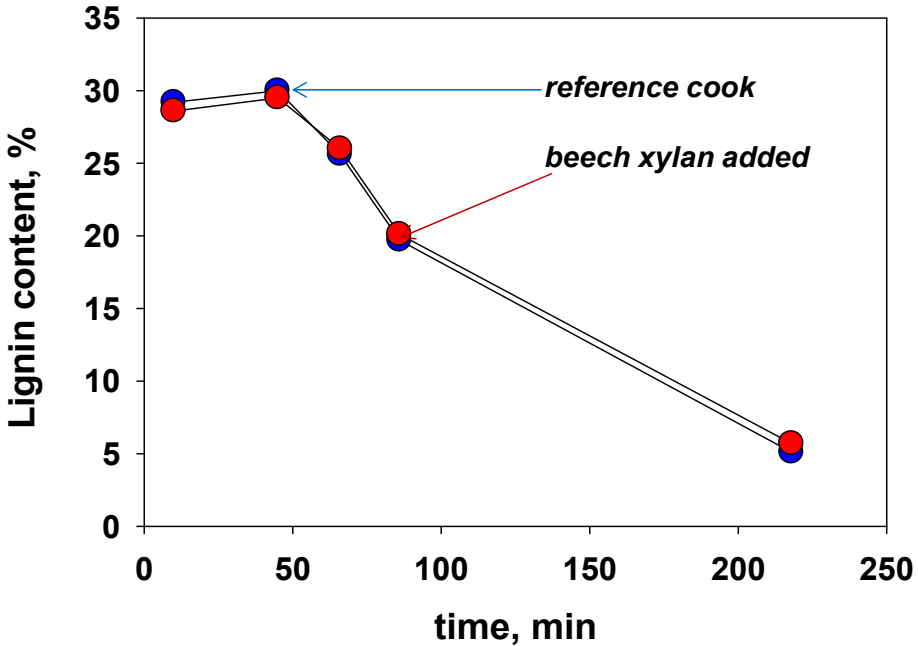

Figure 4. Lignin content as a function of time in the cook for reference and cook with addition of beech xylan to the original cooking liquor at 10g/l; liquor/wood ratio 6:1.
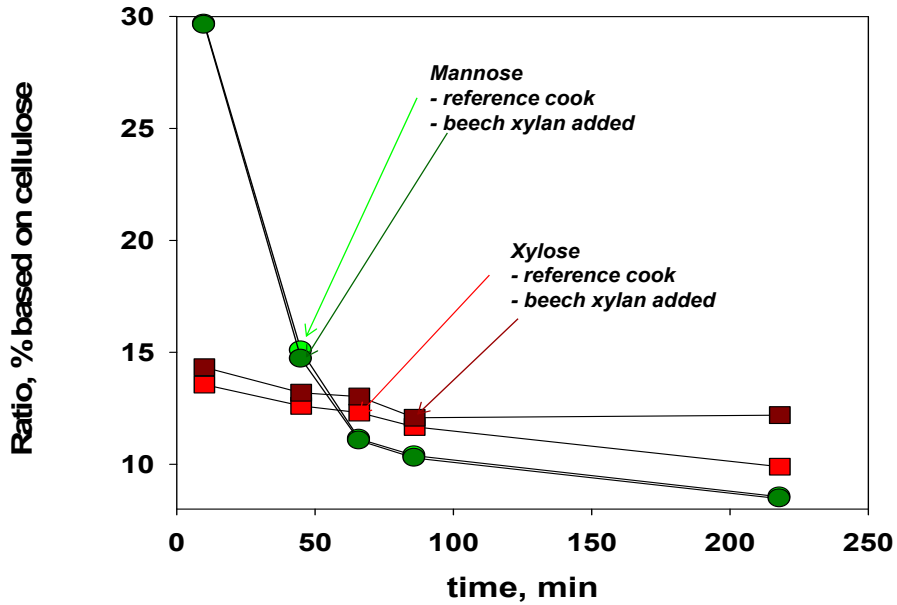
Figure 5. Relative mannose and xylose content based on cellulose as a function of time in the cook for the reference and cook with beech xylan added to the original cooking liquor at 10g/l; liquor/wood ratio 6:1.

Table 2. Carbohydrate and lignin content for the investigated chips/pulp

		Wood	Ref 10 min	Ref 45 min	Ref 66 min	Ref 86 min	Ref 218 min	Beech 10 min	Beech 45 min	Beech 66 min	Beech 86 min	Beech 218 min
Arabinose	Rel %	2.2	2.1	1.0	1.9	1.5	0.7	2.0	1.9	1.8	1.5	0.6
Galactose	Rel %	3.2	2.9	1.8	1.4	1.1	0.4	2.4	1.9	1.5	1.0	0.5
Glucose	Rel %	68.1	68.0	76.1	78.8	80.3	83.9	68.1	75.8	78.4	8+.1	82,3
Xylose	Rel %	8.6	8.5	9.2	9.4	9.1	8.1	9.0	9.6	9.9	9.4	9.8
Mannose	Rel %	18.0	18.6	11.0	8.5	8.1	7.0	18.6	10.7	8.4	8.0	6.8
Arabinose anhydr.	%	1.3	1.3	1.1	1.3	1.1	0.6	1.3	1.3	1.3	1.0	0.6
Galactose anhydr.	%	2.0	1.8	1.1	1.0	0.8	0.3	1.6	1.3	1.0	0.7	0.4
Glucose anhydr.	%	42.5	43.0	45.1	53.5	59.8	76.1	45.2	50.1	55.4	56.6	73.7
Xylose anhydr.	%	5.3	5.2	5.3	6.3	6.6	7.2	5.8	6.2	6.8	6.5	8.6
Mannose anhydr.	%	11.2	11.8	6.5	5.8	6.0	6.3	12.3	7.1	5.9	5.7	6.1
Tot anhydr. sugar	%	62.3	63.1	59.2	67.8	74.3	90.5	66.2	65.9	70.4	70.5	89.4
Hydrolyse remaining	%	28.8	29.2	30.0	25.6	19.7	5.1	28.6	29.5	26.0	20.1	5.7

3.3 VARIATION ON CHIP LEVEL

The distribution of lignin and xylan was followed at the chip level with both histochemical and immunohistochemical methods (*Appendix 2*), as well as FTIR spectroscopy (*Appendix 3*). With both measurements a clear trend was noted in that lignin dissolution occurred from the chip surfaces inwards indicating a rather large variation in the local lignin content with position within the chip (see *Figures 6 and 7*). With FTIR, higher lignin content was also noted for samples cooked with xylan added to the cooks. However, no effect on microdiffusion was noted from FLIP.
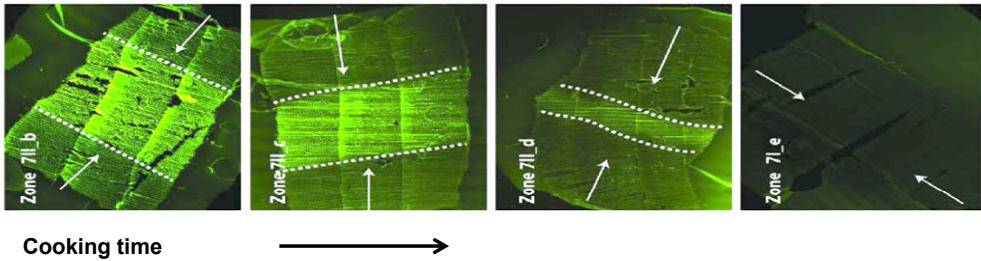
Cooking time ➡

Figure 6. Lignin microdistribution as determined with acridine orange staining and visible as strongly fluorescence green areas in cross sections of chips from cooks with added beech xylan taken from different positions during the course of the cook. The images show how the lignin level is progressively reduced from the outside of the chip inwards. Interestingly, the pathway of delignification does not seem to follow the orientation of the rays within the wood structure indicating that the diffusion of the cooking liquor and presumably dissolved components may not be governed by the native wood structure.
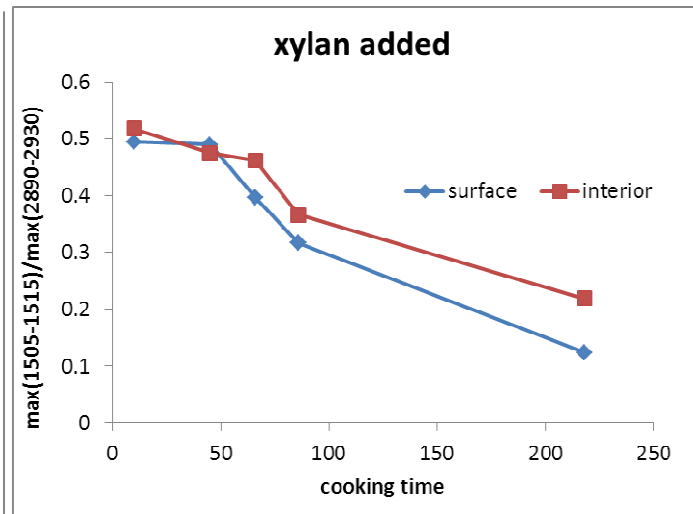

Figure 7. Relative lignin content determined from peak heights of FTIR spectra, as a function of cooking time for two positions of a chip; chip surface 6 III and chip interior 6 I from cooks with beech xylan addition.

For xylan, antibody labelling indicated that the xylan content increased with cooking time. Also more xylan was noted in samples cooked with added beech xylan in accordance with the chemical analysis.

3.4 VARIATION AT THE FIBRE LEVEL

Studies at the fibre level were difficult to interpret. Immunolabeling using specific xylan antibodies indicated that early in cooks the samples where beech xylan had

been added, a more intense signal was observed in the lumen areas compared to cooks with no xylan added. At later stages of cooking, reference chips most often showed xylan signals in the lumen area while chips from cooks with added beech xylan showed signals both from outside of fibres in degraded middle lamella regions as well as from cell lumen areas (see *Figure 8*). The presence of xylan in middle lamella regions is suggesting that a new pathway for xylan penetration into the wood chips during cooking was developed. As diffusion rates, FLIP measurements, did not change significantly with the addition of xylan, therefore it is unlikely that large amounts of xylan penetrated into the S_2 wall.

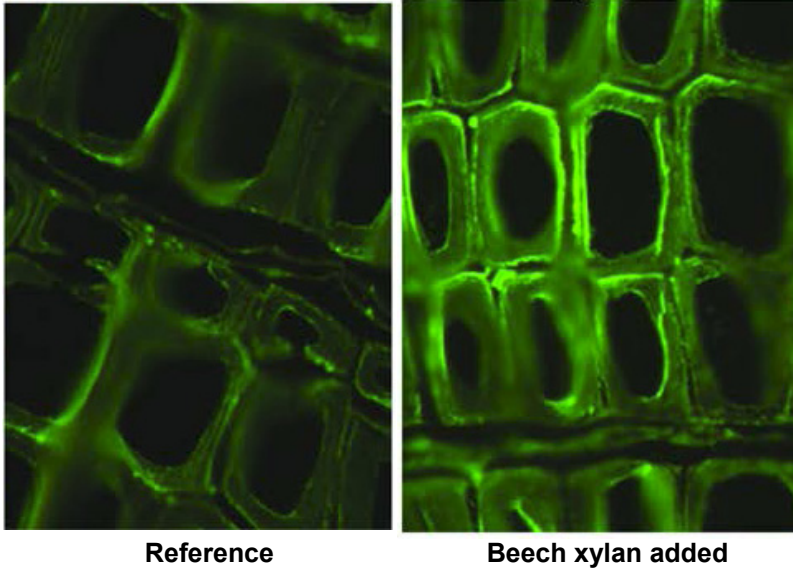**Reference**   **Beech xylan added**

*Figure 8. Xylan localisation at the fibre level as determined by immunolabeling of chips removed at the end of the cook; position **e** from reference cook and cook with beech xylan added respectively.*

4 Conclusions

Results indicated that when xylan was added to the cook, the chips already had after 10 minutes of impregnation an increased amount of xylan. It is likely that this increased amount of xylan comes from xylan entering into the fiber lumens with the cooking liquor and is then attached to the lumen areas. However, only chips at the end of the cook showed a substantial increase in the sorption of xylan.

During cooking, a large gradient in lignin content was noted using fluorescence microscopy and FTIR measurements between the surfaces and the inside of the chips which always showed higher lignin content even in the final cooked chips. Increasing xylan content was also noted within chips as cooking proceeded.

From the studies performed it was not possible to determine if the addition of xylan lead to any increased xylan content within the fibre secondary cell walls. Early on in the cooks with xylan added, increased xylan content was noted in the lumen area while later increased xylan content was also noted in-between fibres in degraded middle lamella regions.

5 Recommendation

These cooking studies indicate that most of the sorption of xylan occurs in the later stages of cooking. Any further studies in this area should thus concentrate on these stages of cooking where in this study data are lacking.

Unfortunately none of the techniques used were able to determine definite changes in xylan or xylan type within fibre cell wall regions. In order to gain better information in this respect, i.e. if added xylan is able to penetrate into the cell wall or if it may hinder native xylan to be dissolved out from the cell wall is a question that requires new- or more refined techniques to be developed.

References

Daniel, G., Olsson, A-M., Ander, P., Salmén, L., Filonova, L. Sjöström, K., Boussard, L., Berg, S., Heijnesson-Hultén, A. and Lindström, C. (2012). The influence of the molecular weight of the added xylan on pulp properties. CRUW Chemical Pulp. CRUW Internal Report 9, 58 pp.

Appendices

APPENDIX 1A. COOKING RESULTS

Reference cook

Sample name	Model chips	Time (min) and temp. (°C) for breaking cook		H-factor	Residual alkali g/l	Alkali consumpt. kg/t	Tot. Yield %
LX.Ref.a	6 & 7	10	120°C	2	33.57	64.6	93.14
LX.Ref.b	1 & 2	45	120°C	7	23.11	127.4	81.73
LX.Ref.c	8 & 9	10	165°C	164	18.75	153.5	72.21
LX.Ref.d	3 & 4	30	165°C	384	16.91	164.6	63.19
LX.Ref.e	10 & 11	162	165°C	1705	12.04	193.8	50.87

Beech xylan cook

Sample name	Model chips	Time (min) and temp. (°C) for breaking cook		H-factor	Residual alkali g/l	Alkali consumpt. kg/t	Tot. Yield %
LX.Beech.a	28-29	10	120°C	2	32.12	73.3	94.6
LX.Beech.b	22-23	45	120°C	7	24.36	119.9	79.4
LX.Beech.c	24-25	10	165°C	162	17.95	158.3	70.1
LX.Beech.d	30-31	30	165°C	374	15.87	170.8	62.9
LX.Beech.e	26-27	162	165°C	1703	10.44	203.4	48.8

Xylan used: Beech-xylan, nr. 4252, lot BCBG5928V from Sigma.

APPENDIX 1B. ANALYSIS OF BEECH XYLAN

Beech xylan		
Galactose	Rel. %	1.3
Glucose	Rel. %	1.8
Mannose	Rel. %	<0.1
Arabinose	Rel. %	0.1
Xylose	Rel. %	88.5
HexA	Rel. %	<1
GlcA	Rel. %	7.5
Rhamnose	Rel. %	0.8
Tot. anhydro sugar	%	100
Molecular weight	Mp	12570
	Mn	9310
	Mw	11095

APPENDIX 2. HISTOCHEMICAL AND IMMUNOHISTOCHEMICAL ANALYSIS OF LIGNIN AND NORMAL/LOW MOLECULAR WEIGHT XYLAN DISTRIBUTION WITHIN MODEL WOOD CHIPS

Lada Filonova and Geoffrey Daniel, SLU, Uppsala

Aim

The aim of the project was to visualize the microdistribution of lignin and xylan in spruce wood model chips during kraft pulping with added beech xylan of different molecular weights (X-HMw; X-LMw xylans). Interest was directed to the possible penetration of xylans into the wood blocks and fibre cell walls as delignification progressed.

Background

One possibility for improving the strength delivery of the kraft process is to better understand the events taking place in time and space at the wood (i.e. chip) structural level –for example the uniformity of cooking and the diffusion in- and out of components. Here the diffusion of the cooking liquor into wood chips and the movement of xylans in and out of the wood chips are of particular interest. Understanding these events could lead to improved retention of native xylans or introduced extraneous xylans thus improving or retaining the strength of fibres during processing. In this study, the microdistribution of lignin and xylans was followed by interrupting laboratory kraft cooks at different stages (i.e. cooks with- and without added beech xylan of high and low molecular weight) and studying the partially degraded chips using fluorescence and immunofluorescence microscopy and specific xylan antibodies.

EXPERIMENTAL

1. Wood model chips

Spruce model wood pieces (20 x 30 x 5 mm) were collected during pulping at five cooking times with addition of 10 g/l of normal (here referred as X-HMw) beech xylan or 10 g/l of low molecular weight beech xylan at the impregnation stage of kraft pulping (*Fig. 1* summary report). Beech xylan (X-HMw) was obtained from Sigma (X4252). High Mw xylan was degraded using endo xylanase to achieve X-LMw of about 1800 (= low Mw xylan). The degradation of high molecular weight xylan under alkaline conditions was difficult to control and therefore endo xylanase was used instead (see Daniel et al., 2013, *Appendix 2*).

All model chips were dehydrated in ethanol and embedded in Technovit 8100 and LR white resin (Daniel et al., 2010a, b) followed by sectioning in three zones and

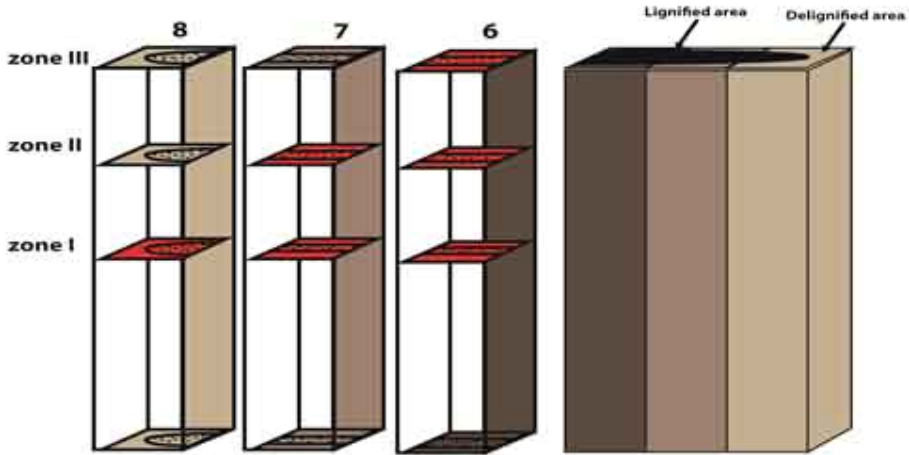six areas of interest within samples: Zone I – areas 8, 7, 6; Zone II - areas 7, 6; Zone III - area 6 (*Figure 1*).

Figure 1. Sampling of analyzed spruce model pieces: lignified (dark brown)/delignified areas; zones (I – III) and areas (8-6) of interest (in red).

2. Lignin and xylan detection by fluorescence specific labeling

Acridine orange was used to visualize the spatial microdistribution of lignin using secondary fluorescence. Lignin was detected in cross-sections cut from the model spruce pieces at the 5 different times of kraft pulping (*Summary report, Fig. 1, page 4*) and in all zones and areas of interest shown in *Figure 1*. Cross sections (*ca* 4 µm thick) of spruce chips embedded in Technovit 8100 resin were stained using aq. 0,05% of Acridine orange for 60 min., room temperature.

Spatial microdistribution of xylan was studied in cross-sections cut from the model spruce chips after 4 different times of kraft pulping (i.e. *Figures 1, 2* ; Summary report b - e) and in all zones and areas of interest (*Figure 1*). For detection of xylan semi-thin cross-sections (3-4 µm) were mounted on object glasses and labeled using two rat monoclonal antibodies against unsubstituted and low-substituted xylans (LM10) and low- and highly substituted xylans (LM11) (PlantProbes) overnight at 4°C (Daniel et al., 2010a). For signal visualization, sections were labeled by FITC-conjugated secondary antibody for 1h at room temperature. Samples labeled with only secondary antibody were used as a negative technical control.

Both acridine orange and LM10/LM11 labeled sections were analyzed using a Leica DMRE fluorescence microscope equipped with BP450-490 –FT510-LP 520 filter combination. Images were recorded using a Leica DC300F CCD camera and

digital imaging system for professional microscopy (Leica Microsystem) at equal settings: exposure time 1,3c and gain 3,2 for antibodies and exposure time 2c and gain 1,7 for acridine orange).

Results

1. Lignin vs xylan distribution
Acridine orange stained a central zone green in the model chips (*Figure 2, images top row*). This central area of green staining decreased as delignification progressed across the wood chips. In contrast, labeling with LM11 for low- and higly substituted xylans increased with cooking time (*Figure 2*). Both the acridine orange staining and the LM11 labeling represented a common trend for both the X-HMw and reference model spruce chips without added xylan (*Figure 2*).
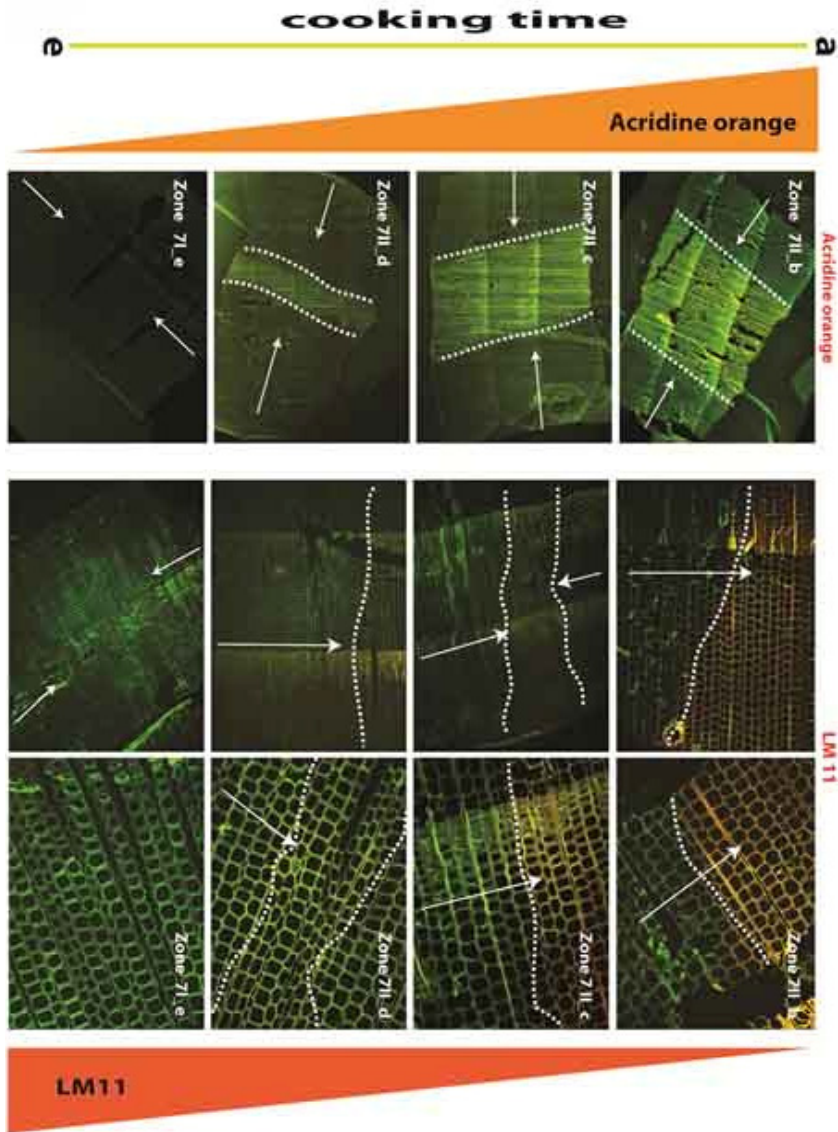
Figure 2. Kraft pulping time dependent distribution of lignin (acridine orange staining, images top row) and xylan-positive (LM11 antibody) signal in sections from the spruce model pieces with added X-HMw and reference sample without added xylan (Figure 2, bottom row). The large arrows on the sides (top and bottom) of the image show the progression of delignification and reduction of acridine orange staining from the edges of the wood blocks towards the centre and increase of LM11 labeling.

The delignification process did not appear controlled by the native structure (i.e. anatomy) of the spruce wood samples. No evidence for example was obtained that delignification followed the rays (e.g. easiest pathway) into the wood as the areas of delignification were produced irrespective of structure with an obvious diffusion of the cooking liquor into the wood (*Figure 2*, upper row of images with time). The images further show that penetration has occurred from the sides of the blocks over time (*Figure 2, arrows*) and that diffusion from the ends of the model chips was not as rapid as that from the edges. Indirectly, this would also indicate that any penetration of xylans into the wood blocks should likely follow the same pathway.

2. Xylan vizualisation in sections from spruce model chips with- and without added X-HMw beech xylan

The intensity of LM10 and LM11 fluorescence signals increased with time of cooking (i.e. delignification) in both the X-HMw and reference model wood chips. Presumably, delignification leads to an increase of xylan available for xylan-specific labeling (*Figures 3, 4*).

Comparison of the fluorescence signal intensity in sections from spruce chips with- and without addition of X-HMw beech xylan showed a higher signal in xylan treated samples both with LM10 and LM11 labeled samples, both in the early- and latewood (*Figures 3, 4*). Interestingly, this tendency was preserved in samples taken from all zones (*Figure 1*) and areas of interest irrespective of cooking time. *Figures 3, 4* show comparison of signal intensity in X-HMw xylan treated- and reference samples at the same cooking time.
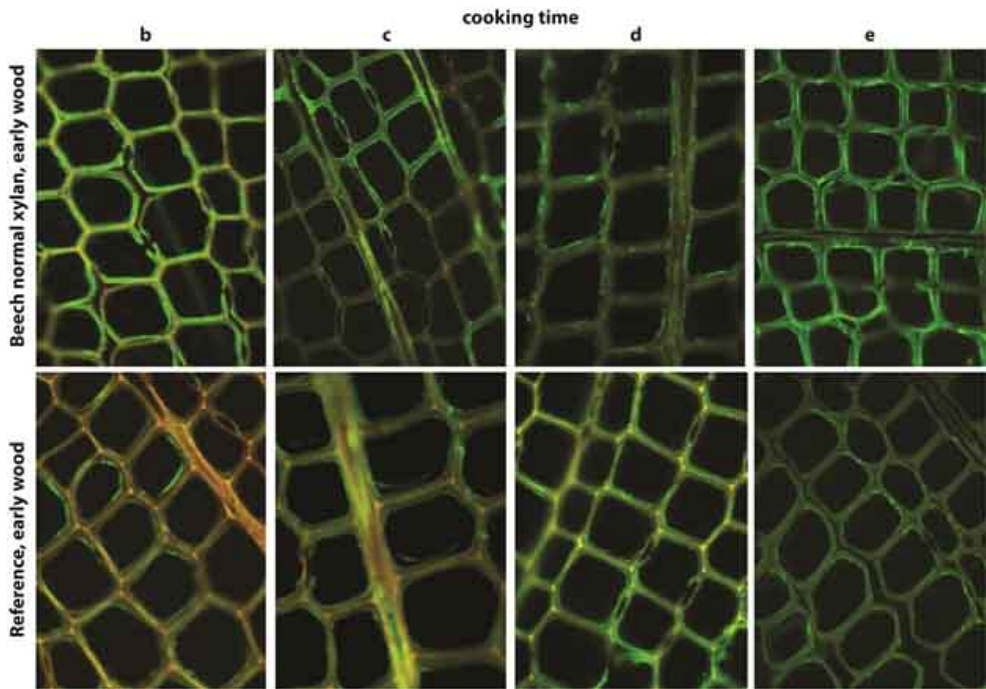
Figure 3. Comparison of the intensity of LM11 labeling of sections from spruce wood chips with X-HMw xylan and reference samples with time of kraft pulping (earlywood).
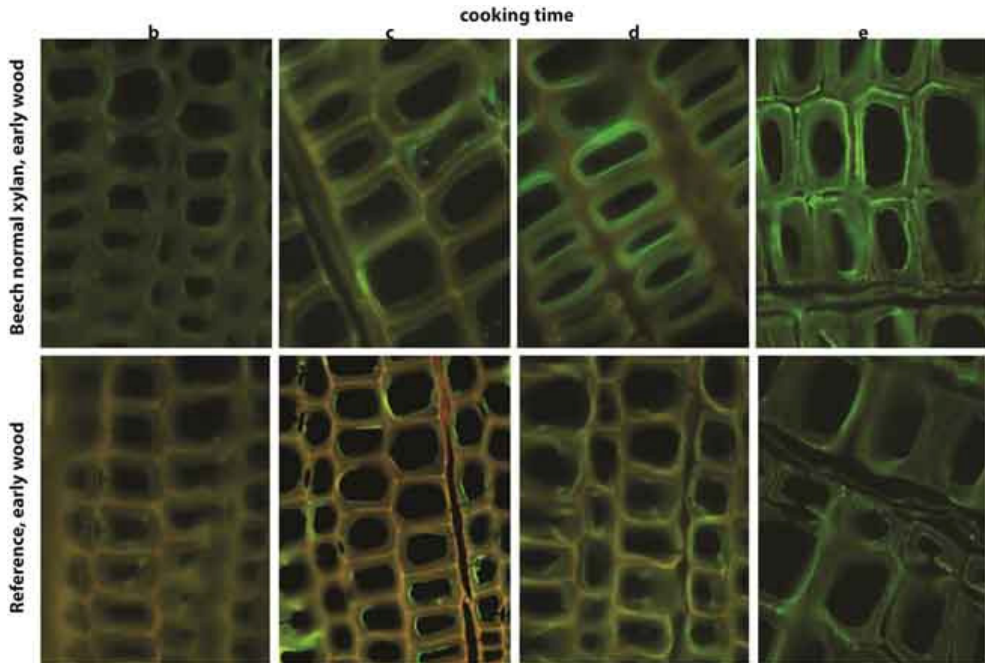
Figure 4. Comparison of the intensity of LM11labeling in sections from spruce wood chips with X-HMw xylan and reference samples depending on time of kraft pulping (latewood).

Following localization of the fluorescence signal we can conclude equal signal intensity within cell walls and abundant signal localization in cell lumen in both chip types (treated and non-treated). However, in samples from cooking time **e**, there is prevalence for an extracellular distribution of the fluorescence signal in both amount and intensity for the X-HMw xylan treated model wood chips compared to reference samples pieces. This is typical for all areas of interest in the **e**-treated model chips (*Figure 5*).
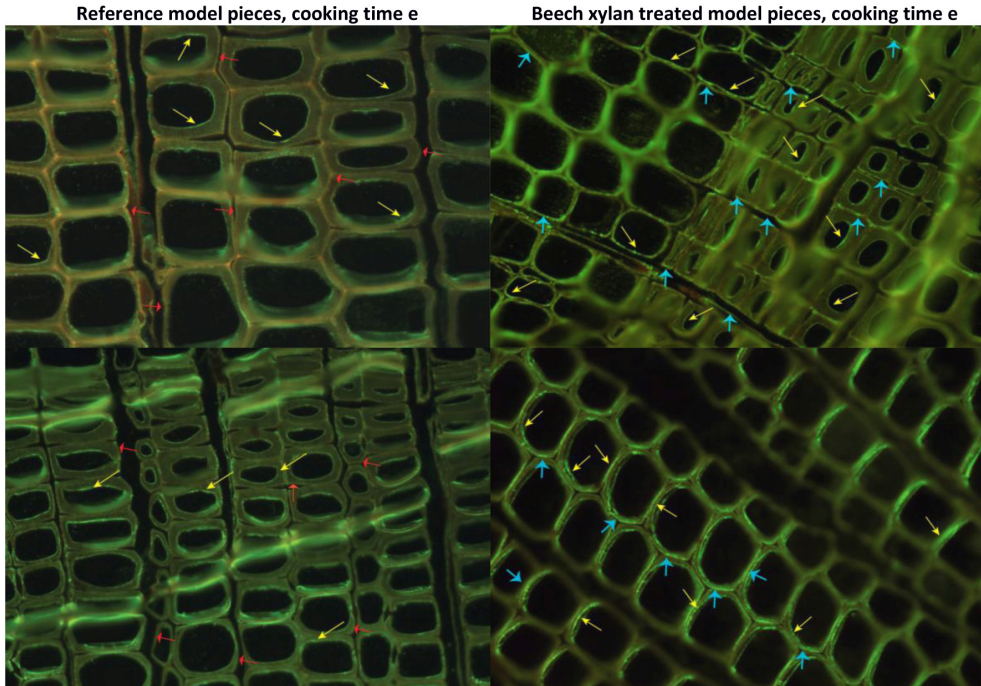
*Figure 5. Spatial microdistribution of xylan in spruce model pieces after cooking time **e**. Yellow arrows show positive signal for xylans using LM11 in the fibre cell lumina; blue arrows on the outer surfaces of the fibre cell walls (i.e. middle lamella and S1/primary wall). Red arrows – indicate no signal on the outer fibre cell wall outer surfaces. Only LM11 is shown as results for LM10 were similar.*

3. Xylan visualization in spruce model pieces with and without added normal beech xylan

Only **"e-cooked"** wood model pieces with added X-LMw beech xylan were available for analysis in this study. Samples were characterized by low lignin content, opened structure (i.e. the middle lamellae were partially or fully degraded) and damaged areas. The intensity of fluorescence signal in samples (i.e. using semi-thin sections) labeled by LM10 was shown to be significantly higher than for samples labeled by LM11 (Figures 6-8). This was also true for signals within cell walls (both early- and latewood). Thus, we have more intensive LM10 signal in the whole section compared with LM11. This was similar for samples taken from all areas of interest (Figure 1).

Figures 6-8 show immunofluorescence images after labeling with LM10 or LM11 antibodies from treatment stage e at different zones of the chips. See Figure 1 for explanation of zones.
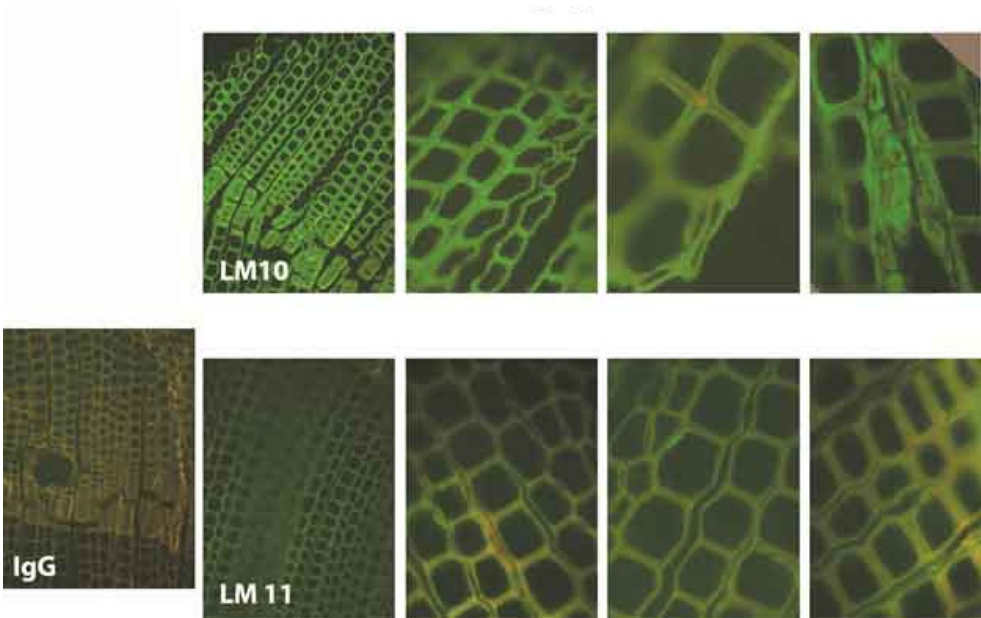
26

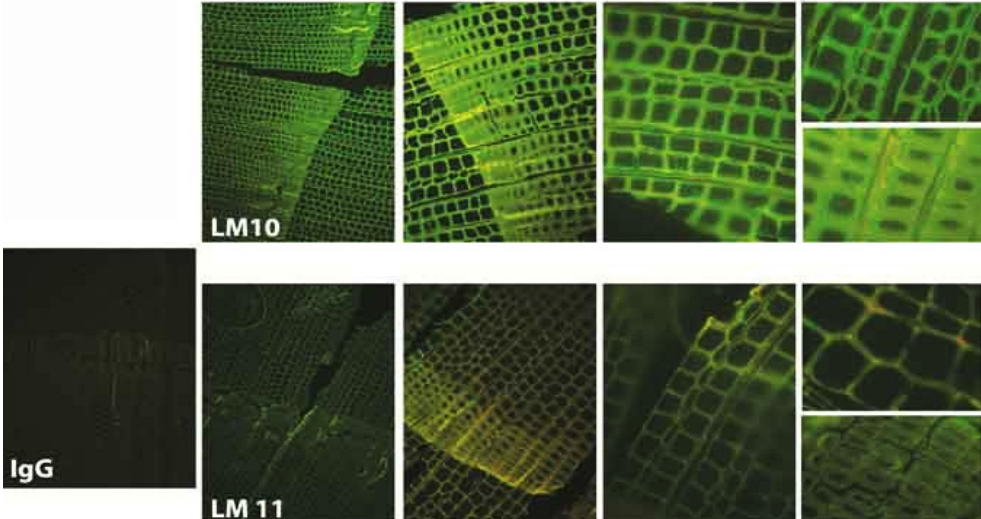*Figure 6. Immunofluorescence from zone **e** (LX low molecular weight zone 6III treatment **e**)*

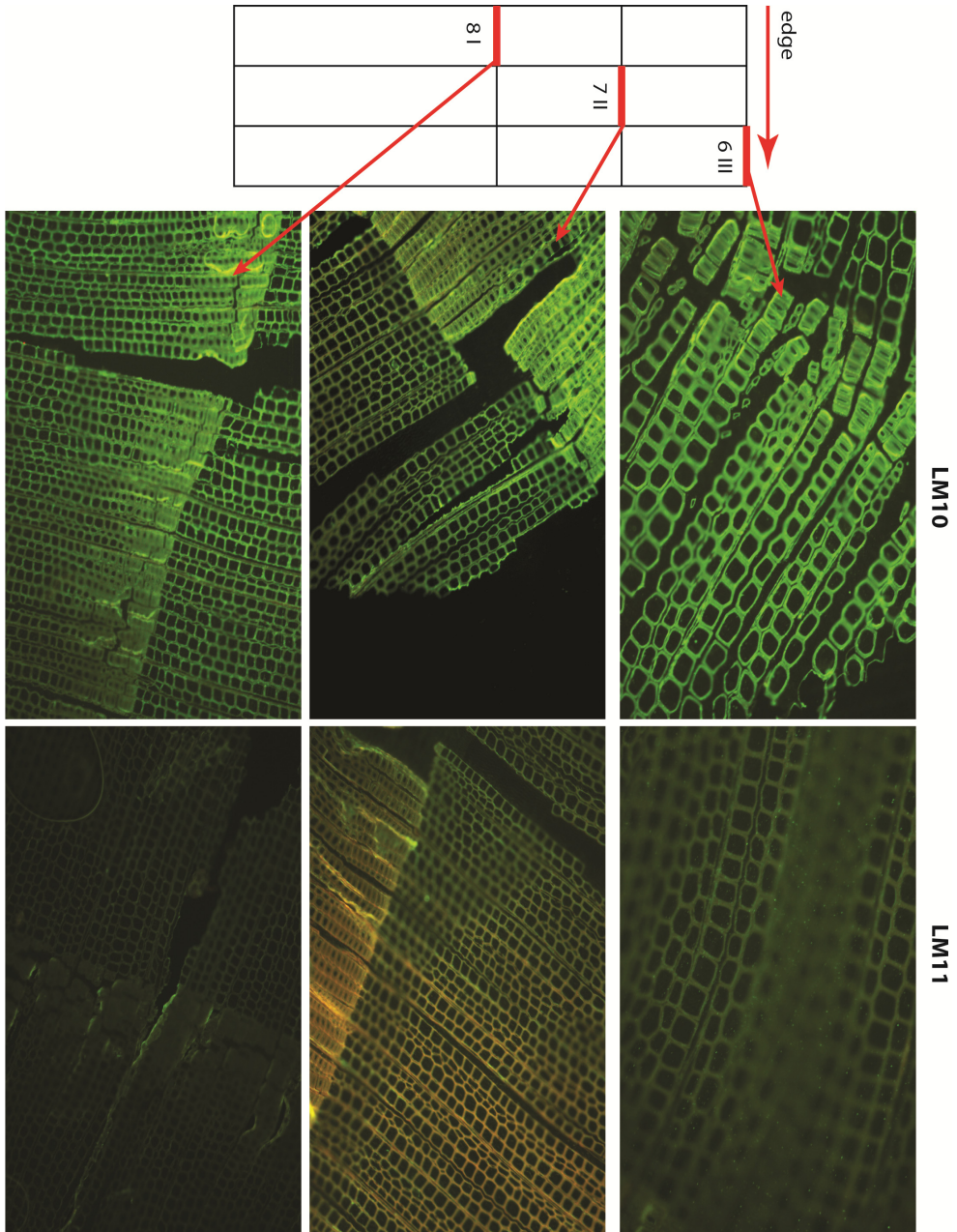*Figure 7. Immunofluorescence from zone **e** (LX low weight zone 8I treatment **e**)*

27

Figure 8. Immunofluorescence for the presence of xylan (C-LMw) using LM10/LM11 in sections taken from 3 zones (8I, 7II, 6III) of the model chips.

Conclusions

1. Sections from X-HMw treated chips showed greater fluorescence intensity starting at the first stage of cooking for both LM10 and LM11 compared with reference chips;
2. That the reference X-HMw treated blocks showed evidence for more intense signals in the cell lumen of fibres at least starting from stage **b**; At later stages of cooking (i.e. stage **e** sometimes **d**.) (*Figure 5*) there is a difference in signal distribution. In the reference chips the signal was most often in the cell lumen areas. With the beech X-HMw xylan treated blocks, the signal was very often outside the fibre in the position of the degraded middle lamellae as well as in the cell lumen areas (*Figure 5*);
3. At stage **e.** with X-LMw xylan there was a significant difference in immunofluorescence with LM10 and LM11. LM10 gave a more intense signal (i.e. *Figures 6-8*). In this case, this is an indication for the penetration of X-LMw xylan into the cell walls of the fibres;
4. Points 1-3 were similar for all zones and areas of interest for the model chips;
5. Concerning the penetration of xylans into the wood chips. Penetration into the wood blocks seems to be feasible, but the pathway has not been confirmed. Presumably, it occurs along the same pathway as the diffusion of the delignification liquor. Regarding penetration of xylans into the fibre wall further studies are needed;
6. One possible problem that may have existed is that during the washing stage after cooking that some of the xylans have been removed so the difference between treated- and non-treated were are not as distinct.

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Daniel, G., Olsson, A-M., Ander, P, Salmén, L., Filonova, L., Sjöström, K, Boussard, L., Heijnesson-Hultén, A. 2013. The influence of the molecular weight of the added xylan on pulp properties. CRUW Chemical Pulp. Intern rapport 9, 58 pp.

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Aims

The purpose of this study was to investigate how the lignin and xylan content changed within a chip and within the fibre cell walls during cooking. By following changes in xylan distribution during cooking both for normal- and cooks with xylan added, it was hoped that knowledge could be gained to understand how addition of extraneous xylan may affect the dissolution of both xylan and lignin during kraft cooking. In order to monitor local changes in chips and fibre walls FTIR-spectroscopy and FTIR microspectroscopy was chosen as this allows local areas and/or small amounts of material to be studied for chemical characteristics.

Background

It is well known that xylan is precipitated onto fibres surfaces during cooking. With addition of extraneous xylan an increase in the total xylan content of pulps have been noted. This additional xylan also improves the strength properties of the pulp. There is an hypothesis that the added xylan could also affect the strength delivery of the pulp, i.e. diminish the detrimental effects on strength properties during industrial pulping due to mechanical forces compared to laboratory handled cooks. So far this hypothesis has not been possible to verify. Therefore to understand how and when addition of xylan may affect the precipitation of xylan, studies were performed to follow the local composition of xylan and lignin as a function of cooking time. FTIR spectroscopy is a suitable technique for characterisation of chemical changes in a material as only small amounts of sample material are required. Also with the use of imaging FTIR microspectroscopy variations in chemical composition may be characterised at the cell structural level (Fackler et al. 2011).

Experimental

WOOD MATERIAL

The model chips were frozen in liquid nitrogen and stored in -70 °C. Half of each model chip was divided into 3 parts with approximate cross section dimensions of 5*5 mm and 30 mm in the fibre direction. From this small wood sticks were taken at 6 positions as shown in *Figure 1*.
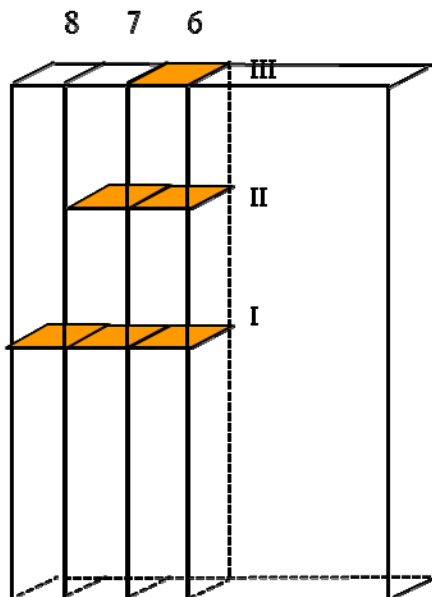
Figure 1. Schematic image of a model wood chip showing the position of sampling.

Overall changes in chemical composition were studied by grinding the wood material from these areas and blending these with KBr at the level of less than 1% of material making tablets for FTIR transmission measurements. Microtome cuts from different positions were made from the frozen model wood pieces. These cuts were dried on glass slides for imaging FTIR microspectroscopy studies.

FTIR ANALYSIS

The composition of the ground material (KBr-tablets) was determined using FTIR spectra taken in transmission by a Varian 680-IR spectrometer (Agilent technologies, Santa Clara, CA, USA) in the range from 700 to 4000 cm^{-1} with a spectral interval of 1 cm^{-1} using a DTGS detector.

The spectra from the ground material were baseline corrected to zero at 813, 1525, 1845, 2505, 1981 and 3750 cm^{-1}. To compensate for different amounts of material in the tablets the spectra were normalised to an intensity of 1 at the peak at 2890 cm^{-1}, assigned to overall carbohydrates. In Figure 2 a baseline corrected and normalized spectrum is shown. The amount of lignin was evaluated as the peak height at 1508 cm^{-1} assigned to the C=C aromatic ring vibration. Xylan appears as a shoulder at 1460 cm^{-1} from CH$_2$ bending. This value was difficult to determine and therefore the xylan estimate is more uncertain than lignin. The peak heights have not been recalculated to an absolute content, therefore they must be seen as relative differences.
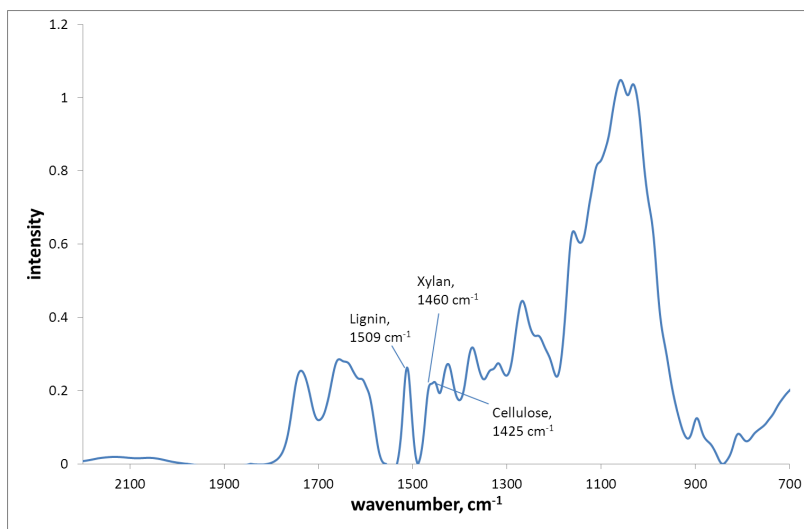
Figure 2. A typical spectrum of wood baseline corrected and normalized. The peaks used for evaluation are indicated.

For imaging, a Perkin Elmer FTIR equipped with a microscope using a Spectrum Spotlight 400 FTIR Imaging System (Perkin Elmer Inc, Shelton, CT, USA) was used. In the microscope using attenuated total reflectance (ATR) with a germanium crystal pressed towards the sample, spectral images with a pixel size of 1.56 μm times 1.56 μm were obtained. These measurements were taken with a spectral resolution of 4 cm^{-1}.

Distribution images were made from one spectrum from every 1.56 μm sized pixel. Spectral images were calculated showing lignin; with peak area from 1510 to 1500 cm^{-1} and xylan with peak area from 1450 to 1440 cm^{-1} normalized with against the peak area 1430 to 1420 cm^{-1} corresponding to cellulose.

Results and discussion

The relative composition of lignin and xylan was studied as a function of cooking time, both at the chip level and the fibre level using FTIR imaging. The two conditions of reference (i.e. no xylan added) and that with addition of 10 g/l of beech xylan to the cooking liquor were studied.

COMPOSITION ON CHIP LEVEL

When comparing the composition on the chip level the amount is given as a relative estimate based on the absorption from carbohydrates. As seen from Figure 3, with xylan added, slightly higher lignin content was obtained. The scatter in the

last point (i.e. longest cooking time) for samples from the interior of the chip 6 I was large but the trend seemed general. This was also consistent with the chemical composition determined on the whole pulps indicating slightly (not significantly) higher lignin content for pulps with added xylan.

By comparing the different positions of the chips -the surface as compared to the interior- it was clear that the lignin content was lower on the surface of the chips for both cooking experiments (Figure 4). The situation was somewhat confused when comparing the end surface of the reference cook (Figure 4); see also Figure 6 from middle of chip)
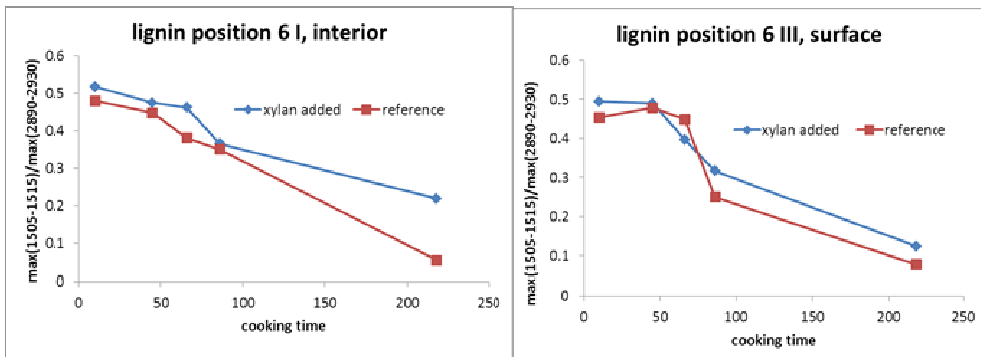

Figure 3. Relative lignin content, determined from peak heights of FTIR spectra, as a function of cooking time for reference cook and cook with addition of beech xylan. Two positions in the chips are compared; chip surface (right), chip interior (left).
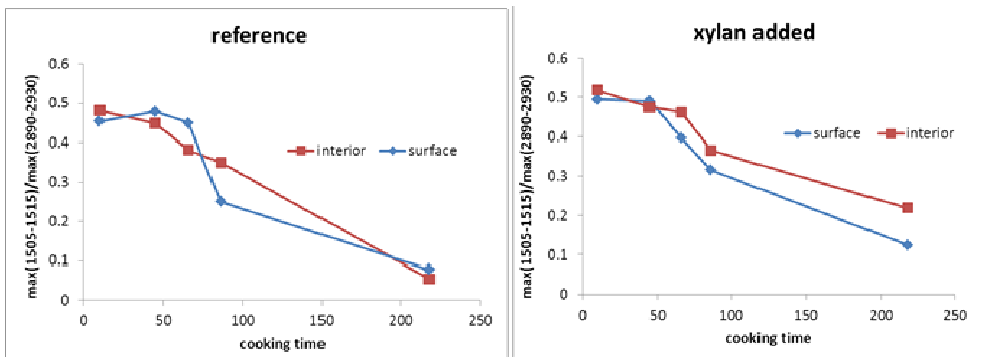

Figure 4. Relative lignin content, determined from peak heights of FTIR spectra, as a function of cooking time for two positions of a chip; chip surface 6 III and chip interior 6 I. Two cooks are illustrated, a reference cook (left) and a cook with addition of beech xylan (right).

For changes in xylan content, the scatter was too large (*Figure 5)* in comparison to the changes occurring as a function of cooking time or between the two cooking series; the reference cook and cook with added beech xylan.
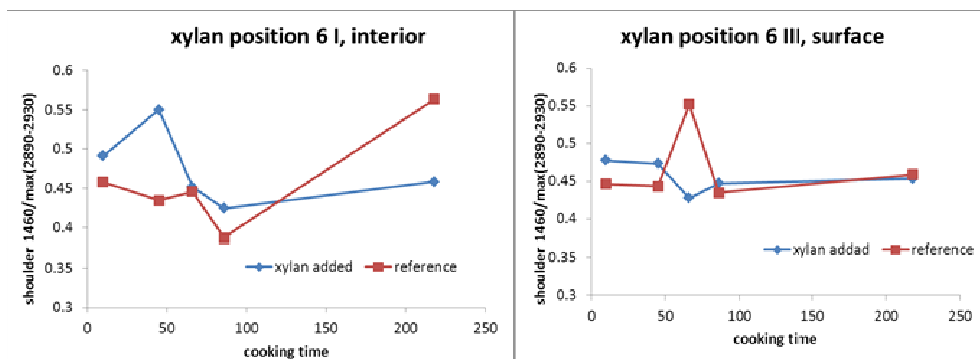

Figure 5. Relative xylan content, determined from peak heights of FTIR spectra as a function of cooking time for a reference cook and a cook with addition of beech xylan. Two positions in the chips are compared; chip surface (right) and chip interior (left).

These trends were also confirmed from measurements from chip areas from position 6I where the surface part was compared to the interior part (*Figure 6)*. At the end of the cook the interior of the chip contained substantially more lignin than the surface. In this case higher lignin content was also noted for the cook with xylan added but only on the surface. For xylan no clear conclusion could be drawn due to the large scatter.
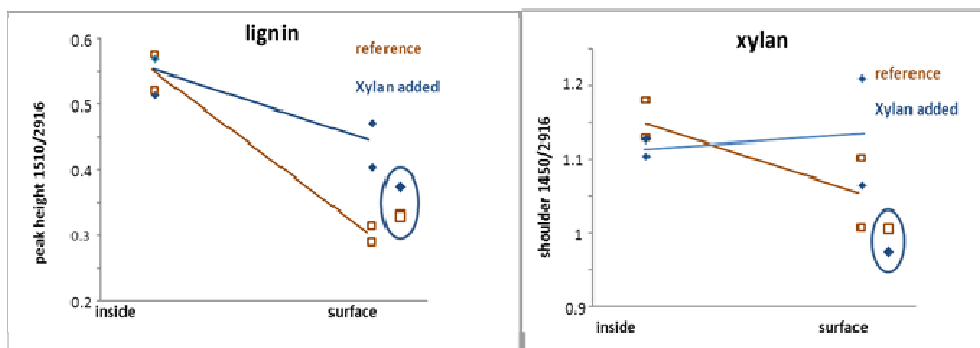

Figure 6. Relative lignin and xylan content respectively (left and right) determined from peak heights of FTIR spectra for two positions of a chip; inside and surface at position 6 I. comparing a reference cook and cook with addition of beech xylan.

34

COMPOSITION ON FIBRE LEVEL

Studies of the composition at the fibre level were made on microtome cuts made on frozen wood samples in order to avoid embedding media that would interfere with the FTIR measurements. In general, the cuts were very difficult to examine due to deformations of the fibre structure from the cuts and also during the measurements performed with ATR (Attenuated Total Reflection) when the crystal was pressed onto the surface of the sample. Good distribution images could only be made for wood samples cooked for 46 and 66 minutes. With shorter times the samples fibres were too stiff and with longer times too weak.

As seen from Figure 7 it is not easy to follow trends in dissolution of the components as a function of cooking time. For lignin clear reduction of the lignin content with cooking time was seen, although the final position, e is difficult to examine. The images also indicate that lignin should be more easily removed from the tangential- than from the radial walls. The higher content of lignin as well as xylan noted in the middle of the images from position c was probably coming from the ray cells. For the xylan content the images were less clear.
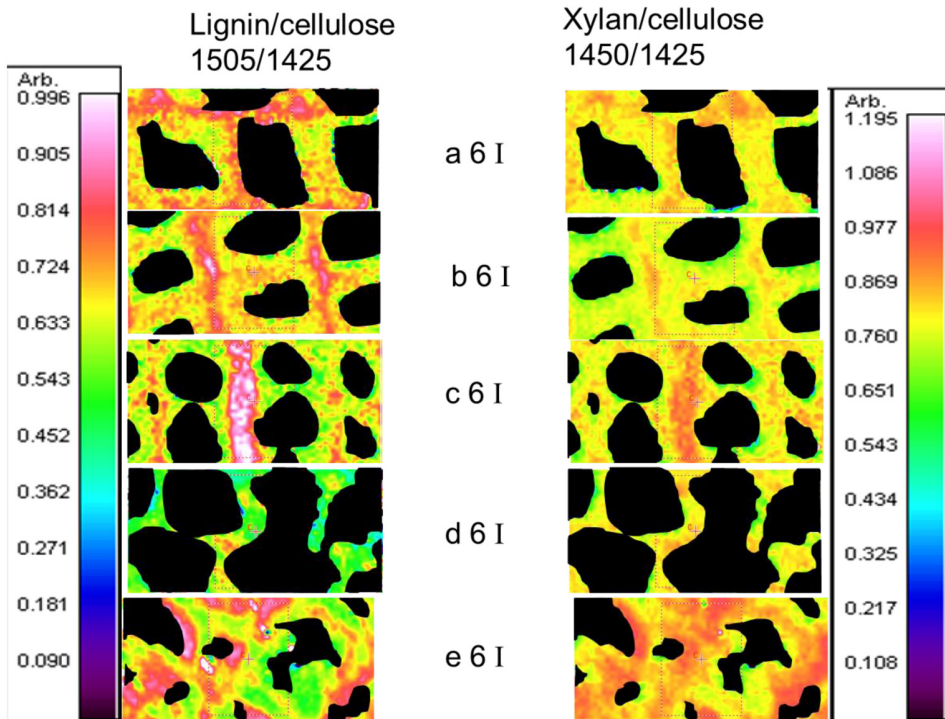
*Figure 7. Images of the relative lignin and xylan content respectively (left and right), determined from peak heights of ATR-FTIR spectra from each position of 1.56 times 1.56 μm as a function of cooking time, **a** to **e**, from chips at position 6 I for a reference cook. Red colour indicates high amounts, green colour, and low amounts of the respective components.*

Conclusions

Dissolution of lignin seems to be possible to monitor to some extent with FTIR spectroscopy. Clearly higher lignin contents were noted in the interior of chips than on the surface. It also seemed clear that, that slightly more lignin remained in chips cooked with the addition of xylan than without. For xylan distribution no significant trends in variations of the content as a function of cooking time or between the cooking with or without addition of xylan were noted due to too high scatter in the absorption peaks used in evaluating FTIR spectra.

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APPENDIX 4. FLUORESCENCE LOSS INDUCED BY PHOTOBLEACHING (FLIP)

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Summary

The micro-diffusion properties on wood and kraft cooked wood fibers, with- and without addition of xylan, have been analyzed using a relatively new method, Fluorescence loss induced by photobleaching (FLIP). The fluorescence microscopy method FLIP has in this study been adopted to track the diffusion properties of a fluorescent marker (FITC) within the wood-fiber cell-wall matrix; the more spacious matrix – the faster diffusion. Wood has anisotropic structure and consequently showed anisotropic diffusion properties. Kraft cooked fibers showed increased diffusion rates (relative to native wood fiber). The addition of xylan to the cook did not significantly affect micro-diffusion in the secondary wood cell wall after the cook.

Introduction

In this study, analyses of micro-diffusion of a green fluorescent marker (FITC) in wood fibers have been performed by tracking the movements of fluorophores within the fiber cell walls using a confocal microscope. Micro-diffusion analysis using a microscope-based technique allows for high spatial-temporal resolution, higher than with other spectroscopic methods, which enables even very small (and fast) events to be recorded and analyzed. Fluorescence loss induced by photobleaching (FLIP) is one of several newly developed methods for analyzing local diffusion properties using fluorescent markers (Ishikawa-Ankerhold et al. 2012)., We employed FLIP for analyzing wood fibers since it allows for studies on samples with great differences in diffusion properties. These methods have been developed, and are most commonly used, for tracking protein movements within single living cells, but can also be used analyzing properties of extracellular matrixes. Little work has previously been performed on heterogeneous matrixes since heterogeneities in shape, size and phases greatly affect the diffusion parameters. However, if the analyzed area is much larger than the heterogeneities the effects of the structure will quickly become averaged and thus allow for valid comparisons between experiments (Lorén et al. 2009). Wood consists of heterogeneous sub-structures of nanometer scale and by analyzing diffusion effects on micrometer scale we average the intrinsic heterogenic structures. Using FLIP, we employed a new approach to probe the effects of cooking and xylan addition on wood cell wall diffusion properties.

Experimental

Fluorescein isothiocyanate (FITC) is a highly green fluorescent molecule that was dissolved in water. The solution was added to cross sections of wood block samples. The wood sections were transferred onto glass-slides and mounted wet under a cover glass. Fluorescence loss induced by photobleaching (FLIP) analyses was performed using a confocal microscope (Zeiss LSM510META Confocal Microscope). The concept of FLIP is basically that a fluorescent marker is present within a sample, and then a defined area in the sample (that display fluorescence) is continuously photo-bleached by short laser-pulses. Diffusible fluorophores will meanwhile diffuse into the photobleaching area and thereby lower the surrounding fluorescence as more and more fluorophores diffuses into the photobleaching pulse and are destroyed. The principles of the technique are shown in *Figure 1*. *Figure 1 a) - c)* are illustrations of samples displaying 100, 50 and 0% diffusion, respectively. The regions of interest (ROI) that are analyzed in the microscope are indicated by yellow squares, and the photo-bleaching ROI is labeled red. At time point t_4, the different diffusions have resulted in very different fluorescence intensities at the yellow ROI, and the intensities can be directly correlated with the diffusion properties. If the bleaching and monitoring areas, and time, is carefully analyzed, also diffusion coefficients can be calculated.
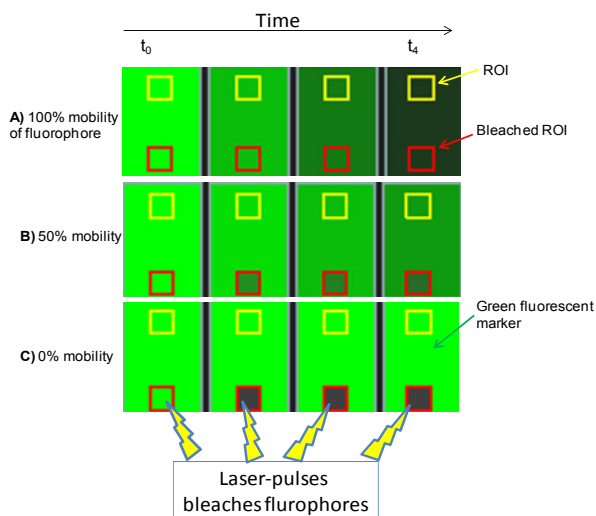

Figure 1. Illustration of the principle behind fluorescence loss induced by photobleaching (FLIP). The green light represents the emitted light of fluorophores distributed throughout a matrix. In Fig. 1a) fluorophores diffuse freely and photobleaching affects the whole sample. Partial diffusion (b) has some general effect on fluorescence but noticeable stronger effect on the photobleached ROI. At 0% mobility (c) the effect of photobleaching is located and restricted to the photobleached ROI.

Results and discussion

MICRO-DIFFUSION IN WOOD

Since FLIP is a new method for spatial-temporal resolved micro-diffusion studies on wood and wood fibers, we first analyzed the diffusion properties of FITC in native wood. The known structural anisotropy of wood needs to be measurable using FLIP for the method to be useful for analyses of introduced chemical of mechanical structural effects. FITC is water soluble and small enough to diffuse within the wood fiber matrix (molecular weight 332 g/mol). FITC is fluorescent with excitation maximum at 494 nm and emission at 521 nm. Therefore, its presence and movements within a matrix can be easily monitored using a fluorescence microscope equipped with appropriate optical filters. *Figure 2a* shows a micrograph of a cross section of wood with FITC. The green fluorescence shows the presence of FITC. The graph in *Figure 2b* shows the fluorescence intensity development in ROIs 1-3. The sample has been exposed to repeated laser pulses at ROI "1", i.e. the laser induces photo-chemical destruction of the FITC molecules within ROI 1 (Song et al. 1995), and after 80 pulses only 40% of the initial fluorescence remains. In the area next to the photo-bleached zone, in ROI 2, there is about 60% fluorescence left, indicating there has been some diffusion from ROI 2 into ROI 1. ROI 3 is located on the other side of the compound middle lamella, and further away from ROI 1, and clearly fewer fluorophores have diffused from ROI 3 into ROI 1. By measuring local diffusion rates we concluded FLIP is useful for micro-diffusion studies of wood. The diffusion clearly showed the expected variations over time and place, for example the diffusion across the compound middle lamella is considerable slower than within the secondary cell wall of a fiber tracheid. And the diffusion rate was slower with time and distance.
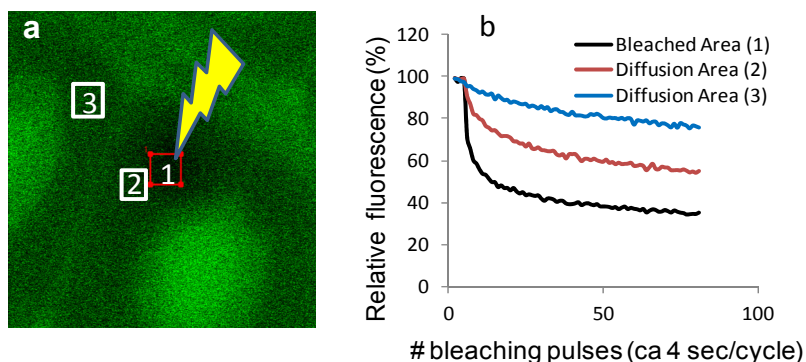

Figure 2. Cross section of wood impregnated with green fluorescent marker molecules (FITC). Figure 1a) shows photobleached ROI (marked with red box and number "1") and two ROI boxes marked "2" and "3". Figure 2b) shows the fluorescent intensities over time in the respective ROIs (marked in 1b).

MICRO-DIFFUSION IN KRAFT COOKED FIBER WALLS

Micro-diffusion analysis of cooked wood and fibers showed an effect of the cooking itself (longer cooking gives more open structure); but, the effects of xylan addition to the cook were less evident (in the different analyses used in the project). However, low molecular weight xylan seemed to effect the relation of tensile strength *vs* hand sheet density (Intern rapport nr 10, chemical pulping). Whether it is due to decreased inter-fiber bonding strength or increased paper or fiber density is unknown. Increased fiber density would be possible to measure by the micro-diffusion properties in the fiber wall matrix. We therefore specifically analyzed wood fibers cooked to time point "E" (2h cooking at 165 °C, see Experimental for this Intern rapport) with, and without, addition of low Mw xylan to the cooking liquor. The samples were impregnated with FITC and analyzed for micro-diffusion using FLIP. Time point E was the final time point of our cooking scheme and has lower lignin content and a more porous structure than lignified fibers. The xylan sample and the reference were analyzed using the same conditions and the diffusion rates were compared at the same relative distance ROI to the photo-bleached areas (*Figure 3*). The diffusion rates did not change significantly with the addition of xylan, which indicates that, at least, no large amount added xylan penetrated the wood fiber secondary cell wall, to fill the gaps lignin removal leave in the wood matrix during cooking. However, the results do not show whether cooking dynamics specifically for native xylan have been affected by the xylan addition. The dissolution rates and properties of native lignin/xylan during the cook might be affected by the addition of extraneous xylan to the cooking liquor. The xylan addition to kraft cooking did result in slightly larger alkali consumption to time point E (*Appendix 1*), more remaining hydrolysate *(Table 2*, Results and discussion for this Intern rapport) and affected lignin fluorescence wavelength distribution (data not shown), which together suggest the cooking and lignin degradation to be somewhat affected, and thereby maybe also lignin-associated hemicelluloses. However, to obtain further information on endogenous-extraneous xylan relationship in wood fibers perhaps more xylan-specific and/or yet higher resolution methods must be developed.
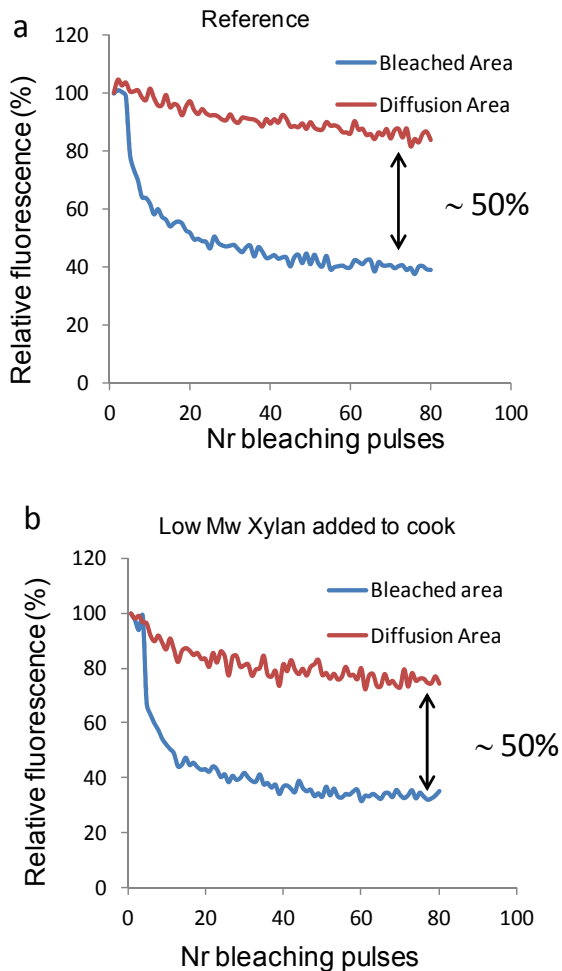
Figure 3. FLIP graphs of kraft cooked wood fibers, a) Reference sample, and b) cooked with addition of low molecular weight xylan.

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Collaborative Research on the Ultrastructure of Wood Fibres (CRUW)

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