Effect of cooking and bleaching on the structure of xylan in conventional pine kraft pulp

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ABSTRACT: Pine chips were cooked with a conventional kraft process, and the structure of the surface xylan as a function of the cooking time was analyzed by enzymatic peeling and ^{1}H-NMR spectroscopy. A brownstock pulp with kappa no. 25.9 was also bleached to about 80% ISO brightness with bleaching sequences OZEP, OPZEP, OPPP, and ODEDED. The structure of the kraft pulp xylan was different from the native wood xylan. During kraft pulping, the 4-O-methylglucuronic acid (MeGlcA) sidegroups were converted to 4-deoxyhex-4-enuronic acid (HexA) groups almost completely, and as a consequence, only about 12% of the carboxylic groups of the accessible xylan were 4-O-methylglucuronic acid groups. During bleaching with ozone or chlorine dioxide, the hexenuronic acid sidegroups of pulp xylan were, however, degraded, whereas the MeGlcA and arabinose sidegroups were relatively stable toward the bleaching chemicals. Thus, both cooking and bleaching affect the carboxylic acid profile and content in kraft pulps.

KEYWORDS: Bleaching, chemical pulping, enzymolysis, kraft pulping, kraft pulps, multistage process, nuclear magnetic resonance, pinus, *uronic acids, xylanase.*

Softwoods contain about two-thirds of O-acetylgalactoglucomannan and one-third of arabino-4-O-methylglucuronoxylan of the total hemicellulose *(1).* In kraft pulping, the structure of hemicelluloses is extensively modified because of the dissolution of polymers and partial degradation of sidegroups due to the high alkalinity and temperature of the cooking liquor. As the cook proceeds, the alkali concentration decreases, and xylans precipitate in a more or less ordered form onto the surface of cellulose microfibrils *(2).*

Conventional kraft pulps are reported to contain about 85–125 mmol/kg of carboxylic groups, depending on the wood species *(3).* The total amount of carboxylic groups in the pulp can be determined by indirect methods, i.e., by ion exchange *(4)* or by different types of titrations *(3).* The chemical composition of the basic constituents of pulp carbohydrates has been previously determined by using acid hydrolysis. This method can, however, lead to a lower concentration of the components than that obtained by indirect methods, due to incomplete hydrolysis or degradation of the carbohydrates.

Specific enzymes hydrolyzing pulp hemicelluloses can be used as analytical tools in fiber characterization *(3, 5, 6).* Due to the selectivity of the hemicellulases, xylanases solubilize only pulp xylan without attacking other carbohydrates in pulp. Correspondingly, mannanases solubilize only pulp glucomannan. In addition, due to the mild hydrolysis

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conditions, no additional destruction of solubilized carbohydrates or fibers occurs. By combining this enzymatic peeling of fibers with powerful techniques of carbohydrate analysis, such as NMR spectroscopy, novel information on the structure of the pulp carbohydrates can be obtained.

In this study, pine chips were cooked using a conventional kraft process, and the structure of the surface xylan was analyzed as a function of the cooking time by using enzymatic peeling and proton NMR. The brownstock pulp was also bleached to brightness values of 50–88% ISO with bleaching chemicals that included oxygen, ozone, chlorine dioxide, and hydrogen peroxide. The effect of these bleaching chemicals on the chemical structure of surface xylan was evaluated with special emphasis on the structure and amount of sidegroups, especially carboxyl groups in pulp xylan.

Materials and methods

Pulps

Pine *(Pinus sylvestris)* logs from Southern Finland were debarked and chipped in the field. The chips were dried and screened.

The cooking experiments were performed at the Research Centre of Enso-Gutzeit Ltd. The liquid-to-wood ratio was 4:1, and the charge of active alkali (as Na_2O) was 18.0 or 22.0%. The sulfidity of the white liquor was 34%, and the rate of heating from 80 to 170°C was 1°C/min. The maximum time at 170°C was 148 min. Ten experiments, representing different stages of cooking, were carried out (**Table I**). The undiluted cooking liquor was recovered, and the chips were washed with tap water, disintegrated, and screened (pulps with kappa number less than 50). The carbohydrate compositions of the pulps are presented in Table I.

The pine kraft pulp with kappa no. 25.9 was cooked in a 600-L digester at the Finnish Pulp and Paper Research Institute. The cooking conditions were active alkali: 18%, sulfidity: 35%, and H-factor: 2033.

Bleaching

The pulp with kappa no. 25.9 was bleached with different sequences to brightness values of 50–89%. The bleaching conditions used are presented in **Table II.**

Analysis of the pulps

The carbohydrate composition of the pulp samples was analyzed as described earlier *(7).* The pulps were also analyzed for kappa number (SCAN C1:1977), viscosity (SCAN 15:1962), and brightness (ISO 3688).

Enzymatic peeling

The xylanase (pI 9) was purified from *Trichoderma reesei* as previously described *(8).* The pulps obtained as a function of the cooking time were peeled with xylanase with an enzyme dosage of 5000 nkat/g for 24 hours. The enzymatic peeling was carried out at 45°C, pH 5.0, and 5% consistency. The pH of the unbleached pulps was adjusted with H_2SO_4. In the enzymatic peeling of bleached pulps, the metal profile of the pulps was modified prior to the enzyme addition to be beneficial for the xylanase action *(9).* The bleached pulps were acidified to pH 2.5 with 0.5 M H_2SO_4 at room temperature to remove the metal cations and were washed twice with distilled water. Afterward, the pH of the pulps was adjusted with 0.1 M $Ca(OH)_2$ to 5.0, and the xylanase peeling was carried out as previously described. After the hydrolyses, the solubilized carbohydrates were filtered before further analysis.

Analysis of the solubilized carbohydrates

The carbohydrates solubilized in the enzymatic peeling were analyzed directly by ^1H nuclear magnetic resonance (NMR) spectroscopy and by high-pressure liquid chromatography (HPLC) after a secondary enzymatic hydrolysis to monomers as previously described *(10).*

I. Characterization of the pulps

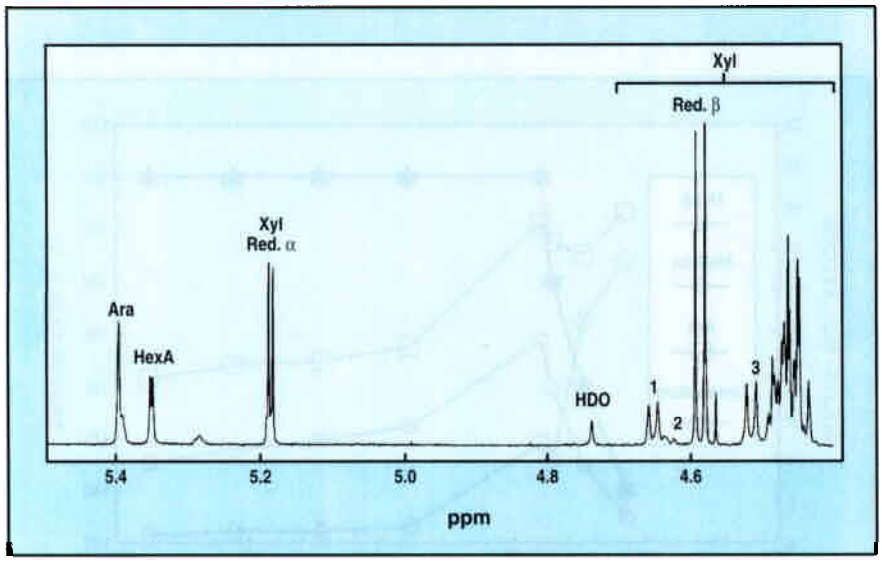
							Carbohydrate composition of the pulps,						Xylanase peeling	
			Residual	Residual	Yield,	Kappa	% of d.w.						% of pulp	% of pulp xylan
Time, min	Temp., °C	H-factor	NaOH, g/L	Na_2S, g/L	%	no.	Glc	Xyl	Man	Ara	Gal	Total	solubilized	solubilized
58	140	11	16.2	17.4	77.4	>50	58.8	6.6	6.3	1.5	1.0	74.0	1.14	12.3
74	150	42	15.7	17.5	73.5	>50	63.1	6.6	6.1	1.6	0.8	78.1	2.00	21.7
86	160	124	12.8	17.1	67.5	>50	66.9	6.3	5.9	1.6	0.9	81.6	3.20	35.6
90	170	204	10.9	16.0	63.0	>50	67.2	6.3	6.0	1.3	0.7	81.6	2.20	25.1
140	170	1007	3.2	12.2	50.4	45.7	76.0	6.6	5.9	1.0	0.5	90.0	2.40	27.8
173	170	1500	0.9	13.6	48.0	27.6	81.7	6.8	6.3	1.1	0.4	96.2	2.22	24.7
206	170	2001	-	12.0	47.4	26.0	83.5	7.2	6.1	1.0	0.4	97.8	1.78	19.1
238	170	2517	-	11.5	46.6	24.2	83.1	7.3	6.0	0.7	0.2	97.2	2.14	23.5
173*	170	1499	5.5	17.3	45.5	21.0	83.6	6.4	6.4	0.6	0.3	97.3	1.78	24.4
206*	170	2017	3.4	16.0	44.7	17.4	81.3	6.4	6.2	0.6	0.5	94.9	1.72	23.9

*Alkali charge 22%

II. Bleaching conditions used. Q=chelating stage, O=oxygen, Z=ozone, E=alkaline, P=peroxide. Symbols D1–D3 and E1–E3 refer to sequence ODEDED.

Stage	Initial/final temp., °C	pH	Consistency, %	Residence time, min	Dosage, %	NaOH, %	Additional chemicals, %
Q	50	4.6	3	30	0.3 EDTA	-	-
O	100	10	10	60	8 bar	2.5	1.0 $MgSO_4$
Z	25/39	3.0	10	10–15	0.6	-	-
E	80	10.2–10.4	10	60	-	0.8	-
P	80	10.5	10	180	3	1.5–2.0	0.25 DTPA/ 0.25 Epsom
D1	50	2.5	3	60	2.4	-	-
E1	60	11.5	10	60	-	1.8	-
D2	60	10.5	9	60	1.5	0.15	-
E2	60	11.2	9	60	-	0.8	-
D3	70	8.7/7.2	9	180	0.4	-	-

1. Anomeric proton region of a typical ^1H NMR spectrum of xylanase hydrolyzate. (1) Internal xylose unit (H1), which contains HexA as sidegroup; (2) internal xylose unit (H1), which contains MeGlcA as sidegroup; (3) internal xylose unit (H1), which contains arabinose as side group.



NMR analyses were carried out using a Varian UNITY 600 spectrometer operating in the pulsed Fourier transform mode. Lyophilized samples (0.5–5 mg) were dissolved in 0.7-mL D_2O (99.8% isotopic purity). The pH was adjusted to 7.0 with 2 M NaOH (99.5% isotopic purity). ^1H NMR spectra were recorded at 600 MHz. Typical parameters for the spectral acquisition were as follows: a 90° pulse of 14 μs, a spectral width of 8000 Hz, and a repetition time of 30 seconds. The spectra were recorded at 27°C. A typical ^1H spectrum is shown in **Fig. 1.** The resonances were assigned according to Teleman *et al. (11).*

Results and discussion

Kraft cooking of pine wood

Pine chips were cooked using a conventional kraft process with different cooking times (1–4 hours). With the lower alkali charge (18%), the alkalinity of the cooking liquor decreased to a level where, after about 80 min at the maximum temperature, only a slight dissolution of lignin occurred. The final kappa number was 24.2. With the higher alkali charge (22%), a kappa number of 17.4 was reached (Table I).

Most of the glucomannan in the chips was dissolved early during the warmup period. The dissolution of arabinoglucuronoxylan was less extensive, and during the last stages, the absolute yield of xylan increased slightly due to its readsorption onto the fibers (Table I).

Effect of cooking on the structure of xylan

The structure of the pulp xylan was analyzed after xylanase hydrolysis. As a result of the xylanase peeling, about 12–36% of the pulp xylan was selectively solubilized without any solubilization of other carbohydrates in the pulp (Table I). These oligomeric carbohydrate fractions obtained in the enzymatic hydrolyses were then analyzed by ^1H NMR spectroscopy. Quantitative data were obtained by integrating the signals arising from the anomeric protons between 4.4 and 5.5 ppm. The molar ratios of carboxylic acid and arabinose sidegroups to xylose were calculated.

The ^1H NMR analysis of the hydrolyzates revealed that the amount of 4-O-methylglucuronic (MeGlcA) acid sidegroups of xylan decreased in the early phases of the cook (**Fig. 2**). However, concomitantly, the amount of another type of acidic group, 4-deoxyhex-4-enuronic acid (HexA), increased. Hexenuronic acid (**Fig. 3**) is a ß-elimination product of 4-O-methylglucuronic acid and has been recently detected in xylans isolated from kraft cooking liquors and from a pine kraft pulp *(11).* Previously, the presence of hexenuronic

III. Characterization of the bleached pulps

Bleaching sequence	Brightness, %	Kappa no.	Carbohydrate composition of the pulps, % of d.w. Glu	Xyl	Man	Ara	Gal	Total	Xylanase peeling % of pulp solubilized	% of pulp xylan solubilized
Q	32.6	25.9	84.2	8.5	6.1	1.2	0	104.0	2.76	28.5
QO	49.8	11.0	83.7	8.6	6.7	1.0	*	101.0	2.68	27.9
QOQZ	62.5	6.2	84.2	8.5	6.5	0.8	*	101.0	3.06	32.9
QOQZE	63.8	4.7	84.4	8.3	6.4	0.9	0	102.0	2.44	26.5
QOQZEP	80.4	3.2	84.9	8.1	6.1	0.9	0	104.0	2.82	31.3
QOQP	75.5	7.3	84.2	8.5	6.5	0.8	0	99.7	3.04	32.7
QOQPZ	80.4	2.3	84.3	8.2	6.3	0.8	0.4	100.0	3.74	41.5
QOQPZE	80.8	1.6	84.7	8.1	5.9	0.8	0.5	103.0	3.18	35.7
QOQPZEP	88.5	1.2	84.9	8.2	6.1	0.8	0	100.0	3.56	39.6
QOQPPP	82.2	5.6	84.6	7.8	6.8	0.8	*	96.3	2.90	33.7
QOQDEDED	88.1	0.9	84.0	8.1	7.0	0.9	*	94.5	3.98	44.2

*<0.3

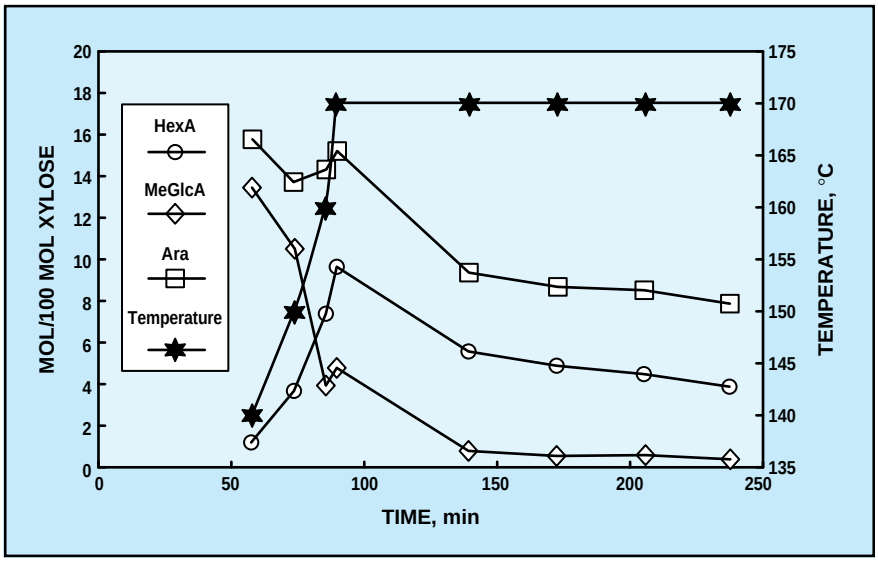
acid had not been detected in kraft pulps, although Johansson and Samuelson *(12)* found this acid after alkaline treatment of 2-O-(4-O-methyl-α-D-glucopyran-osyluronic acid)-D-xylitol. Apparently, in conventional acid hydrolysis of kraft pulp, the hexenuronic acids are readily degraded and therefore cannot be detected, as suggested by Johansson and Samuelson *(12)*. Hence, by using specific enzymes, the drawbacks of the acid hydrolysis can be overcome, and qualitative and quantitative analyses of labile components can be obtained.

The highest amount of hexenuronic acid, i.e., about 10% of xylan, was detected at the end of the heating period, when the temperature had reached 170°C. During continuation of the delignification, about 60% of the formed hexenuronic acids were, however, degraded (Fig. 2). Despite the partial degradation, hexenuronic acids form the major part of the carboxyl groups present in pulp xylan, even after the final phase of cooking. Surprisingly, a small amount of MeGlcA was detected in all pulps, indicating that part of the initial MeGlcA groups do not react with the cooking chemicals. The reason for this phenomenon remains to be studied.

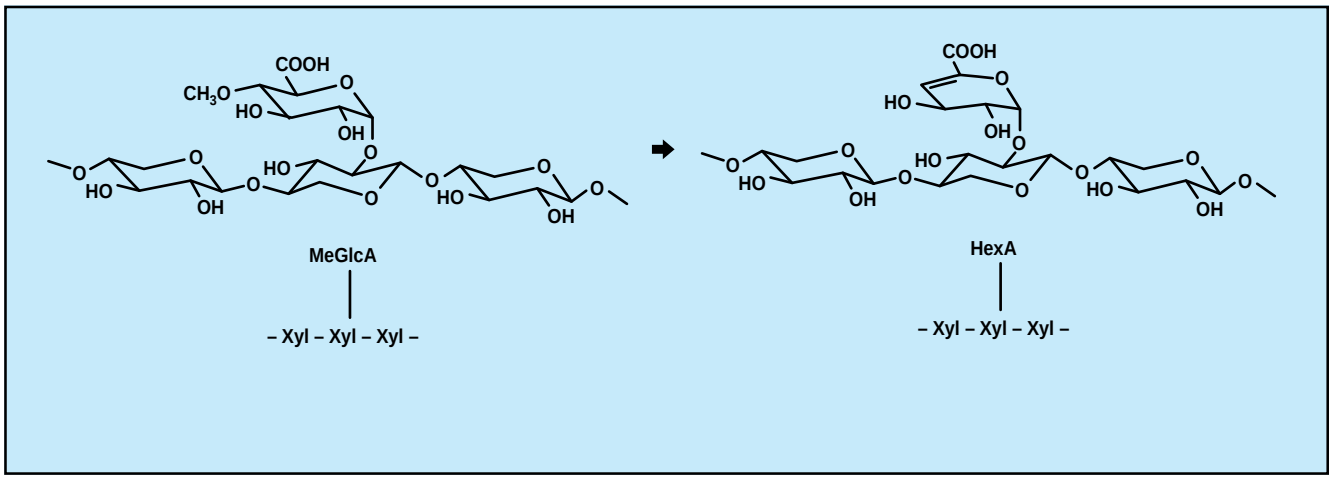
Using this one-stage enzymatic peeling procedure, about 12–36% of the pulp xylan was characterized due to the inaccessible location of the rest of the xylan in the pulps (Table I). The accessible xylan in pine kraft pulp with kappa no. 25.6 is known to contain reduced amount of carboxylic groups *(3)*, and it is estimated that only about 10% of the total amount of carboxylic acids in the pine kraft pulp was analyzed by the combination of enzymatic peeling and ^{1}H NMR spectroscopy. In the most accessible xylan in the pine kraft pulp with kappa no. 26.0, the ratio of xylose to uronic acids was 20:1; hence, about 75% of the initial uronic acid sidechains of xylan present in the native wood was degraded. Of the remaining uronic acids, about 88% were hexenuronic acids (Fig. 2).

Arabinose sidegroups are more stable during the cook, and about 46% of the initial arabinose sidegroups survived in the final pulp (Fig. 2). The degradation of arabinose occurred simultaneously with the degradation of hexenuronic acid during the pulping (Fig. 2). As a result of the degradation of uronic acids and arabinose during the kraft cook, the degree of substitution of the accessible xylan in pulp was reduced from 0.3 to 0.13 (Fig. 2).

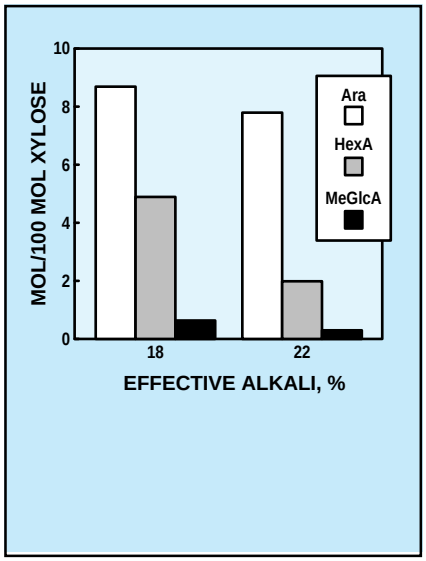
2. Structure of accessible xylan as a function of the kraft cook



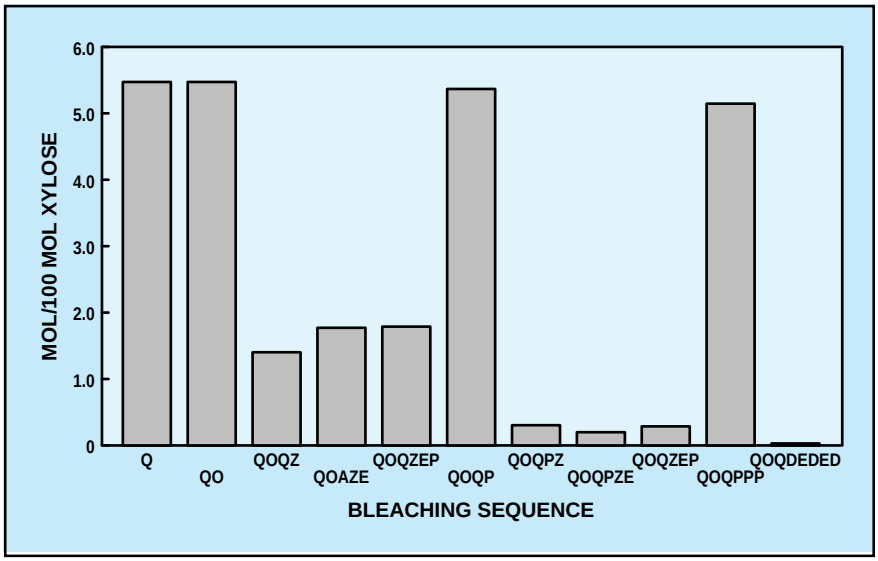
3. 4-O-methylglucuronoxylan is converted to hexenuronoxylan during kraft pulping



4. Effect of active alkali (18, 22%) on structure and amount of carboxylic acids in accessible xylan. Cooking time was 173 and 206 min, respectively.



5. Effect of bleaching chemicals on the amount of hexenuronic acid in the accessible xylan



The alkalinity of the cooking liquor strongly affected the amount of carboxyl groups in the xylan. When the charge of active alkali was increased from 18 to 22%, the total amount of carboxyl groups in the accessible xylan was decreased from 5.5 to 2.3% at a constant H-factor of 1500 (**Fig. 4**). The increase in the alkalinity of the cooking liquor decreased the amount of residual arabinose sidegroups by 10% and decreased the 4-O-methylglucuronic acid and hexenuronic acid sidegroups by 50–65%. Hence, the alkalinity and alkaline profile of the kraft cook strongly affect the amount of carboxylic groups in pulp. Therefore, pulps cooked with modified methods are expected to differ profoundly from conventional kraft pulp with respect to the amount and type of uronic acids in pulp xylan. The structure of xylan in these modified pulps remains to be studied.

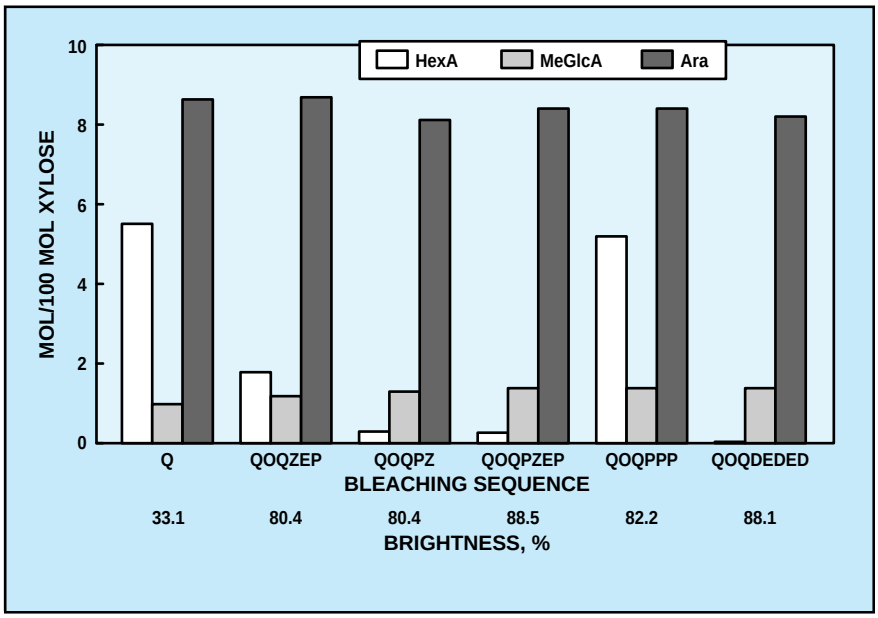
Effect of bleaching on the structure of xylan

The pine kraft pulp (kappa no. 25.9) was bleached with different chemicals, and the amount of hexenuronic acids in the accessible xylan was analyzed by enzymatic peeling and ^{1}H NMR spectroscopy. The accessibility of pulp xylan increased with the decreased amount of lignin in the pulp, and in the pulps with brightness values above 80%, about 31–44% of pulp xylan could be solubilized with the xylanase treatment (**Table III**). The hexenuronic acid sidegroups of xylan were sensitive to the bleaching chemicals. Ozone and chlorine dioxide destroyed the hexenuronic acids almost completely, whereas peroxide and oxygen had no effect (**Fig. 5**). Bleaching with chlorine gas also resulted in a

loss of hexenuronic acids (results not shown).

Further analysis of the chemical structure of the accessible xylan in pine kraft pulps bleached to brightness values of 80–88% ISO with different elemental chlorine-free (ECF) and totally chlorine-free (TCF) bleaching sequences revealed that 4-O-methylglucuronic acid and arabinose sidegroups are stable in the bleaching irrespective of the bleaching sequence used, whereas the ECF pulp and the TCF pulps, in which ozone was used, contained reduced amounts of hexenuronic acid (**Fig. 6**). Thus, although 4-O-methylglucuronic acid groups were rather unstable during kraft cooking, the residual 4-O-methylglucuronic acid groups in the brownstock pulps were stable during bleaching. This degradation of hexenuronic acids explains the observed differences in the total amount of carboxyl groups in pulps bleached with different sequences *(13)*. Hence, the carboxylic acid profiles differ profoundly, not only with respect to the amount of carboxylic acids present, but also with respect to the chemical structure in different types of bleached pulps.

6. Effect of bleaching on the chemical structure of the accessible xylan



Conclusions

The results obtained in this study show that the structure of kraft pulp xylan, especially that of the acidic groups, undergoes extensive modifications compared to the native wood xylan. The 4-O-methylglucuronic acid sidegroups were almost completely converted to 4-deoxyhex-4-enuronic acid groups during kraft pulping. In the conventionally cooked pine kraft pulp with kappa no. 26.0, 4-O-methylglucuronic acid groups constituted only about 12% of the carboxylic acids in the accessible xylan. Furthermore, the increased alkalinity in the cook decreased the amount of carboxyl groups in the pulp.

The chemical structure analysis of the xylo-oligosaccharides obtained by xylanase hydrolysis of ECF- and TCF-bleached pine kraft pulps revealed that 4-O-methylglucuronic acid and arabinose sidegroups were stable in the bleaching irrespective of the bleaching sequence used. Thus, although 4-O-methylglucuronic acid groups were rather unstable during the kraft cooking, the residual 4-O-methylglucuronic acid groups in the brownstock pulps were stable during the bleaching. Chlorine dioxide degraded the hexenuronic acids completely, and ozone reduced the amount of this acid significantly. Hence, it can be concluded that both cooking and bleaching affect the structure of pulp xylan, especially the content and type of the different carboxylic acid sidegroups in the pulp xylan. The chemical composition of the xylan in turn is expected to affect the technical properties of the pulps.

In this work, less than 45% of the total pulp xylan was analyzed, and variations most likely exist in the structure of xylan in different parts of the fibers. The development of a complete enzymatic hydrolysis method enabling a detailed analysis of the overall pulp composition is underway. TP

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