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Hydrothermally treated xylan rich by-products yield different classes of xylo-oligosaccharides

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Abstract

Four xylan rich by-products, namely wheat bran, brewery's spent grain, corn cobs and *Eucalyptus* wood, were characterised and subjected to a mild hydrothermal treatment in order to release and degrade the xylan from the starting materials. The chemical characterisation of the feedstock materials, with emphasis on the extracted xylan fractions and using enzymatic degradation of these xylans, resulted in rather detailed pictures of the xylans present. Depending on the feedstock material studied, the xylan present was substituted with arabinose, 4-*O*-methylglucuronic acid and acetyl groups. During the hydrothermal treatment, arabinose was rather easily removed from the xylan-backbone (wheat bran, brewery's spent grain and corn cobs). The acetyl groups were partly released from the feedstocks, becoming available to catalyse the depolymerisation of the xylan. Also, part of the uronic acids were released, mainly during the treatment of *Eucalyptus* wood. Due to the partial release of the substituents and cleavage of the xylan by the treatment performed, a wide variety of xylo-oligosaccharides with different structural features corresponding to the xylan-structure of the original feedstock were obtained. Xylo-oligosaccharides branched with arabinose were identified in the hydrolysate from brewery's spent grain, while in the hydrolysate of corn cobs and *Eucalyptus* wood xylo-oligosaccharides substituted with 4-*O*-methylglucuronic acid were present as well. Additionally, a series of partially acetylated (acidic) xylo-oligosaccharides was identified in the *Eucalyptus* wood hydrolysate.

Keywords: Xylan-oligosaccharides; Wheat bran; Corn cob; Brewery's spent grain; Eucalyptus wood

1. Introduction

Agro-industrial, agricultural and forest by-products, like wheat bran, brewery's spent grain, corn cobs and *Eucalyptus* wood are rich in cellulose and hemicelluloses. Currently, more and more effort is directed towards the re-use of such by-products, considering economic values and environment. Fractionation of these by-products into their main components could be of interest to obtain separate streams useable for different product applications (Garrote, Dominguez, & Parajo, 1999).

Since the main component of the hemicellulose in the by-products mentioned is xylan, fractionation of these by-products may result in both xylose, an intermediate for the production of xylitol, and a variety of differently substituted xylo-oligosaccharides. Because of its anti-cariogenic properties xylitol has already been used in food applications, e.g. chewing gum or tooth paste (Pepper & Olinger, 1988). Xylo-oligosaccharides are reported to enhance growth of bifidobacteria and they are frequently defined as prebiotics (Fooks, Fuller, & Gibson, 1999; Modler, 1994).

Xylan as present in the cell walls of Gramineae (grasses) consists of a β-D-(1,4)-linked xylopyranosyl backbone, which can be substituted with α-L-arabinofuranosyl on *O*2 and/or *O*3, α-D-glucopyranosyl uronic acid, or its 4-*O*-methyl derivative on *O*2, and acetyl on (some of) the arabinose or xylose residues. In spite of these general characteristics, the source from which the xylan is extracted strongly determines the specific features with regard to the type, the amount, position and distribution of glycosylic side-chains over the xylan-backbone (Huisman, Schols, & Voragen, 2000; Ishii, 1997). For example, xylan purified from wheat bran is mainly substituted with arabinose residues

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at O-2 and/or O-3, while some side-chains of (4-*O*-methyl-) α-D-glucuronic acid are present as well (Brillouet, Joseleau, Utille, & Lelievre, 1982; Schooneveld-Bergmans, Beldman, & Voragen, 1999). This latter is also the case for corn cobs where even the unusual side-chain 2-*O*-β-D-xylopyranosyl-α-L-arabinofuranose has been described to occur (Ebringerova & Hromadkova, 1995; Ebringerova, Hromadkova, & Alfodi, 1992). Xylan from brewery's spent grain resembles that of wheat bran, although the proportion of arabinose residues at the O-2 position is much higher compared to wheat bran (Han, 2000; Vietor, Angelino, & Voragen, 1992).

In contrast with the xylan from Gramineae, xylan from hardwoods is an acetylated 4-*O*-methyl-α-D-glucuronoxylan almost without any arabinose substitution. On the average every tenth xylose residue carries an α-4-*O*-methylglucuronic acid substituted to O-2 (Puls & Poutanen, 1989). A more complex glucuronoxylan purified from *Eucalyptus globulus* Labill has been described by Shatalov, Evtuguin, and Neto (1999), who reported linkages of both 4-*O*-methyl-α-D-glucuronic acid and 4-*O*-methyl-α-D-glucuronic acid substituted at O-2 with α-D-galactose.

An environmental friendly way to fractionate xylan rich materials is to perform a mild hydrothermal treatment. Such a hydrothermal treatment will result in a selective release and degradation of the xylan of the used resource, leaving the cellulosic residue available for other purposes (Garrote et al., 1999; Koukios, Pastou, Koullas, Sereti, & Kolisis, 1999). Furthermore, since the xylans in the various by-products are expected to possess rather diverse structures, differences in size and structural features of the oligosaccharides released are expected upon treatment for each material.

In our study wheat bran, brewery's spent grain, corn cobs and *Eucalyptus* wood, were subjected to a mild hydrothermal treatment. The hydrolysates were characterised and the results obtained were related to the composition of the starting materials and the structural features of the xylans present.

2. Experimental

2.1. Feedstock materials

Wheat bran was obtained from Germen Moagens de Cereais, SA, Vila Franca de Xira (Portugal) and harvested in France (autumn 1997). Brewery's spent grain was supplied from the Brewery Central de Cervejas, Vialonga (Portugal). Corn cobs were supplied by Casa Agrícola Monte Real, Salvaterra de Magos (Portugal) and harvested in Portugal (autumn 1998). Chips of *Eucalyptus* wood were obtained from ENCE Complejo Industrial de Pontevedra Puentenolinos, Lourizan (Galicia, Spain; July 1998).

2.2. Characterisation and fractionation of feedstock materials

To remove starch, first the feedstock material (40 g) was suspended in maleic-buffer (360 ml), containing 0.01 M maleic acid, 0.01 M sodium chloride, 0.001 M calcium chloride, 0.05% (w/v) sodium azide, pH 6.5 and stirred for 1.5 h at 100 °C. The suspension was cooled till 30 °C and porcine alpha-amylase (Merck; 150 U/g feedstock), amyloglucosidase of *Aspergillus oryzae* (Sigma; 420 U/g feedstock) and pullulanase of *Bacillus acidopullulyticus* (Megazyme; 60 U/g feedstock) were added to degrade most of the starch of the feedstock material (20 h; 30 °C). To remove the glucose formed and other low molecular weight material, ethanol was added till a concentration of 70% was reached. The final alcohol insoluble solids (AIS) were washed with acetone and dried in the air.

AIS was suspended in distilled water (1:20 w/v) and extracted for 3 h at 65 °C under continuous stirring. After centrifugation (10,000 g; 30 min) the residues were re-extracted twice with distilled water and the corresponding supernatants were collected as water soluble solids (WSS). The corresponding residues were recovered as water un-extractacle solids (WUS).

WUS was suspended in 200 ml of 4 M potassium hydroxide (KOH)/0.26 M $NaBH_4$, for 16 h at 25 °C under continuous stirring. After centrifugation (10,000 g; 30 min) the residues were reextracted twice with 4 M KOH (+0.26 M $NaBH_4$). The final residues were neutralised with acetic acid, dialysed against distilled water and freeze-dried (KOH res). The corresponding supernatants were collected, neutralised, dialysed and freeze-dried (KOH ss).

2.3. Enzymatic degradation of the alkali-extractable-fractions (KOH ss)

A solution of alkali-extractable xylan (4 mg KOH ss) or wheat arabinoxylan (4 mg; Megazyme) in 50 mM sodium acetate buffer pH 5 (1 ml) was incubated with endo-(1,4)-β-D-xylanase I (0.2 μg/ml) for 24 h at 30 °C, according to Gruppen et al. (1992) and Kormelink, Gruppen, Vietor, and Voragen (1993). After inactivation of the enzyme the digests were analysed by HPAEC, HPSEC and MALDI-TOF MS.

2.4. Hydrolysis of feedstock materials by hydrothermal treatment

The wheat bran was autoclaved (85 °C; 1 h) first, with a ratio of 1 g of feedstock per 10 g of water and starch was substantially removed with the aqueous phase. The autoclave-residue was used to prepare the hydrolysate and corresponding residue of wheat bran by hydrothermal treatment; 155 °C for 60 min with a ratio of 1 g of feedstock per 10 g of water (Technical University of Athens, Greece). The brewery's spent grain was autoclaved (100 °C; 1 h), with a ratio of 1 g of feedstock per 8 g of water, and starch was substantially removed with the aqueous phase. The autoclave-residue was used to prepare the hydrolysates and residue of brewery's spent grain by hydrothermal treatment; 150 °C for 60 and 120 min, both with a ratio of 1 g of

feedstock to 8 g of water (INETI, Portugal). To obtain a hydrolysate and residue from corn cobs, the cobs were hydrothermal treated at 160 °C for 75 min with a ratio of 1 g of feedstock to 8 g of water (University of Vigo, Spain). The hydrolysate and residue (Garrote et al., 1999) of treated *Eucalyptus* wood were prepared at 160 °C for 60 min with a ratio of 1 g of feedstock to 8 g of water (University of Vigo, Spain).

2.5. Neutral sugar composition

The neutral sugar composition was determined by gas chromatography according to Englyst and Cummings (1984), using inositol as an internal standard. The samples were treated with 72% w/w H_2SO_4 (1 h, 30 °C) followed by hydrolysis with 1 M H_2SO_4 for 3 h at 100 °C and the constituent sugars released were analysed as their alditol acetates.

2.6. Uronic acid content

The uronic acid content was determined as anhydrouronic acid (AUA) by an automated *m*-hydroxydiphenyl assay (Blumenkrantz & Asboe-Hansen, 1973; Thibault, 1979) using an autoanalyser (Skalar Analytical BV, Breda, The Netherlands).

2.7. Acetic acid content

The degree of acetylation was determined on a SP 8800 system HPLC (Thermo Quest), using an Aminex HPX column (Voragen, Schols, & Pilnik, 1986). The level of acetyl substitution was corrected for the free acetic acid in the sample.

2.8. Starch content

Starch was determined enzymatically using the test kit supplied by Boehringer.

2.9. HPSEC

High-performance size-exclusion chromatography was performed on three TSKgel columns (7.8 mm ID × 30 cm per column) in series (G4000, G3000, G2500; Tosohaas), in combination with a PWX-guard column (Tosohaas). Elution took place at 30 °C with 0.2 M sodium nitrate at 0.8 ml/min. The eluate was monitored using a refractive index (RI) detector (Shodex RI-71). Calibration was performed using pullulans (Polymerlabs).

2.10. HPAEC (pH 12)

High-performance anion-exchange was performed on a Dionex system equiped with a CarboPac PA-1 column (4 mm ID × 250 mm) in combination with a CarboPac PA guard column (3 mm × 25 mm) and PAD-detection (Lee, 1996). Elution (1 ml/min) of the oligomers on the hydrolysates was performed with a combination of linear gradients of 50–90 mM sodium acetate in 100 mM NaOH during 0–5 min, 90–130 mM sodium acetate in 100 mM NaOH during 10 min, followed by a linear gradient to 520 mM sodium acetate in 100 mM NaOH in 15 min. For the analysis of arabinose and xylose in the hydrolysates, an isocratic elution (1 ml/min) of 20 minutes was carried out with a solution of 16 mM NaOH. Each elution was followed by a washing and equilibration step. The eluate was monitored using PAD detection.

2.11. MALDI-TOF mass spectrometry

For matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) a Voyager-DE RP Biospectrometry workstation (PerSeptive Biosystems Inc., Framingham, MA, USA) was used, operated as described by Daas, Meyer-Hansen, Schols, Ruiter, and Voragen (1999). The mass spectrometer was calibrated with a mixture of maltodextrins (mass range 365–2309).

The samples were mixed with a matrix solution (1 μl of sample in 9 μl of matrix), after desalting the samples with anion-exchange material (AG 50W-X8 Resin; Biorad). The matrix solution was prepared by dissolving 9 mg of 2,5-dihydroxybenzoic acid and 3 mg 1-hydroxyisoquinoline in a 1-ml mixture of acetonitrile/water (300 μl:700 μl). Of the prepared (sample + matrix) solutions 1 μl was put on a gold plate and allowed to dry at room temperature.

2.12. Miscellaneous

The content of furfural, hydroxymethylfurfural (HMF), formic and levulinic acid was determined by HPLC, with use of an Aminex HPX-87H column and UV-/RI-detection.

3. Results and discussion

3.1. Characterisation of the four by-products and corresponding alkali extracted fractions

Four xylan rich by-products, namely wheat bran, brewery's spent grain, corn cobs and *Eucalyptus* wood, were characterised. The composition of both the feedstock and their alcohol insoluble solids (AIS) is presented in Table 1.

In wheat bran 41% of non-starch polysaccharides (neutral sugars + uronic acids + non-starch glucose), 17% of protein and 20% of starch were found, corresponding with the 41–60, 15–22 and 10–30%, respectively, reported in the literature (Brillouet & Mercier, 1991; Schooneveld-Bergmans, 1997). In general, the composition of the brewery's spent grains depends on the brewery's conditions and ingredients used for brewing. In spite of this, the contents found for non-starch polysaccharides (38%), starch (4%) and protein (30%) for the spent grains used in this study fitted within the range as described in the literature by Angelino (1992). The sugar and protein composition of corn cobs as presented in Table 1 was similar as described

Table 1
Yield and composition of xylan rich feedstock materials (FS) and corresponding alcohol insoluble solids (AIS)

	Wheat bran		Brew. SG		Corn cob		*Eucalyptus*	
	FS	AIS[a]	FS	AIS[a]	FS	AIS[a]	FS	AIS[a]
Yield[b]	100	55	100	84	100	93	100	95
Protein[a]	17	9	30	25	3	2	1	1
Sugars (total)[c]	61	36	42	40	71	64	68	67
Ara[a]	9	7	8	7	5	3	1	0
Xyl[a]	16	14	15	16	28	26	14	13
Man[a]	0	0	0	0	0	0	1	1
Gal[a]	1	1	1	1	1	1	1	2
Rha[a]	0	0	0	0	1	1	1	1
Glc[a](of which starch)	33(20)	12(2)	16(4)	13(3)	33(0)	31(0)	44(0)	44(0)
Uronic acid[a]	2	2	2	3	3	3	6	6
Acetic acid[a]	0.4	0.3	0.8	0.8	3	3	3	3

[a] Expressed as g of recovered protein, sugar (-residues) or acetic acid from 100 g of feedstock.
[b] Neutral sugars + uronic acids expressed as weight percentage (dry matter) of each fraction.
[c] Yield as weight percentage per 100 g feedstock material (dry matter).

by Pellerin, Gosselin, Lepoutre, Samain, and Debeire (1991). The content of glucose (44%) and other sugars (24%) in the *Eucalyptus globulus* wood resembled that of *Eucalyptus goniocalyx* wood (Timell & Syracuse, 1967).

3.2. Structural characteristics of the alkali-extracts (KOH ss) from the four feedstock materials

To study the xylan present in the four materials in more detail, alkali-extracts (KOH ss) were prepared. Table 2 shows the yield of the extracts and molar sugar composition of the feedstock materials and of their extracts. Furthermore, the molar ratios of arabinose to xylose, uronic acid to xylose, and acetyl groups to xylose are presented, since these ratios are considered to be a measure for the branching of the xylan.

The yield of glucuronoarabinoxylan (GAX) for the alkali-extraction was calculated as the sum of weights of the recovered arabinose, uronic acid and xylose. For wheat bran, brewery's spent grain and corn cob, most of the GAX was recovered in the KOH-extract (62, 45 and 67%, respectively), while part of the GAX remained in the corresponding residues (25, 17 and 16%, respectively). The alkali extractions of *Eucalyptus* wood resulted in a lower yield of xylans in the alkali-soluble fractions (23%). The latter extraction was probably hindered by the high lignin content of the wood (Timell & Syracuse, 1967), since 56% of the GAX was recovered in the KOH-residue. Still, we consider our results on the extracted xylan as being representative for most of the (insoluble) xylans in the *Eucalyptus globulus* wood, since our results are rather similar to those obtained by Shatalov et al. (1999) for the xylan extracted from *Eucalyptus globulus Labill* wood.

Comparing the different by-products several remarks regarding the structural characteristics of the GAX present can be made (Table 2). In the KOH-extracts of wheat bran, brewery's spent grains and corn cobs, GAX was found having a molar ratio of arabinose to xylose of 0.40, 0.48 and 0.11, respectively, while only small amounts of uronic acids were found. On the other hand, the alkali-extract of *Eucalyptus* contained almost no arabinose and much higher levels of uronic acids (UA/Xyl = 0.16). The ratio of acetyl groups to xylose was determined for the feedstocks and AISs. This ratio was found to be much higher for *Eucalyptus* wood (0.64) and corn cobs (0.35) than for wheat bran (0.06) and brewery's spent grain (0.15).

To further study the structural characteristics of the four extracted GAX's, a purified and well characterised endo-xylanase (Kormelink et al., 1993) was used. The molecular weight (MW) distribution of the alkali-extracts before and after enzymatic degradation by this endoxylanase I is shown in Fig. 1.

Seen from Fig. 1, about one-third of the wheat bran and brewery's spent grain xylan was degraded by endoxylanase I to fragments having a MW $< 10^4$. Knowing both the sugar composition of the KOH-extracts and the mode of action of the endoxylanase used, it was suggested that the less degraded xylans present were highly branched with arabinose. Corn cob xylan and *Eucalyptus* xylan were degraded almost completely by endoxylanase I, suggesting a relatively low substitution of most of the xylan. However, the remaining high MW material of the *Eucalyptus* xylan (Fig. 1) probably constitutes a highly substituted acidic xylan. This was substantiated by our finding that a hydrothermal treatment of the *Eucalyptus* wood resulted in a series of xylo-oligosaccharides (DP 4-12) containing two 4-*O*-methylglucuronic acid substituents (Kabel, Schols, & Voragen, 2001).

The xylo-oligosaccharides formed by endoxylanase I treatment of the KOH-fractions were monitored using HPAEC and MALDI-TOF mass spectrometry. The elution patterns and the main masses from the MALDI-TOF mass spectra (not shown) are presented in Fig. 2.

The HPAEC-elution pattern of the endoxylanase I-digest

Table 2
Yield and molar composition of xylan rich feedstock materials and corresponding extracts

	Yield[a]	Total sugar content[b]	Molar composition							Ara/Xyl[c]	UA/Xyl[c]	Acetyl/Xyl[c]
			Ara	Xyl	Man	Gal	Rha	Glc	UA			
Wheat bran		61	17	30	0	1	0	49	3	0.56	0.10	0.07
AIS	100	66	23	44	0	1	0	28	4	0.52	0.09	0.06
Wss	10	39	19	30	1	4	2	41	3	0.63	0.10	–
KOH ss	49	72	22	55	0	2	1	18	2	0.40	0.04	–
KOH res	28	78	25	24	1	2	1	42	5	1.04	0.21	–
Brew. SG		43	21	39	0	2	0	33	5	0.54	0.13	0.17
AIS	100	49	21	42	0	1	0	31	5	0.50	0.12	0.15
Wss	2	28	23	26	2	8	3	30	8	0.88	0.31	–
KOH ss	34	50	26	54	0	3	2	11	4	0.48	0.07	–
KOH res	24	81	11	14	2	1	2	66	4	0.79	0.29	–
Corn cob		74	7	43	0	2	1	43	4	0.16	0.09	0.34
AIS	100	72	5	46	0	2	1	42	4	0.11	0.09	0.35
Wss	1	24	13	18	4	14	4	29	18	0.72	1.00	–
KOH ss	38	67	9	81	0	2	3	1	4	0.11	0.05	–
KOH res	43	82	4	12	0	0	2	79	3	0.33	0.25	–
Eucalyptus		71	1	25	1	1	2	63	7	0.04	0.28	0.67
AIS	100	73	1	24	1	2	2	63	7	0.04	0.29	0.64
Wss	1	13	7	12	14	15	8	21	23	0.58	1.92	–
KOH ss	7	74	1	81	1	3	2	1	13	0.01	0.16	–
KOH res	80	68	1	15	1	2	2	74	7	0.07	0.47	–

[a] Expressed as weight percentage (dm).
[b] Neutral sugars + uronic acids (UA) + acetyl-groups expressed as weight percentage (dry matter) of each fraction.
[c] Ratio (mol/mol).

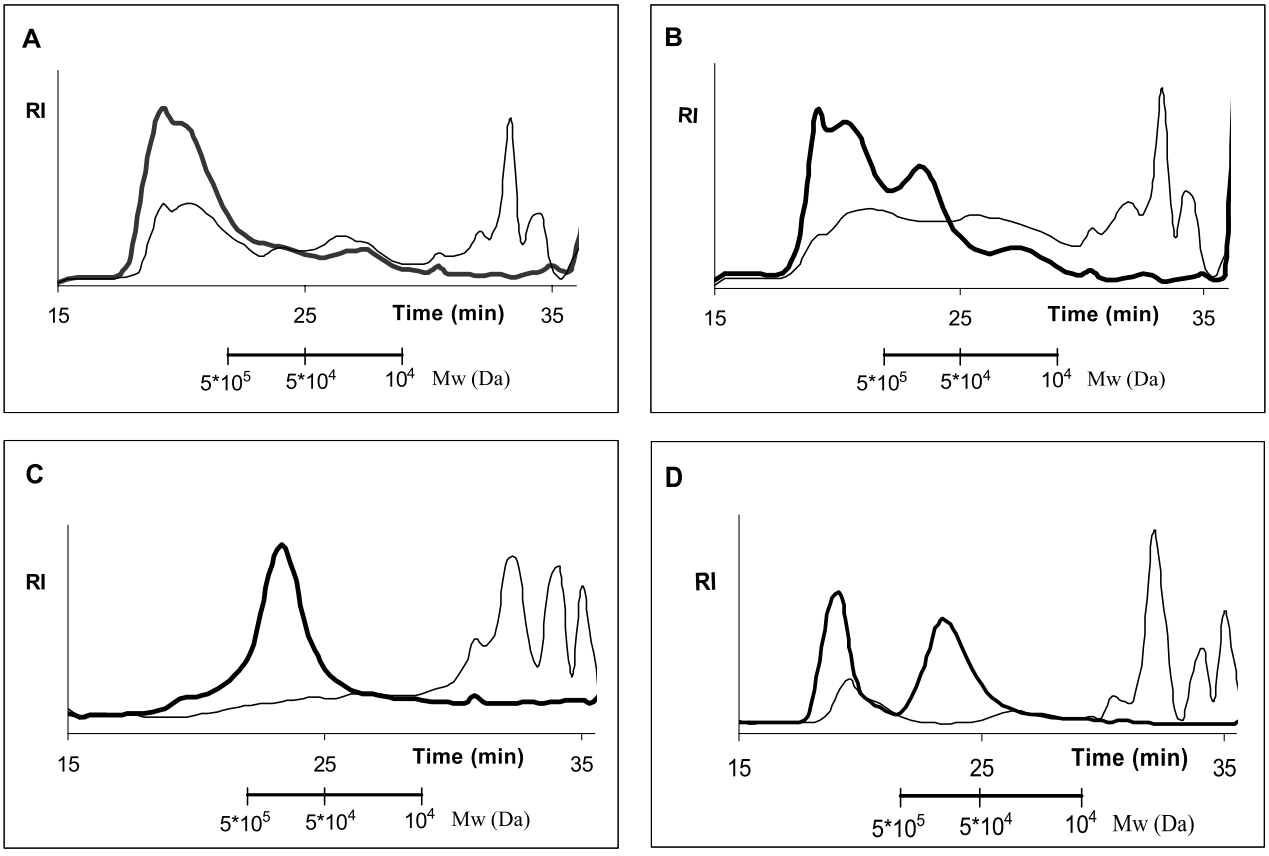

Fig. 1. HPSEC elution profiles of the alkali-soluble fractions (KOH ss) of wheat bran (A), brewery's spent grain (B), corn cobs (C) and *Eucalyptus* wood (D); before (bold line) and after (thin line) degradation by endoxylanase I.

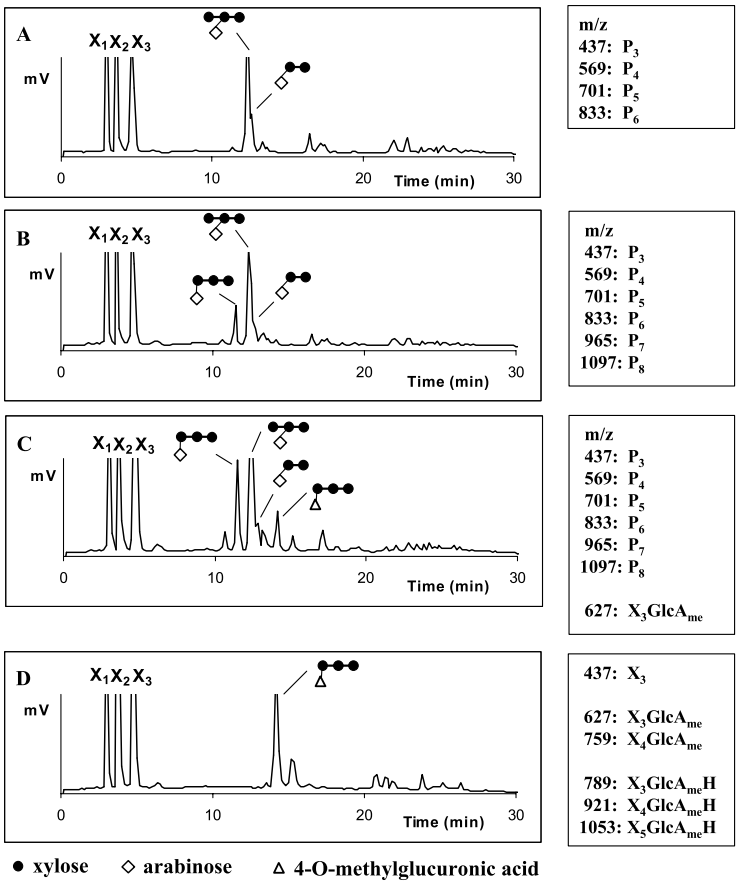

Fig. 2. HPAEC elution patterns (on the left) and the main molecular masses (as sodium-adducts) as found by MALDI-TOF mass spectrometry (on the right) of the alkali-soluble fractions (KOH ss) of wheat bran (A), brewery's spent grain (B), corn cobs (C) and *Eucalyptus* wood (D) after degradation by endoxylanase I (P = pentose; X = xylose; $GlcA_{me}$ = 4-*O*-methylglucuronic acid; H = hexose).

of wheat flour arabinoxylan, extensively described by Gruppen et al. (1992) and Kormelink et al. (1993), was used as a 'standard' for the identification of arabinoxylo-oligosaccharides in our xylan-digests together with the data obtained by MALDI-TOF MS. Endoxylanase I released xylose (X_1), xylobiose (X_2) and xylotriose (X_3) from the xylan-fractions of all materials. Furthermore, in the elution patterns of the degraded wheat bran arabinoxylan, two xylo-oligosaccharides linked with arabinose at O-3 were well distinguishable (X_3A_1 and X_2A_1), of which the masses were confirmed by MALDI-TOF MS as well. Both structures were also present in the endoxylanase-digest of brewery's spent grain xylan and corn cob xylan, in addition to a xylo-oligosaccharide substituted at O-2 with arabinose (X_3A_1) (Vietor et al., 1994). The major peak in the elution pattern of the digest of *Eucalyptus* wood corresponded to the acidic oligosaccharide X_3GlcA_{me}, confirmed by the MALDI-TOF mass 627 (sodium adduct), present in the digest of corn cob xylan as well (Verbruggen, Beldman, & Voragen, 1998). Remarkable was the detection of the masses 789, 921 and 1053 (sodium adducts) in the digest of the *Eucalyptus* wood xylan (Fig. 2). These masses indicate the presence of a series of xylo-oligosaccharides containing a 4-*O*-methylglucuronic acid *and* an additional hexose as substituent. Shatalov et al. (1999) have already described the linkage 2-*O*-α-galactopyronosyl-4-*O*-methyl-α-D-glucuronic acid to be present in xylan from *Eucalyptus globulus labill* wood. In addition, we do not expect the masses 789, 921 and 1053 to be the sodium adducts of X_3GlcA_2, X_4GlcA_2 and X_5GlcA_2, respectively, which is another possibility according to the masses, because we do not have any evidence for the presence of the same series of xylo-oligosaccharides but containing only *one* glucuronic acid.

3.3. Hydrolysates from the four by-products obtained after hydrothermal treatment; yield and sugar composition

In order to evaluate the use of hydrothermal treatment to produce xylose and xylo-oligosaccharides from the four by-products, hydrolysates were analysed in detail and the yield of total sugar and of sugar residues was calculated (Table 3). The figures as presented in Table 3 include the sugar-residues from both monomeric and oligomeric origin.

After hydrothermal treatment, xylose (present as poly- and oligomeric material) was almost completely recovered in the hydrolysates and residues, which indicates that xylose is quite stable during the hydrothermal treatment (Table 3).

Table 3
Yield of sugars and sugar residues in the hydrolysates and corresponding residues after hydrothermal treatment of wheat bran, brewery's spent grain, corn cobs and *Eucalyptus* wood

	Total[a]	Total sugar[b,(c)]	Xyl[b,(c)]	Ara[b,(c)]	GlcA[b,(c)]	Glc[b,(c)]	Acetyl[b,(c)]
Hydrolysates:							
WB II = 155 °C; 60 min; 10 g/g	33	13 (22)	7.2 (27)	2.3 (16)	1.0 (33)	2.6 (11)	0.1 (25)
BSG E1 = 150 °C; 60 min; 8 g/g	34	17 (35)	7.9 (42)	4.1 (45)	1.0 (33)	2.8 (16)	0.3 (38)
BSG J = 150 °C; 120 min; 8 g/g	40	19 (40)	9.4 (49)	4.3 (47)	1.2 (40)	3.1 (18)	0.3 (38)
CC. A = 160 °C; 75 min; 8 g/g	37	24 (33)	17 (61)	2.2 (44)	1.5 (50)	1.5 (4)	1.1 (37)
Euc. B2 = 160 °C; 60 min; 8 g/g	20	15 (21)	9.0 (64)	0.2 (40)	2.3 (38)	0.4 (1)	1.2 (40)
Residues:							
WB II = 155 °C; 60 min; 10 g/g	69	31 (47)	8.3 (31)	1.4 (10)	0.7 (23)	21 (95)	0.3 (75)
BSG J = 150 °C; 120 min; 8 g/g	61	23 (48)	7.3 (38)	1.2 (13)	0.6 (20)	13 (76)	0.3 (38)
CC. A = 160 °C; 75 min; 8 g/g	62	44 (59)	9.9 (35)	0.6 (12)	0.6 (20)	32 (95)	0.8 (25)
Euc. B2 = 160 °C; 60 min; 8 g/g	78	50 (70)	5.5 (39)	0 (0)	1.6 (27)	42 (94)	0.8 (25)

[a] Yield (dry matter) of 100 g of material subjected to treatment (w/w%).
[b] Yield of sugar (residue) of 100 g of material subjected to treatment (w/w%).
[c] Recovery of sugar (residue) as g sugar (residue) of sugar (residue) present in 100 g of material subjected to treatment (w/w%).

However, the recovery of arabinose expressed as the sum of arabinose found in hydrolysates and residues was quite low. This loss of arabinose was reflected in the recovery of GAX (%) from the GAX originally present in the destarched materials used (AIS), calculated as the sum of arabinose, uronic acid and xylose, which is illustrated by the following data (Table 3): wheat bran 24% of (G)AX in the hydrolysate and 24% in the residue; brewery's spent grain 48% of (G)AX in the hydrolysate and 30% in the residue; corn cobs 58% of (G)AX in the hydrolysate and 31% in the residue; *Eucalyptus* wood 55% of (G)AX in the hydrolysate and 34% in the residue. The total loss of (G)AX during the hydrothermal treatment of wheat bran, brewery's spent grains and corn cobs was mainly due to a loss of arabinose, while of *Eucalyptus* wood it was mainly due to a loss of uronic acids (Table 3).

The degradation of the sugars, mainly arabinose, in the hydrothermal treated feedstock contributed most likely to the increase in furfural and HMF (5-hydroxymethyl-2-furfural) (results not shown). For brewery's spent grain it was measured that formic acid (0.6 g/l) and levulinic acid (0.05 g/l) were present in the hydrolysates resulting from further degradation of part of the furfural (0.2 g/l) and HMF (0.02 g/l).

HPAEC analysis of the hydrolysates (not shown) revealed that quite a proportion of the arabinose and xylose was present as monomer. Therefore, the sugar composition of the hydrolysates was corrected for the presence of monomeric arabinose and xylose (Table 4 versus Table 5). In the wheat bran hydrolysate the arabinose was *only* present as monomer. Also in the brewery's spent grain hydrolysate, a significant part of the arabinose was present as monomer, but still a ratio of arabinose to xylose of 0.3 was calculated for the oligomers. For the corn cobs and the *Eucalyptus* hydrolysate the ratio of linked arabinose to xylose was remarkably

Table 4
Sugar composition (mol%) of the hydrolysates and corresponding residues obtained after hydrothermal treatment of wheat bran, brewery's spent grain, corn cobs and *Eucalyptus* wood

	Total sugars[a]	Molar composition							Ara/Xyl[b]	UA/Xyl[b]	Ac/Xyl[b]
		Rha	Ara	Xyl	Man	Gal	Glc	UA			
Hydrolysates:											
WB II	41 (0)	0	17	58	0	3	17	5	0.29	0.09	0.04
BSG E1	48 (1)	0	27	50	0	3	15	5	0.54	0.08	0.11
BSG J	48 (1)	0	24	53	0	3	16	4	0.45	0.08	0.11
CC A	63 (3)	0	10	74	0	5	6	5	0.14	0.07	0.20
Euc. B2	71 (6)	2	2	70	2	8	2	14	0.03	0.20	0.42
Residues:											
WB II	45 (0)	0	5	31	0	0	62	2	0.16	0.06	0.10
BSG J	38 (1)	0	7	35	0	0	55	3	0.2	0.09	0.14
CC A	69 (1)	0	1	27	0	0	70	2	0.04	0.07	0.24
Euc. B2	63 (1)	0	0	13	1	0	82	4	0	0.31	0.44

[a] Neutral sugars + uronic acids (UA) and between parentheses the total content of acetyl-esters expressed as weight percentage of each fraction (dm).
[b] Ratio (mol/mol).

Table 5
Sugar composition (mol%) of the hydrolysates obtained after hydrothermal treatment of wheat bran, brewery's spent grain, corn cobs and *Eucalyptus* wood; corrected for the presence of monomers

Hydrolysates	Total sugars[a]	Molar composition							Ara/Xyl[b]	UA/Xyl[b]	Ac/Xyl[b]
		Rha	Ara	Xyl	Man	Gal	Glc	UA			
WB II	33 (0)	0	4	64	0	4	21	7	0.06	0.11	0.05
BSG E1	44 (1)	0	18	56	0	5	16	6	0.32	0.11	0.11
BSG J	44 (1)	1	19	56	0	4	15	5	0.34	0.09	0.11
CC A	57 (3)	0	3	78	0	5	7	7	0.04	0.09	0.22
Euc. B2	65 (6)	2	0	68	2	9	2	17	0	0.25	0.48

[a] Neutral sugars + uronic acids (UA) and between parentheses the total content of acetyl-esters expressed as weight percentage of each fraction (dm).
[b] Ratio (mol/mol).

lower (0.04 and 0, respectively). Furthermore, for the hydrolysate from *Eucalyptus* wood the highest ratio of uronic acids to xylose was found (0.25), together with a noticeable amount of (linked) galactose.

Finally, the ratio's of acetyl groups to xylose in the four hydrolysates were calculated. In the hydrolysates from wheat bran and brewery's spent grain this ratio was much lower than the corresponding ratio for the hydrolysates from corn cobs and *Eucalyptus* wood. However, from all four by-products part of the acetyl groups were released during the hydrothermal treatment. This release is quite desirable, since the liberated acetic acid catalyses the depolymerisation of the xylan and contributes to an increase in soluble xylan (Garrote et al., 1999).

3.4. Molecular weight (MW) distribution during and after hydrothermal treatment

The MW distribution of the soluble material after hydrothermal treatment was monitored using HPSEC (Fig. 3). The treatment performed, designed to obtain a high proportion of oligosaccharides and a low concentration of furfural, resulted in material with masses lower than 10^4 Da (Rt > 30 minutes; based on pullulans). For the hydrolysate from brewery's spent grain also a remarkable part of the material eluted before 30 min. This suggested that, although about 50% of all GAX became soluble upon treatment, a significant proportion was still having a high MW.

To show the presence of a whole range of oligosaccharides

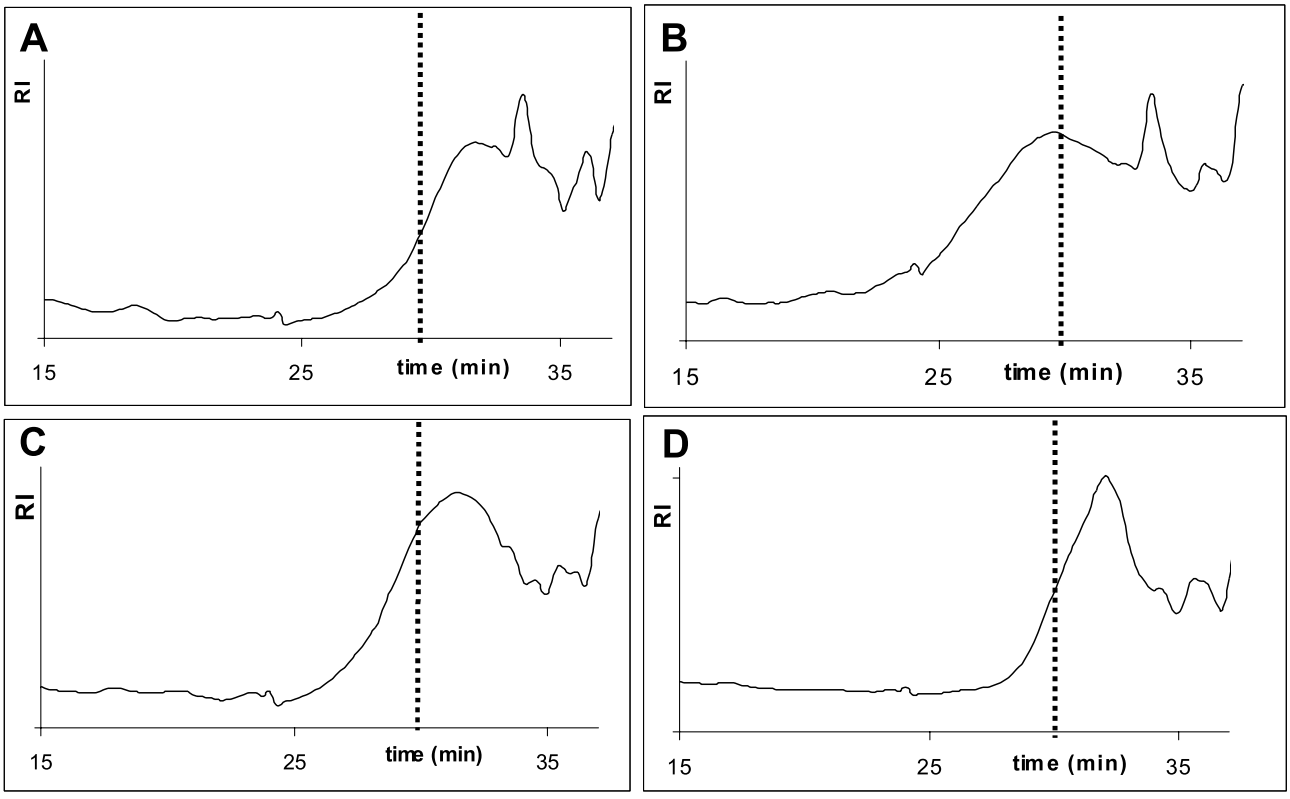

Fig. 3. HPSEC elution profiles of the hydrolysates from wheat bran (A), brewery's spent grain (B), corn cobs (C) and *Eucalyptus* wood (D).

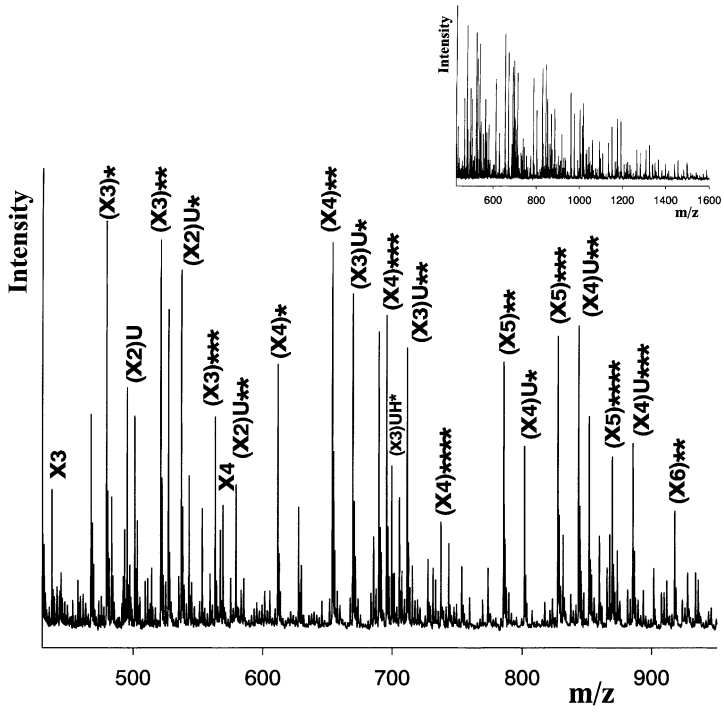

Fig. 4. Part of the MALDI-TOF mass spectrum of the hydrolysate from *Eucalyptus* wood (X = xylose; U = 4-*O*-methylglucuronic acid; H = hexose; asterix = acetyl group). As illustration the complete spectrum is inserted.

in the hydrolysates, a typical MALDI-TOF mass spectrum is presented in Fig. 4. Several series of xylo-oligosaccharides substituted with acetyl groups and 4-*O*-methylglucuronic acid residues were identified (based on the sugar composition of the hydrolysate). In future, research will be carried out to identify the various oligomers present in more detail (Kabel et al., 2001).

4. Conclusions

A rather detailed picture of the structural features of the xylans present in the four feedstock materials was obtained. Our results corresponded well with results previously reported in the literature. For wheat bran xylan mainly substituted at O-3 and both O-2 and O-3 with arabinose were detected. The same substitution was present in xylan from brewery's spent grain, but also substitution at O-2 with arabinose was found. In addition to substitution at O-2, O-3 or both O-2 and O-3 with arabinose, also linkages at O-2 with 4-*O*-methylglucuronic acid were found for the xylan from corn cobs. For the *Eucalyptus* wood xylan mainly substitution with 4-*O*-methylglucuronic acid was detected and some indications for the presence of the linkage 2-*O*-α-galactopyronosyl-4-*O*-methyl-α-D-glucuronic acid were obtained.

The four agrobased by-products appeared to be very suitable for studying the effect of hydrothermal treatment on structurally different xylans as well as to recover different series of xylo-oligosaccharides. Arabinose was rather easily split off by hydrothermal treatment from the xylan-backbone of wheat bran, brewery's spent grain and corn cobs. The acetyl groups were partly released from the feedstocks, becoming available to catalyse the depolymerisation of the xylan. Also, part of the uronic acids were released during the treatments performed, mainly concerning the treatment of *Eucalyptus* wood.

Due to the partial release of these substituents and cleavage of the xylan by the treatment performed, a wide variety of xylo-oligosaccharides with different structural features depending on the xylan-structure of the original feedstock were obtained. In the hydrolysate from brewery's spent grain xylo-oligosaccharides linked with arabinose were identified, while in the hydrolysate of corn cobs and *Eucalyptus* wood also xylo-oligosaccharides with 4-*O*-methylglucuronic acid residues were present. Additionally, in the *Eucalyptus* wood hydrolysate a series of acetylated xylo-oligosaccharides was identified. Further structural characterisation of these oligosaccharides will be helpful in studying the mechanism and improving the hydrothermal treatment in the release of xylose and xylo-oligosaccharides. Moreover, these fractions with structurally different xylo-oligosaccharides are very suitable (after purification) to study the effect of different substitution of xylo-oligosaccharides in several biological activity tests and their fermentability by the human intestinal flora. Both the further structural characterisation and the fermentability of the discussed xylo-oligosaccharides will be the subject of further publications.

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