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Characterization of arabinose and ferulic acid rich pectic polysaccharides and hemicelluloses from sugar beet pulp

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Abstract

Pectic polysaccharides were extracted from sugar beet pulp to yield fractions representing homogalacturonans, rhamnogalacturonans, arabinans and relatively small amounts of glucomannans and xyloglucans. The homogalacturonans had an apparent molecular weight of 21 kDa and contained relatively high amounts of methyl esters and relatively low amounts of acetyl groups as compared with the ramified 'hairy' regions. Three populations which originated from the ramified 'hairy' regions of pectin were distinguished. Two of these were rhamnogalacturonans with high apparent molecular weights of 1300 and 120 kDa, respectively. These populations had a high Ara and ferulic acid content. Despite the high neutral sugar content, these rhamnogalacturonans strongly bound to a DEAE column. The third population which originated from the ramified 'hairy' regions was a neutral population, which did not interact with the DEAE column and had a low apparent molecular weight and a high Ara and ferulic acid content. The arabinan side-chains of the rhamnogalacturonans were heavily branched in all populations. Enzymatic degradation of the xyloglucans showed similarities with apple xyloglucans with respect to the substitution with Fuc and Gal.

Keywords: Sugar beet pulp; Pectin; Rhamnogalacturonan; Homogalacturonan; Xyloglucan; Autoclave extraction; Anion-exchange chromatography; Enzymes

1. Introduction

Like all pectins, sugar beet pectins are considered to consist of 'smooth' homogalacturonan regions and ramified 'hairy' regions in which most of the neutral sugars are located [1]. The homogalacturonan regions of sugar beet pectin consist of a backbone of (1→4)-linked GalA, with an estimated minimum length of 72–100 GalA residues [2]. Both methyl esters and acetyl groups are present in the homogalacturonan. 80–90% of the acetyl groups are found on the homogalacturonic backbone and are located at the C-2 and/or C-3 position of the GalA residues [3]. A study using acid hydrolysis of sugar beet pectin showed a maximum length of 20 residues of alternating Rha and GalA for the rhamnogalacturonan backbone, although it is stated that breakdown in the backbone might have occurred [4]. The rhamnogalacturonans in sugar beet are substituted with highly branched (1→5)-linked arabinans and linear (1→4)-linked galactans of low DP [5]. Ferulic acid is attached to the O-2 position of (1→5)-linked Ara residues in the arabinan side-chains as well as to the O-6 position of Gal

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residues in (1→4)-linked galactans [5–9]. Approximately 50–55% of the feruloyl groups in sugar beet pulp are linked to Ara residues and approximately 45–50% to Gal residues [8].

In a previous study, the extraction of pectic polysaccharides from sugar beet pulp using autoclaving and extraction with 4 M NaOH was described [10]. Extracts obtained by autoclaving contained pectic polysaccharides with highly branched arabinan side-chains [10]. The average length of the side-chains appeared to be much higher for the autoclaved extract (61–118) [10] than for acid extracted material (18) [5]. In the current study we describe the further structural elucidation of the most prominent pectic and hemicellulosic fractions. The information obtained will be used in future studies concerning the structure–function relationship of the pectic polysaccharides.

2. Experimental

Materials.—Wet beet pulp (8.9% dry weight) was obtained from CSM Suiker bv (Breda, The Netherlands). Pectic and hemicellulosic polysaccharides were extracted from sugar beet pulp by two sequential autoclave treatments (121 °C, 40 min) and an alkali treatment (4 M NaOH + 0.02 M $NaBH_4$, 80 °C, 2 h) as described [10]. The extracts were named Autoclave 1 and 2, and 4 M NaOH 1, respectively.

Analytical methods.—The uronic acid content was determined by the automated *m*-hydroxy biphenyl assay [11]. The neutral sugar composition was determined after hydrolysis with 2 M TFA (1 h, 121 °C) as described previously [10]. Methylation analysis was performed as described previously [10], without reduction of GalA residues to Gal. Feruloyl groups were determined spectrophotometrically at 375 nm in freshly prepared pectin solutions adjusted to pH 10 with a 0.1 M NaOH solution. A molar extinction coefficient of 31,600 was used [3]. The degrees of methylation and acetylation were determined as described previously [12]. Diferulate cross-links of the Autoclave 2 extract were released by saponification with 0.4 M NaOH at 4 °C over a 16 h period.

Chromatography.—High-performance size-exclusion chromatography (HPSEC) was performed on three Bio-Gel TSK columns in series (60XL–40XL–30XL) as described [5], using a combined RI detector and viscometer (Viscotek, model 250), a Right Angle Laser Light-Scattering detector (RALLS, Viscotek, LD 600) and a UV detector (Kratos, Spectroflow 773). Molecular weights (M_w), intrinsic viscosities ($[\eta]_w$) and radii of gyration (Rg_w) were calculated using the light-scattering and universal calibration modules of the Trisec software (Viscotek). Also, a calibration was performed comparing the elution times with those of pectin and dextran standards. The pectin standards were obtained by mechanical degradation through ball milling and the molecular weights were calculated from their viscosities [13]. The dextrans were obtained from Pharmacia.

High-performance anion-exchange chromatography (HPAEC) was performed on a Dionex Bio-LC system with a (4 × 250 mm) PA100 column. The gradients were obtained by mixing 0.1 M NaOH and 1 M NaOAc in 0.1 M NaOH as shown in the corresponding figures. Rhamnogalacturonan [14] and xyloglucan [15] oligomers prepared from apple cell-wall polysaccharides were used as standards.

Preparative size-exclusion chromatography was performed on two columns (50 × 10 cm) of Sephacryl S 500 (Pharmacia) in series, using a Biopilot system (Pharmacia). The sample (1.0 g) was eluted with 0.05 M NaOAc pH 5.0 at a flow rate of 37 mL/min.

Preparative anion-exchange chromatography was performed on a column (54 × 2.6 cm) of DEAE Sepharose Fast Flow (Pharmacia) using a Hiload System (Pharmacia). Samples (0.4–0.5 g) were applied onto the column at a flow rate of 2.6 mL/min (0.005 M NaOAc, pH 5.0). After 60 min, the flow rate was increased to 13.2 mL/min and a gradient was applied as indicated in the corresponding figures.

The fractions obtained by preparative size-exclusion chromatography (120 mL) and preparative anion-exchange chromatography (20 mL) were assayed for total neutral sugar [16] and uronic acid [11] content, using Ara and GalA as standards. The anion-exchange

column was calibrated using pectins with degrees of methylation ranging from 30 to 70 (Obi Pektin), and soluble starch (E. Merck). The presence of ferulic acid in the fractions was monitored spectrophotometrically at 335 nm [8]. Pooled fractions were dialyzed and freeze-dried.

Enzymatic degradation.—All the pools obtained by DEAE anion-exchange chromatography were treated with the following (combinations of) enzymes: arabinofuranosidase B (AF) [17], arabinofuranosidase B (AF) plus *endo*-arabinanase (EA) [17], *endo*-polygalacturonase (PG) [18] combined with pectin methyl esterase (PE) [19], and rhamnogalacturonase (RG) [20] in combination with rhamnogalacturonan acetyl esterase (RGAE) [21]. Measurement of activity towards a broad range of polysaccharides showed that these enzymes had no measurable activity on pectic substrates other than their indicated activity.

The DEAE pool 4 M NaOH 1a was treated with *endo*-glucanase I (EG I) [22] to degrade the excess of glucans/mannans/glucomannans. Following this treatment the pools were dialyzed, using microconcentrators, and treated with *endo*-glucanase V (EG V) [22]. The samples were analyzed by HPAEC to examine the presence of xyloglucan fragments.

The DEAE pools (5 mg/mL) were dissolved in 0.04 M sodium acetate buffer pH 5.0. All enzymes were added to obtain a final concentration of 1 μg of protein/mL, sufficient to obtain the maximum degradation possible. Incubations were carried out at 30 °C for 20 h. The reactions were stopped by heating at 100 °C for 5 min. The digests were analyzed by HPSEC and HPAEC.

3. Results and discussion

Polysaccharides as present in sugar beet extracts.—In the previous paper we described the extraction of pectic polysaccharides from sugar beet pulp by autoclaving and treatment with 4 M NaOH [10]. This method yielded pectic material containing highly branched arabinan side-chains. Furthermore, the extracts obtained by autoclave treatment also contained relatively high amounts of feruloyl groups, acetyl groups and methyl esters. The presence of multiple polysaccharide populations in all extracts was shown. In order to separate these populations, the extracts named Autoclave 1, Autoclave 2, and 4 M NaOH 1 were fractionated using anion-exchange chromatography on DEAE–Sepharose (Fig. 1(A–C)). The populations which appeared to be present in the extracts and some of their characteristics are summarized in Table 1. Analysis of the pools revealed the presence of the following polysaccharides.

Arabinans.—The arabinans were found in the neutral pools (pool a, Fig. 1(A–C)) of Autoclaves 1 and 2 and made up more than 75 mol% of these pools. Since the neutral populations of Autoclave 1 and 2 represented only a small part of the total amount of Ara present, it is concluded that most of the arabinans were present in charged polymers. The neutral sugar linkage composition of pool a of Autoclave 2 (Table 3) shows that Ara was predominantly present as terminal, (1→5)-linked, (1→3,5)-linked and (1→2,3,5)-linked residues. Furthermore, the presence of small amounts of (1→2,4)-linked Rha and GalA in the neutral population indicates that it originates from rhamnogalacturonans. Pool a from both extract Autoclave 1 and 2 showed two populations based on molecular weight (Fig. 3A & B, RI and UV detection), one which eluted at 35 min with a high absorption at 335 nm (ferulic acid) and an average molecular weight of ~20 kDa and one at 36 min. Treatment of these pools with EA + AF resulted in a shift of the population at 35 min towards lower molecular weights (data not shown), indicating that this pool contains the arabinan. Based on these results it was concluded that the arabinans were highly branched and highly feruloylated, and contained around 100 Ara residues per molecule, approximately one third of which was present as side-chains of the arabinans, assuming that the population was homogeneous.

HPSEC data of pool III show that the intrinsic viscosity of this pool was 0.2 dL/g (Table 4). This low value is in agreement with the highly branched nature and low molecular weight of the arabinans. The high amount of branches, which increases solubility, and its low molecular weight make this arabinan un-

suitable as a thickening or gelating agent. Cooper et al. [23] showed that the removal of the side-chains of the arabinans improves the thickening properties, although relatively high amounts of the arabinans were needed in comparison with other thickening agents (10–20% w/w). However, we found that cross-linking of the arabinans through the ferulic acid groups results in gel formation at lower concentrations (4% w/w) [24].

Glucomannans.—Besides Ara, relatively high amounts of Glc and Man were found in the neutral pools (pool a) of Autoclaves 1 and 2, in a ratio of approximately 1:1 (Table 2). This is possibly indicative of the presence of glucomannan or a mixture of glucan and mannan. It was found that Glc and Man were present as (1→4)-linked residues in these pools (Table 3). High performance size-exclusion chromatography showed that pool a

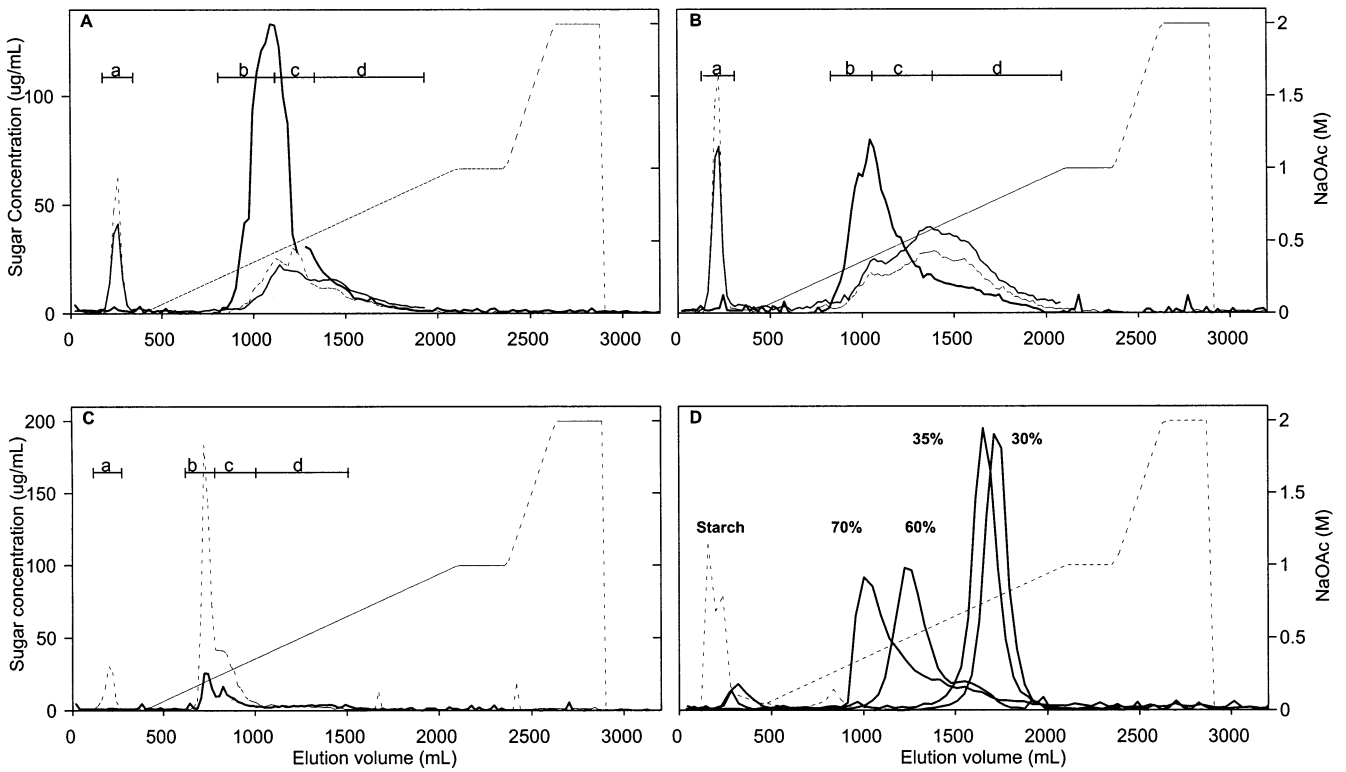

Fig. 1. Preparative anion-exchange chromatography on DEAE–Sepharose of the extracts obtained by autoclaving and extraction with 4 M NaOH. (A) Autoclave 1, (B) autoclave 2, (C) 4 M NaOH 1, (D) calibration of DEAE with pectins of various degree of methylation. Thin lines: ferulic acid, thick lines: uronic acid, dashed lines: neutral sugars.

Table 1
Characteristics of the populations present in the extracts obtained by autoclaving and extraction with 4 M NaOH upon anion-exchange chromatography

Sample	Population	Elution volume (mL)	[NaOAc] (M)	Ferulic acid	AUA:ns
Autoclave 1	1	150–250	0.0	+++	−
	2	800–1250	0.4	+	+++
	3	1250–2000	0.6	+++	++
Autoclave 2	1	150–250	0.0	+++	−
	2	800–1250	0.4	+	+++
	3	1250–2200	0.6	+++	+
4 M NaOH 1	1	150–250	0.0	−	−
	2	700–800	0.2	−	+
	3	1000–1500	0.3	−	++

Table 2
Sugar compositions (mol%) of the DEAE pools of the extracts obtained by autoclaving and extraction with 4 M NaOH

	Rha	Ara	Xyl	Man	Gal	Glc	UA	DA	DM	Relative weight (%)	Ferulic acid (%w/w)	Relative FA content (%w/w)
DEAE												
Autoclave 1	2.6	29.8	0.4	0.4	3.9	1.1	61.9	45	73	–	0.4	–
Pool a	2.6	76.9	0.7	7.2	1.7	7.1	4.0	–	–	6.7	–	19.6
Pool b	2.2	12.1	0.5	2.4	3.6	2.0	77.3	–	–	62.6	–	11.5
Pool c	4.3	27.2	1.0	1.2	7.2	1.8	57.5	–	–	16.5	–	31.8
Pool d	4.4	43.9	1.9	2.6	9.3	1.9	35.9	–	–	14.3	–	37.1
Autoclave 2	3.6	60.8	0.0	0.4	6.6	0.9	27.7	52	60	–	1.1	–
Pool a	2.9	84.1	0.5	4.0	1.8	4.3	2.7	–	–	10.3	–	15.5
Pool b	3.1	22.4	0.4	2.5	4.9	2.0	64.8	–	–	28.1	–	9.0
Pool c	8.4	41.3	0.3	1.7	8.3	1.3	38.8	–	–	39.8	–	34.5
Pool d	7.1	56.1	0.2	0.9	11.5	0.8	23.3	–	–	21.9	–	41.0
4 M NaOH 1	6.2	57.3	6.2	2.4	11.0	5.1	11.7	0	0	–	0.0	–
Pool a	2.1	33.6	10.0	16.1	4.7	30.0	3.5	–	–	13.6	–	–
Pool b	5.8	69.1	2.7	0.5	11.1	2.2	8.8	–	–	55.0	–	–
Pool c	9.3	57.8	1.9	0.4	13.8	1.8	15.2	–	–	23.3	–	–
Pool d	6.6	24.6	1.6	0.1	7.5	1.4	58.4	–	–	8.1	–	–
SEC												
Autoclave 2												
Pool I	6.8	58.8	0.8	0.0	13.2	0.6	19.9	51	29	19.6	–	26.7
Pool II	5.3	51.5	0.5	0.7	9.9	0.4	31.8	56	33	33.1	–	44.3
Pool III	1.5	36.9	0.4	1.9	3.2	1.3	54.9	26	56	47.3	–	28.9

Table 3
Neutral sugar linkage composition of polysaccharides of the DEAE pools of the extracts obtained by autoclaving and extraction with 4 M NaOH.

Linkage Pool	Glycosidic linkage composition [a]				
	Autoclave 1 b	Autoclave 2 a	Autoclave 2 d	4 M NaOH 1 a	4 M NaOH 1 b
Rhamnose					
T-Rha*p* [b]	0.9	0.1	1.3	0.0	0.3
(1,2)-Rha*p* [b]	2.8	0.0	1.8	0.4	0.8
(1,2,4)-Rha*p*	2.1	0.9	1.8	0.0	1.5
Total	5.7	1.0	4.9	0.4	2.6
Arabinose					
T-Ara*f*	27.2	31.5	30.9	19.4	30.4
(1,3)-Ara*f*	0.7	0.7	1.0	0.0	1.0
(1,5)-Ara*f*	21.4	31.8	27.3	13.3	25.8
(1,2,5)-Ara*f*	1.2	0.0	1.6	0.0	1.7
(1,3,5)-Ara*f*	17.5	24.2	16.8	10.2	23.0
(1,2,3,5)-Ara*f*	5.3	3.7	3.7	1.4	4.1
Total	73.3	92.2	80.6	44.2	86
Xylose					
T-Xyl*p*	1.9	0.2	0.9	6.7	0.5
(1,2)-Xyl*p*	0.0	0.0	0.0	6.1	0.0
(1,4)-Xyl*p*	2.5	0.6	0.0	0.0	2.6
(1,2,4)-Xyl*p*	0.7	0.0	0.3	0.0	0.1
(1,3,4)-Xyl*p*	0.6	0.0	0.2	0.0	0.1
Total	5.7	0.8	1.3	12.9	3.3
Galactose					
T-Gal*p*	4.1	0.2	4.8	2.2	2.5
(1,4)-Gal*p*	2.7	0.3	4.1	1.8	2.9
(1,6)-Gal*p*	1.8	0.0	2.3	0.1	1.7
(1,4,6)-Gal*p*	5.4	0.1	0.0	0.0	0.4
Total	13.9	0.6	11.1	4.0	7.5
Glucose					
(1,4)-Glu*p*	0.5	2.5	0.5	21	0.0
(1,4,6)-Glu*p*	0.8	0.0	1.2	10.1	0.5
Total	1.3	2.5	1.7	31.1	0.5
Mannose					
(1,4)-Man*p*	0.0	2.8	0.3	7.3	0.1
Total	0.0	2.8	0.3	7.3	0.1

[a] Linkage types in mol %.
[b] T, terminal; 1,2-linked Rha, etc.

from both extract Autoclaves 1 and 2 (Fig. 3(A and B), RI and UV detection) contained a population which eluted at 36 min, which was present in higher amounts in Autoclave 1 than in Autoclave 2. This material could be degraded by *endo*-glucanases, but not by EA + AF, RG + RGAE or PG + PE. From these observations it was concluded that this low molecular weight population was the glucomannan or a mixture of glucan and mannan.

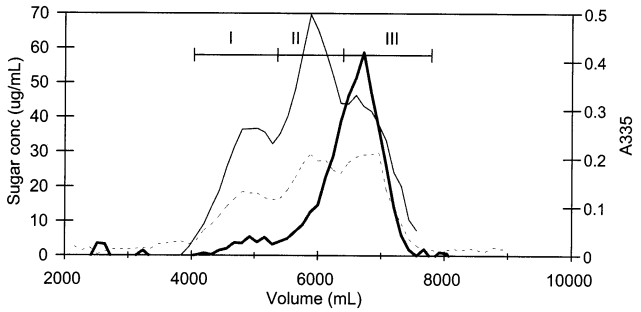

Fig. 2. Preparative size-exclusion chromatography (S 500) of Autoclave 2. Thin lines: ferulic acid, thick lines: uronic acid, dashed lines: neutral sugars.

Homogalacturonans.—Homogalacturonans were found in all the extracts. Pool b obtained by anion-exchange chromatography of Autoclave 1 and 2 consisted predominantly of GalA, indicating that it represents homogalacturonan (Fig. 1(A and B)). Besides a high amount of GalA, pool b of Autoclave 2 also contained 22 mol% of Ara which was probably caused by contamination with the rhamnogalacturonan population (Table 2). In pool d of 4 M NaOH 1 (Fig. 1(C)) a relatively high amount of GalA was also found, as expected from the elution pattern on DEAE of this saponified pectin fraction. Preparative size-exclusion chromatography of Autoclave 2 showed that the homogalacturonans (Fig. 2, pool III) eluted fairly late, and therefore had a low molecular weight. The DM of pool III was 56, which was comparable with that of the parental extract. Comparable values were reported for CDTA and buffer soluble pectins from beet pulp [25]. The degree of acetylation (DA) of the homogalacturonans was much lower than in the whole extract Autoclave 2. On HPSEC, the homogalacturonans of both Autoclave 1 and 2 (pool b) showed a refractive index response at 34 min (Fig. 3(A and

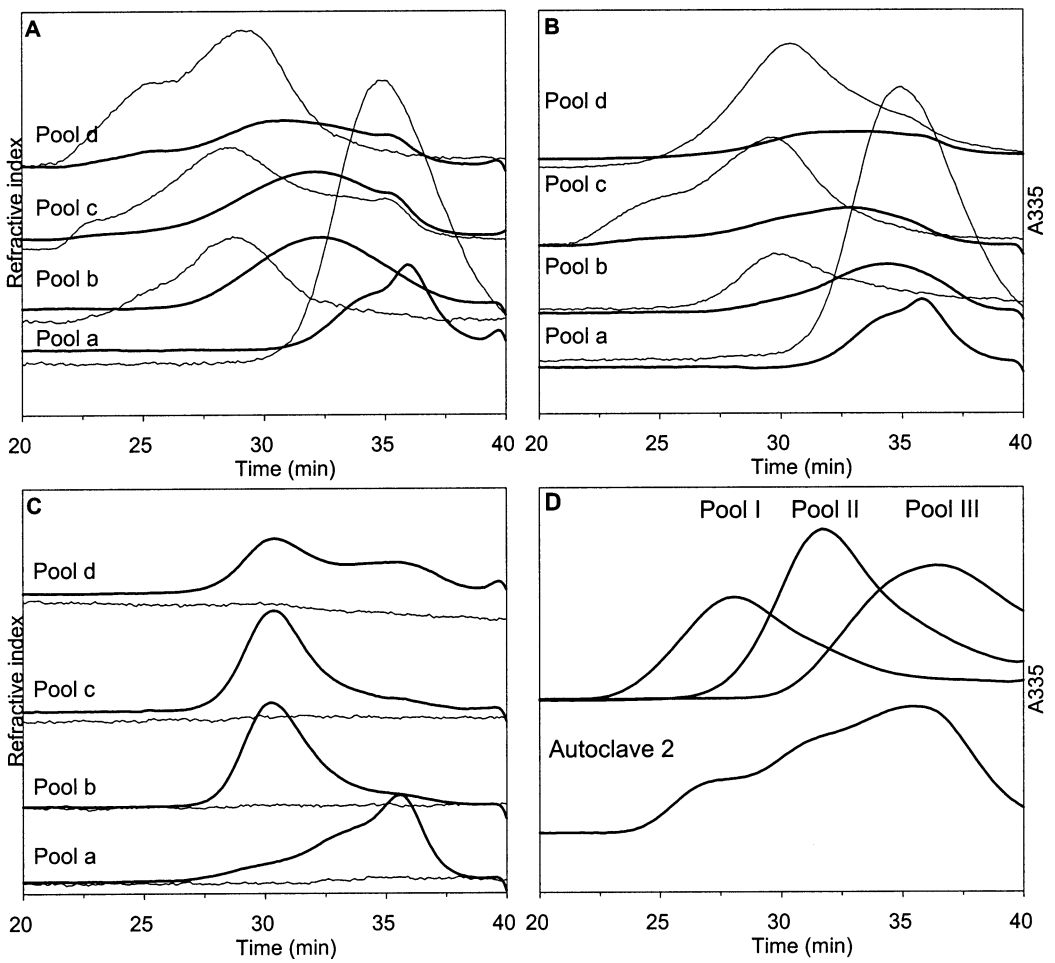

Fig. 3. High-performance size-exclusion chromatography of the DEAE pools of Autoclave 1 (A), Autoclave 2 (B) and 4 M NaOH (C) and of the S 500 pools of Autoclave 2 (D). Thin lines: UV detection, thick lines: RI detection.

B)), which disappeared after treatment with PG + PE (not shown). Also a UV response at 28–29 min was observed, which only changed after treatment with RG + RGAE (not shown) or EA + AF and not with PG + PE. These results indicate that the homogalacturonans and rhamnogalacturonans are present as separate populations in the extracts. The apparent molecular weights of the pools present in Autoclave 2 were calculated from the whole extract after separation by HPSEC, using light-scattering detection. The apparent molecular weight of the homogalacturonans in Autoclave 2 was found to be 21 kDa, implying a degree of polymerization of 120, which is comparable with the value found by Thibault et al. [2]. The intrinsic viscosity of pool III was only 0.2 dL/g. However, in both studies the backbone had been subjected to degradative conditions. Therefore, higher degrees of polymerization are expected for the native homogalacturonans. The assumption that degradation occurred was supported for the extracts obtained by autoclaving by the lower molecular weight of the homogalacturonans of Autoclave 2 than those of Autoclave 1 as was observed by HPSEC (Fig. 3(A and B)).

The low intrinsic viscosity and molecular weight, as well as the high level of acetylation of this homogalacturonan obtained by autoclave extraction results in poor physicochemical properties. This is in contrast to acid extracted beet pectins, especially after removal of the acetyl groups [26]. These results show that that autoclave extraction is not a suitable method for the extraction of beet homogalacturonans with gelling capabilities.

Rhamnogalacturonans.—Pool d as obtained by anion exchange chromatography of Autoclave 1 and 2 mainly consisted of Ara and Gal, besides smaller amounts of Rha and GalA (Table 2). This composition suggests the presence of rhamnogalacturonans. Also, pool b and c of 4 M NaOH 1 contained rhamnogalacturonans based on their composition (Fig. 1(C)). Preparative size-exclusion chromatography of Autoclave 2 showed that the rhamnogalacturonans had a high ferulic acid content (Fig. 2, pools I and II) and eluted early in the chromatogram, indicating a high molecular weight. The DA in these populations was high, which is in agreement with results found for modified hairy regions from apples [27]. The degree of methylation (DM) was relatively low as compared with the parental extract, explaining a strong binding to the anion-exchange column (Fig. 1). In most of the pools Rha was determined in approximately equal amounts of (1→2)-linked and (1→2,4)-linked residues (Table 3). Ara was predominantly present as terminal, (1→3)-linked and (1→3,5)-linked residues. Also some (1→2,3,5)-linked Ara was found. Terminally and (1→4)-linked Gal residues were present in most pools in almost equal quantities. Also (1→6)-linked and, to a lesser extent (1→4,6)-linked Gal was found. The rhamnogalacturonans (pools I and II, Fig. 2) had higher Ara contents than reported before for the rhamnogalacturonans present in acid extracted beet pectins [6,7].

For both pools c and d from Autoclave 1 and 2 peaks were observed on HPSEC at 28–30 min by both RI and UV detection (Fig. 3(A and B)). An additional RI response was seen at 33 min, as well as an additional UV response at approximately 24 min. The populations at 24 and 28–30 min were degradable with RG + RGAE, resulting in a shift of both RI and UV to 34 min, as is shown in Fig. 4(A) for Autoclave 2, pool d. A decrease in intensity was found for both the RI and UV response at 24 and 30 min after treatment with EA + AF. As a result the amount of Ara oligomers as detected by HPAEC increased (data not shown). The population which eluted at 33 min could be degraded with PG + PE and shifted to 37 min. This revealed the presence of two high molecular weight populations based on RI detection, a minor population at 24 min and a population at 30 min. The HPAEC results of the treatment of pool d with RG + RGAE are shown in Fig. 5(A). The products were identified based on their retention times, which were compared with standard rhamnogalacturonan oligomers from apple 'hairy' regions [14]. It shows that after treatment of pool d with RG + RGAE several oligomers of various degrees of polymerization were formed with a backbone of alternating Rha and GalA residues. Most of these oligomers are branched with Gal

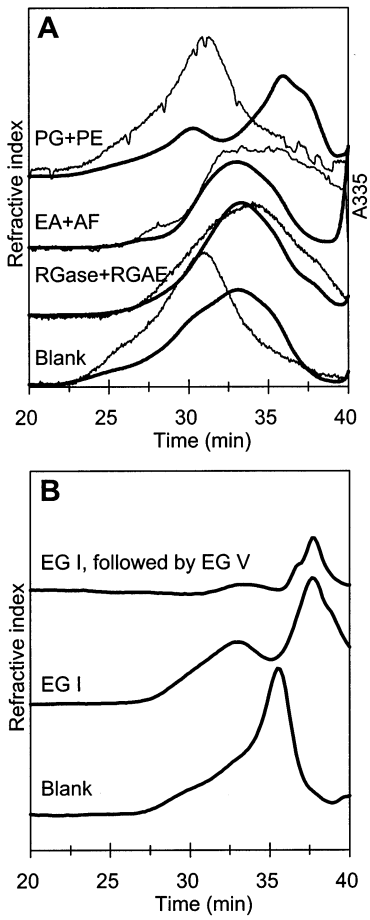

Fig. 4. High-performance size-exclusion chromatography of pool d from Autoclave 2 after treatment with EA + AF, RG + RGAE and PG + PE (A) and of pool a from 4 M NaOH 1 after treatment with EG I and EG V (B). Thin lines: UV detection, thick lines: RI detection.

residues linked to a Rha residue at the C-4 position. From the results of the methylation analysis it could be calculated that the arabinan side-chains had an average DP of 45 residues or more, assuming that all (1→2,4)-linked Rha residues carry an arabinan side-chain. The oligomers released during incubation with RG + RGAE indicated that a part of the (1→2,4)-linked Rha residues are substituted with Gal. Therefore, the average DP of the arabinan side-chains must be higher than 45. A large part of the rhamnogalacturonans was only released with 4 M NaOH. This shows that they are firmly embedded in the cell wall, possibly through diferulate cross-links.

Fig. 3(D) shows the HPSEC patterns of the pools obtained after separation of Autoclave 2 on Sephacryl S 500. The apparent molecular weights of the rhamnogalacturonans present in the extracts obtained by autoclaving were very high, 1300 kDa for the high molecular weight population and 120 kDa for the lower molecular weight population. Since it is known that, in comparison to other techniques, often higher molecular weights are found for pectic polysaccharides when light-scattering techniques are used, two other methods to determine the molecular weights by SEC were also applied. It was found that the results obtained by laser light-scattering were similar to those obtained with universal calibration (1600, 120 and 12 kDa, respectively) and to those obtained by comparison with dextran standards (pools I and II) and pectin standards (pool III) (1400, 110 and 20 kDa, respectively). This means that the 'hairy' regions from sugar beet pectins have higher apparent molecular weights than reported be-

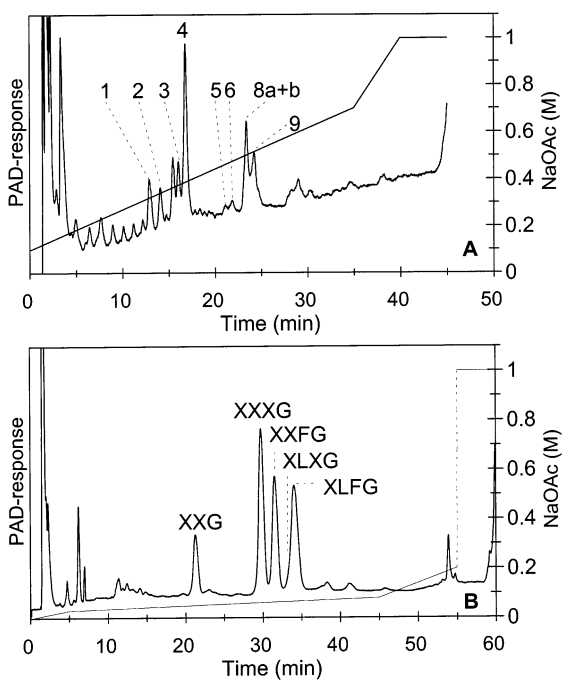

Fig. 5. High-performance anion-exchange chromatography of the enzyme digests of pool d from Autoclave 2, treated with RG + RGAE, (A) and of pool a from 4 M NaOH 1 after treatment with EG V (B).

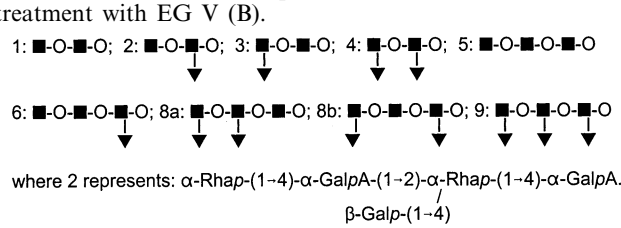

Table 4
Molecular weight, intrinsic viscosity, and radius of gyration of pools from Autoclave 2 obtained by preparative size-exclusion chromatography before and after saponification (sap)

	M_w	$[\eta]_w$	Rg_w
Pool I	1300	0.97	36
Pool II	120	0.32	11
Pool III	21	0.20	5
Pool I sap	658	0.42	21
Pool II sap	203	0.28	13
Pool III sap	60	0.09	6

fore for 'hairy' regions from beet [6,10] or apple [28] and for acid extracted beet pectins [29–31]. This was supported by the fact that the backbone length, which can be calculated for the rhamnogalacturonans (Rha and GalA make up approximately 10% of the total weight, resulting in a backbone weight of 120 kDa), was in agreement with the value found by McNeil et al. for suspension cultured sycamore cell walls [32] (100 kDa). A probable cause for the low molecular weights found in other studies is the use of (rather linear) pectin standards in some of these studies [10,28] to calculate the molecular weight of the highly branched rhamnogalacturonans. Furthermore, in many studies [6,29–31] the pectin has been subjected to acid conditions, thus removing Ara and decreasing the molecular weight.

Another explanation for the high molecular weight found for pool I is the possibility of the presence of diferulate cross-links, connecting several rhamnogalacturonan molecules. Based on the molecular weight, the sugar composition, and the ferulate composition [33], it can be calculated that on average every molecule contains five diferulates, which shows that cross-linking might occur to some extent. To investigate this hypothesis, the extract Autoclave 2 was subjected to alkali conditions in order to break the diferulate ester bonds. The molecular weights, intrinsic viscosities and radii of gyration before and after saponification are shown in Table 4. It appears that saponification leads to a decrease in molecular weight of pool I by a factor 2, whereas the molecular weights of pools II and III increase, probably as a result of a shift towards lower molecular weights of a part of the material originally present in pool I. Interestingly, the intrinsic viscosity of pool III decreased, probably as a result of the degradation of the homogalacturonans by β-elimination. These results indicate that ferulic acid cross-links might have been present, although there is still the possibility that degradation of the backbone has occurred as a result of the alkali conditions. The presence of ferulic acid cross-links is supported by our findings that hydrolysis of the arabinan side-chains in pool I with EA + AF resulted in a decrease in M_w to 99 kDa [34]. Since $\sim$60 mol% of this pool consisted of Ara, which was present as side-chain of the rhamnogalacturonan, a maximum decrease in M_w of $\sim$60% was expected. Therefore, the resulting low M_w after treatment with EA + AF was ascribed to the degradation of the arabinans that participate in cross-links between rhamnogalacturonan molecules.

No additional evidence was found for the presence of xylogalacturonan subunits in the backbone of the ramified 'hairy' regions, as has been described for apple pectins [27], besides a small amount of (1→3,4)-linked GalA which was found previously in both autoclave extracts and in the alkali extract [10].

Since A_{335} is quite selective for ferulic acid and comparable chromatograms were obtained when detection was done by neutral sugar determination or A_{335} (on SEC as well as DEAE), it can be concluded that ferulic acid is evenly distributed amongst the neutral sugar-containing populations. Degradation of the rhamnogalacturonans with EA + AF resulted in a sharp decrease of the UV response at 335 nm, therefore it was concluded that most of the ferulic acid was attached through the arabinan side-chains as opposed to the results found by Guillon et al. for acid and alkali soluble pectins from sugar beet pulp [6,7], who indicated that only 30% of the ferulic acid was removed by EA + AF. However, this might be caused by the degradation of arabinan side-chains as a result of the acid treatment during their extraction.

Although the molecular weight of the rhamnogalacturonans was high, it did not result in a high intrinsic viscosity ($\sim$1 dL/g). The compact nature of the rhamnogalacturonans is caused by the high amount of arabinan and

galactan side-chains, which make up 70 mol% of pool I. This, together with a relatively high acetyl content [35] results in poor physicochemical properties. However, the high ferulate content makes it possible to cross-link these polysaccharides resulting in gel formation at low concentrations [24].

Xyloglucans.—The high content of Xyl, Glc and Man in pool a (neutral population) of extract 4 M NaOH 1 (Fig. 1(C)) was thought to be an indication for the presence of xyloglucans and glucomannans. The presence of xyloglucans was supported by the observation that the Xyl in this pool was mainly present as terminally and (1→2)-linked residues (Table 3). In order to confirm the presence of arabinans, xyloglucans and glucomannans, the material was subsequently treated with EG I, EG V and EA + AF (Fig. 4(B)). Incubation with EG I, which is known to have a high activity towards glucans and mannans, and a much lower activity towards xyloglucans [36], resulted in degradation of the material which eluted at 35.5 min towards lower molecular weight (38 min) on HPSEC. From this it was concluded that this material consisted of glucomannans, glucans or mannans. A population remained at 33 min. This population was treated with EG V, which has a high activity towards xyloglucans [36]. The enzyme degraded the population at 33 min to a lower molecular weight (38 min). Analysis of the reaction products by HPAEC showed the presence of typical xyloglucan oligomers (Fig. 5(B)). As concluded from the elution behavior, the oligomers found were: XXG, XXXG, XXFG and XLFG, named according to the nomenclature of Fry et al. [37], where G, X, L and F represent different substituted β-glucosyl residues of xyloglucan: G, β-Glc*p*; X, α-Xyl*p*-(1→6)-β-Glc*p*; L, β-Gal*p*-(1→2)-α-Xyl*p*-(1→6)-β-Glc*p*; F, α-Fuc*p*-(1→2)-β-Gal*p*-(1→2)-α-Xyl*p*-(1→6)-β-Glc*p*. It was found that three out of four Glc residues carry a side-chain, as was also seen for xyloglucans from many species such as apple, sycamore, and tamarind [15,38]. This in contrast to xyloglucans from potato and tomato, both belonging to the *Solanaceae*, for which the presence of two adjacent unbranched Glc residues is characteristic [39]. In comparison with apple [40], Xyl is only present in low amounts in sugar beet pulp (~ 2 mol%) [10], and therefore it can be concluded that xyloglucan plays a limited role in making up the architecture of sugar beet cell walls. After treatment of pool a of 4 M NaOH 1 with EG I and V an arabinan population at 33 min remained, which could be degraded with EA + AF.

4. Conclusion

Three types of pectic polysaccharides were present in both extracts obtained by autoclave treatment of beet pulp; homogalacturonans, rhamnogalacturonans and arabinans. It was concluded that homogalacturonans and rhamnogalacturonans were present as separate populations in the extracts, since changes in the A_{335} pattern were not observed in any of the incubations with enzymes active on the homogalacturonans, while enzymes active on the rhamnogalacturonans never changed the elution patterns of the homogalacturonans upon HPSEC. Nevertheless, it is believed that the rhamnogalacturonans and homogalacturonans are integral parts of the original pectin molecule [41]. The molecular weight of the material which was formed after treatment of the rhamnogalacturonans with RG + RGAE was similar to the neutral arabinan population, which may indicate that the neutral population originates from degradation of the rhamnogalacturonans during autoclaving.

The populations of pectic polysaccharides in sugar beet pulp (high and low molecular weight rhamnogalacturonan, arabinan and homogalacturonan) are believed to be a result of degradation during the extraction. Also, compared with acid extracted commercial pectins the apparent molecular weight of the homogalacturonan was relatively low, indicating degradation of the pectins, as confirmed by the low intrinsic viscosity. Additional experiments showed that autoclaving of sugar beet pulp at higher temperatures results in larger amounts of low molecular weight material, which coelutes with this population upon HPSEC (data not shown). These results indicate that during autoclave extraction degrada-

tion primarily occurs in the homogalacturonan regions, whereas during acid extraction the arabinan side-chains are mainly degraded.

Some evidence was found that a part of the rhamnogalacturonans present in the extracts obtained by autoclaving were linked to other rhamnogalacturonans by diferulate cross-links, thus explaining their high molecular weight (1300 kDa).

We showed that besides pectic polysaccharides, some hemicelluloses could also be extracted from sugar beet pulp: Glc- and Man-containing polysaccharides, as well as xyloglucans. To our knowledge this is the first report describing the presence of such hemicelluloses in sugar beet pulp. Enzymatic degradation of the xyloglucans showed some similarities with apple xyloglucans with respect to the substitution with Fuc and Gal.

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