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Arabinose and ferulic acid rich pectic polysaccharides extracted from sugar beet pulp

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Abstract

Arabinose and ferulic acid rich polysaccharides were extracted from sugar beet pulp using two extraction methods: a sequential extraction with H_2O (2 times), NaOH/EDTA (2 times), and 4 M NaOH (2 times; method A) and a sequential extraction in which the NaOH/EDTA extraction was replaced by an autoclave extraction (2 times; method B). Both extraction method A and B yielded approximately 45% of the sugar beet pulp polysaccharides. For both method A and B, three extracts with increasing neutral sugar content were obtained: two NaOH/EDTA extracts and a 4 M NaOH extract for method A next to two autoclave extracts and a 4 M NaOH extract for method B. The sugar linkage composition indicated the presence of arabinose rich rhamnogalacturonan ('hairy regions') and homogalacturonan ('smooth regions') in all extracts. The sugar compositions of the NaOH/EDTA extracts were very similar to the autoclave extracts. A remarkable difference was the higher amount of ester groups in the autoclave extracts: next to methyl esters and acetyl groups, they also contained relatively high amounts of feruloyl groups. Size-exclusion chromatography using RI- and UV-detection showed that all extracts were heterogeneous with respect to molecular weight distribution. Ferulic acid was particularly located in the high molecular weight populations of the autoclave extracts. © 1996 Elsevier Science Ltd.

Keywords: Sugar beet; Pectin; Autoclave extraction; Alkali extraction; Ferulic acid; HPSEC

1. Introduction

Sugar beet pulp consists mainly of polysaccharides (approximately 67% of the dry matter), of which pectin (±19%), pectin associated arabinan (±21%), and cellulose

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(±24%) are prevailing. It is therefore a potential source of pectin and arabinan [1]. However, sugar beet pectins have a relatively low viscosity and a poor gelling capacity compared to citrus and apple pectins, which narrow its area of application. These poor physico-chemical properties have been attributed to the high amount of acetyl groups and the relatively low molecular weight [2]. The presence of ferulic acid, which is ester-linked to the arabinan and (arabino) galactan side chains [3–7], makes it possible to cross-link sugar beet pectins [8].

Traditionally, commercial pectins are being extracted under acidic conditions [1,9]. This generally results in degradation of the arabinan side chains [10] and therefore in a loss of feruloyl groups. Arabinans or arabinan rich pectic substances are generally extracted using hot alkali [11,12], releasing the feruloyl groups by saponification. Arabinans from sugar beet pulp can be used as a gelling product and fat replacer, after enzymatic treatment to reduce branching [11]. Also extraction procedures for pectic substances using hot water [8,13], EDTA [1,9], oxalate [1,9] or chlorite/acetic acid [13] have been reported in literature. These extraction procedures often consist of several steps, each of which have a low yield. Autoclave treatment of beet pulp has been described as a means to improve its properties as dietary fiber, although no information was given about the feruloyl content of the water soluble fractions, which were obtained after autoclaving [14].

The aim of this study was to develop an extraction procedure for beet pulp pectic polysaccharides with a maximal yield and a minimal degradation of the arabinan side chains accompanied by a maximal preservation of the feruloyl groups. Since both acid and strong alkali extraction result in a loss of feruloyl groups, we used a sequential extraction procedure introducing an autoclave treatment to obtain ferulic acid and arabinan rich pectins. This was compared with a sequential extraction procedure including mild alkali treatment.

2. Experimental

Materials.—Wet beet pulp (harvest 1991, 8.9% dry weight) was obtained from CSM Suiker bv (Breda).

Alkali extraction of polysaccharides.—Sugar beet pulp was extracted according to the procedure, presented in Fig. 1A. The pulp (1 kg) was ground in a Waring blender with 2 L distilled water (3 min, maximal speed). The mixture was successively extracted twice with distilled water, twice with 0.15 M NaOH in the presence of 0.05 M EDTA, and twice with 4 M NaOH in the presence of 0.02 M $NaBH_4$, in all cases using a total volume of 5 L. Between extractions the residues were filtered over a glassfilter (type 3, ∅ 17 cm). Where applicable, the extracts were neutralized to pH 5.0. All extracts were dialyzed and freeze dried.

Autoclave extraction of polysaccharides.—The second extraction procedure (method B), was similar to method A, except for the third and fourth step (Fig. 1B) in which the sugar beet pulp was autoclaved twice for 40 min at 121 °C (pH 5.2).

Analytical methods.—The uronic acid content was determined by the automated m-hydroxybiphenyl assay [15]. Neutral sugar compositions of the extracts were deter-

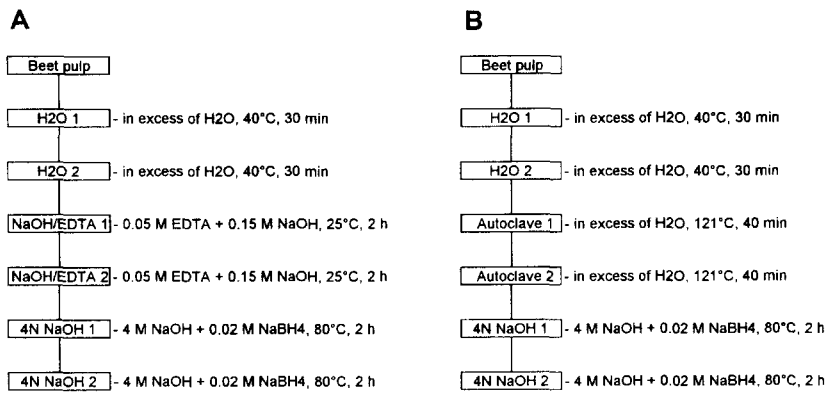

Fig. 1. Scheme for the extraction of polysaccharides using an alkali (A) and an autoclave (B) extraction method.

mined on a Carlo Erba 4200 system GLC, using a J&W DB 225 column, after hydrolysis with 2 M trifluoroacetic acid (1 h, 121 °C) and conversion of the monomers to alditol acetates [16]. After pretreatment with 72% H_2SO_4, hydrolysis with 1 M H_2SO_4 was used for the determination of neutral sugars in the sugar beet pulp. Inositol was used as internal standard. The degrees of methylation and acetylation were determined on a Spectra Physics 8800 system HPLC, using an Aminex HPX 87H column [17]. Feruloyl groups were determined spectrophotometrically (Beckman DU-62 spectrophotometer) 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 [8]. High-performance size-exclusion chromatography (HPSEC) was performed using Bio-rad Bio-Gel TSK 60XL, 40XL, and 30XL columns in series in combination with a TSK XL guard column on a Spectra Physics 8700 XR system HPLC. Elution was carried out with 0.4 M sodium acetate buffer (pH 3.0) at a flow rate of 0.8 mL/min at 30 °C. The eluate was monitored using refractive index (RI) detection (Shodex RI-71) and to monitor the presence of ferulic acid, UV-detection (Kratos, Spectroflow 773) at a wavelength of 335 nm [6] was used. Molecular weights were determined using pectin standards obtained by mechanolysis. The molecular weights of these standards were calculated from their viscosities [18].

Methylation analysis.—Prior to methylation, carboxyl reduction was effected by the method of Taylor and Conrad [19] and was repeated three times. Polysaccharides were methylated according to the Hakomori [20] method, as modified by Sanford and Conrad [21], followed by hydrolysis with 2 M trifluoroacetic acid (1 h, 121 °C), and conversion of the monomers into partially methylated alditol acetates [22]. The partially methylated alditol acetates were identified by GLC–MS using a Hewlett Packard mass selective detector 5970-B coupled to a HP 5890 GLC equipped with a Chrompack CPsil 19CB column and the partially methylated alditol acetates were quantified on a Carlo Erba HRGC 5160 system GLC using a J&W DB 1701 column.

3. Results

Alkali extraction of polysaccharides.—Beet pulp was extracted twice with distilled water to remove some residual water soluble and suspendable material. Both extracts had a low polysaccharide content and the second H_2O wash gave a very low yield (Table 1). Extraction A NaOH/EDTA 1 yielded 9.7% of the total solids of beet pulp and the material consisted for 79.7% of polysaccharides. Based on the composition of the original beet pulp, this fraction represented 12% of the pulp polysaccharides (Table 1). The second extraction with mild alkali yielded another 5.4% of the total solids of beet pulp, containing 6% of the pulp polysaccharides. The highest yield was found in the first strong alkali extract, which contained 23% of the pulp polysaccharides. The second 4 M NaOH extraction resulted in a very low yield, which indicated that nearly all the alkali extractable material had been removed.

The polysaccharides in extract A H_2O 1 consisted mainly of galacturonic acid, arabinose, and galactose. The polysaccharides in extract A H_2O 2 contained less galacturonic acid and more glucose. The polysaccharides in extract A NaOH/EDTA 1 were mainly composed of galacturonic acid and arabinose. Galactose and rhamnose were only present in minor quantities. The second NaOH/EDTA extraction yielded material containing relatively more arabinose and less galacturonic acid compared to the first extraction. The sugar compositions of the A 4 M NaOH 1 and A 4 M NaOH 2 extracts were very similar. They consisted mainly of neutral sugars and 17–18% of galacturonic acid. Compared to the NaOH/EDTA extracts, xylose, mannose, and glucose were found in higher quantities, next to the pectic neutral sugars arabinose, rhamnose, and galactose.

The degrees of acetylation (DA) of the H_2O extracts were relatively high (Table 2).

Table 1
Yield and sugar composition of polysaccharides obtained with alkali (A) and autoclave extraction (B)

Extract	Sugar composition [a]							Yield [b]	Total sugar content [c]
	Rha	Ara	Xyl	Man	Gal	Glc	AUA		
Sugar beet pulp	2.0	32.4	2.3	1.6	7.6	34.6	19.5	100.0	66.7
A H_2O 1	1.7	37.1	2.1	2.1	16.6	7.9	32.4	5.2	21.3
A H_2O 2	1.1	41.3	1.2	2.9	18.8	14.0	20.8	1.2	27.1
A NaOH/EDTA 1	3.6	37.7	0.0	0.0	5.0	0.1	53.6	9.7	79.7
A NaOH/EDTA 2	5.3	59.5	0.0	0.0	7.4	0.0	27.8	5.4	68.8
A 4 M NaOH 1	4.7	53.7	5.5	2.7	10.9	5.7	16.8	23.3	61.4
A 4 M NaOH 2	6.4	60.1	4.8	0.7	7.4	2.4	18.2	3.0	50.9
B H_2O 1	3.1	36.7	2.1	0.0	7.9	25.2	25.0	6.8	21.5
B H_2O 2	0.7	24.3	1.6	2.6	10.9	37.6	22.2	1.3	25.5
B Autoclave 1	2.6	29.8	0.4	0.4	3.9	1.1	61.9	12.0	66.8
B Autoclave 2	3.6	60.8	0.0	0.4	6.6	0.9	27.7	5.8	88.3
B 4 M NaOH 1	6.2	57.3	6.2	2.4	11.0	5.1	11.7	19.0	76.0
B 4 M NaOH 2	6.4	57.7	8.5	1.6	9.5	4.1	12.3	1.8	48.9

[a] Expressed as mole percentage.
[b] Expressed as percentage dry weight of beet pulp.
[c] Expressed as weight percentage of the extract.

Table 2
Amount of acetyl, methyl, and feruloylgroups present in extracts obtained with alkali (A) and autoclave extraction (B). Between brackets the degree of acetylation (DA), methylation (DM), and feruloylation (DF) is given

Extract	Ac [a]	(DA [b])	Me [a]	(DM [c])	FerA [a]	(DF [d])
A H_2O 1	1.1	(48)	0.6	(48)	0.00	(0.0)
A H_2O 2	0.9	(42)	0.4	(31)	0.00	(0.0)
A NaOH/EDTA 1	0.0	(0)	0.0	(0)	0.18	(0.4)
A NaOH/EDTA 2	0.0	(0)	0.0	(0)	0.09	(0.1)
A 4 M NaOH 1	0.0	(0)	0.0	(0)	0.03	(0.0)
A 4 M NaOH 2	0.1	(0)	0.0	(0)	0.03	(0.0)
B H_2O 1	1.1	(51)	0.4	(40)	0.00	(0.0)
B H_2O 2	1.0	(38)	0.5	(39)	0.00	(0.0)
B autoclave 1	7.0	(45)	6.0	(73)	0.39	(1.5)
B autoclave 2	5.4	(52)	3.2	(60)	1.05	(1.3)
B 4 M NaOH 1	0.0	(0)	0.0	(0)	0.04	(0.1)
B 4 M NaOH 2	0.1	(3)	0.0	(0)	0.06	(0.1)

[a] Expressed as weight percentage of the extract.
[b] Moles of acetyl groups/100 moles anhydrogalacturonic acid residues.
[c] Moles of methyl groups/100 moles anhydrogalacturonic acid residues.
[d] Moles of feruloyl groups/100 moles arabinose + galactose residues.

The degree of methylation (DM) was found to be 48% for A H_2O 1 and 31% for A H_2O 2. Only traces of acetyl and methyl were found in the alkali extracts. Despite the alkaline conditions ferulic acid was still present in the first and to a lesser extent in the second NaOH/EDTA extract, indicating the greater resistance of feruloyl esters to alkali conditions as compared to acetyl groups and methyl esters.

Autoclave extraction of polysaccharides.—Compared to the extraction method A, similar yields and sugar contents were found for the H_2O extractions of method B. The first autoclave extraction yielded 12.0% of the dry weight of beet pulp, containing 12% of the pulp polysaccharides. A second autoclave treatment yielded another 5.8%, representing 8% of the beet pulp polysaccharides. Again the highest yield was found for the 4 M NaOH extraction (19.0%) and represented 22% of the pulp polysaccharides. Similar to extraction method A, the second 4 M NaOH extraction resulted in a very low yield.

Although the H_2O extractions of method A and B were performed in the same way, both extract B H_2O 1 and B H_2O 2 contained more glucose and somewhat less galactose compared to A H_2O 1 and A H_2O 2. The sugar composition of extract B autoclave 1 shows that this consisted mainly of galacturonic acid (61.9%) and arabinose (29.8%). Galactose and rhamnose were present in small quantities. Extract B autoclave 2 contained relatively more arabinose (60.8%) and galactose (6.6%) and less galacturonic acid (27.7%). The sugar compositions of the material released during the 4 M NaOH extractions of both methods were similar. Only the relative amounts of galacturonic acid were slightly lower in the extracts of method B.

The degree of acetylation of the extract B autoclave 1 was found to be 45% (Table 2), and was even higher for B autoclave 2 (52%). Due to the alkali conditions only

traces of acetyl groups and methyl esters were found in extract B 4 M NaOH 1&2. The degree of methylation of extract B autoclave 1 (73%) was higher than that of extract B autoclave 2 (60%). Based on the dry weight, extract B autoclave 1 consisted of 0.39% ferulic acid. This amount was much higher for B autoclave 2, although the degree of feruloylation, expressed as mole ferulic acid per mole of galactose plus arabinose, did not change significantly. Traces of ferulic acid were found in extract B 4 M NaOH 1.

Size exclusion chromatography.—The molecular weight distribution as determined

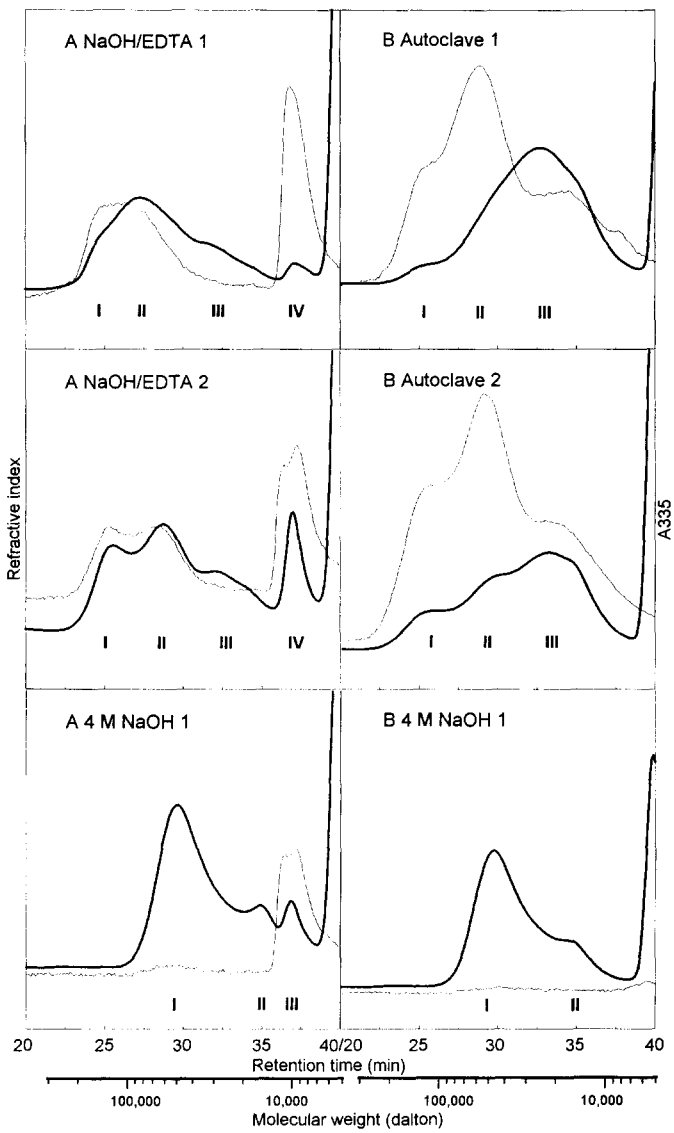

Fig. 2. High-performance size-exclusion chromatography of polysaccharides obtained with alkali (A) and autoclave extraction (B); thin line: UV-detection, thick line: RI-detection.

Table 3
Sugar linkage composition of polysaccharides obtained with alkali (A) and autoclave extraction (B)

Linkage	Glycosidic linkage composition [a]					
	A NaOH/EDTA		A 4 M NaOH 1	B Autoclave		B 4 M NaOH 1
	1	2		1	2	
Rhamnose						
T-Rha *p* [b]	0.4	0.5	0.5	0.5	0.3	0.2
1,2-Rha *p* [b]	0.4	0.8	1.1	0.4	1.0	1.0
1,2,4-Rha *p*	0.4	1.0	1.0	0.4	1.2	0.9
	1.2	2.3	2.6	1.3	2.5	2.1
Arabinose						
T-Ara *f*	17.6	27.3	25.3	17.4	25.7	25.4
1,3-Ara *f*	0.6	0.8	0.8	0.5	0.6	0.8
1,5-Ara *f*	15.4	21.7	23.1	14.2	22.5	21.7
1,2,5-Ara *f*	1.2	2.0	1.5	0.9	1.5	1.5
1,3,5-Ara *f*	13.4	19.0	16.9	11.8	18.8	16.7
1,2,3,5-Ara *f*	3.1	4.8	3.4	2.2	3.7	3.4
	51.2	75.6	71.0	47.0	72.8	69.5
Xylose						
T-Xyl *p*	0.4	0.4	1.2	0.8	0.4	1.4
1,4-Xyl *p*	0.4	0.3	5.3	0.3	0.3	5.9
1,2,4-Xyl *p*	0.1	0.1	0.2	0.4	0.1	0.1
1,3,4-Xyl *p*	0.1	0.1	0.2	0.4	0.1	0.1
	1.0	0.9	6.9	1.9	0.9	7.5
Galactose						
T-Gal *p*	1.7	2.7	2.7	1.8	2.2	3.1
1,4-Gal *p*	2.8	2.6	2.6	0.9	3.3	2.9
1,6-Gal *p*	1.4	1.4	1.5	0.4	1.0	1.4
1,3,6-Gal *p*	0.8	0.5	0.3	0.3	0.5	0.5
1,4,6-Gal *p*	0.3	0.5	0.8	0.3	0.3	1.3
	7.0	7.7	7.9	3.7	7.3	9.2
Glucose						
1,4-Glc *p*	0.4	0.1	2.8	0.9	0.6	2.9
1,4,6-Glc *p*	0.0	0.6	0.4	0.1	0.1	0.1
	0.4	0.7	3.2	1.0	0.7	3.0
Mannose						
1,4-Man *p*	0.5	0.2	2.2	0.8	0.6	2.3
	0.5	0.2	2.2	0.8	0.6	2.3
Galacturonic acid [c]						
T-Gal *p* A	1.9	1.0	0.6	2.3	1.3	0.6
1,4-Gal *p* A	33.1	9.3	4.2	39.4	12.8	4.3
1,3,4-Gal *p* A	3.6	2.2	1.3	2.5	1.3	1.4
	38.6	12.5	6.1	44.2	15.4	6.3

[a] Linkage types in mol%.
[b] T, terminal; 1,2-linked Rha, etc.
[c] Determined as galactose residues after carboxyl reduction.

by HPSEC of the most important extracts is shown in Fig. 2. The concentration of the polymers in the eluate was determined using RI-detection and the presence of ferulic acid was monitored by UV-detection at 335 nm. Based on RI-detection three overlapping high molecular weight populations could be distinguished for extract A NaOH/EDTA 1 with molecular weights of approximately 100 (I), 65 (II), and 24 kDa (III), based on calibration with pectin standards. Population I and II contained most of the ferulic acid. Also some low molecular weight material (population IV), probably EDTA, was observed by UV-detection at a retention time of approximately 37 min. Extract A NaOH/EDTA 2 consisted of three populations with molecular weights of 93 (I), 46 (II), and 24 kDa (III), respectively. Again ferulic acid was mainly found in population I and II. Extract A 4 M NaOH 1 consisted of two populations with molecular weights of 36 (I) and 15 kDa (II). No UV-response was detected, except for peak III at 37 min (EDTA). Using RI-detection two populations could be distinguished in B autoclave 1; a small population (I) with a molecular weight of 96 kDa and the main population (III) with a molecular weight of 20 kDa. Using UV-detection an additional population (II) with a molecular weight of 44 kDa showed up. B autoclave 2 showed three populations on both RI- and UV-detection with molecular weights of 96 (I), 44 (II), and 20 kDa (III). The UV-elution pattern of the second autoclave extract was very similar to the corresponding pattern of the first autoclave extract, however, the concentration of ferulic acid was higher. Extract B 4 M NaOH 1 gave similar results compared to extract A 4 M NaOH 1 mentioned earlier.

Sugar linkage composition.—Table 3 shows the results of the methylation analysis of the most prominent extracts. Reduction of galacturonic acid to galactose was carried out to include the linkage composition of this acid sugar. Not all galacturonic acid residues were recovered after reduction and methylation. This also influences the molar ratios of the other sugars. This might be related to the β-eliminative degradation of galacturonan during the methylation treatment [23]. The amount of rhamnose was also found to be rather low in the non-reduced samples, which has been contributed to the relatively high resistance of the galacturonosyl-1,2-rhamnose linkage towards acid [23]. Little variation in sugar linkage composition was found between the extracts of the alkali extraction method (A) and those of the autoclave extraction method (B). Arabinose residues were mainly terminally linked, 1,5-linked or 1,3,5-linked. Also small amounts of 1,2,5-linked and 1,2,3,5-linked arabinose were present. Rhamnose was terminally linked, 1,2-linked or 1,2,4-linked. Galactose was found to be mainly present as terminally or 1,4-linked residues. Galacturonic acid was in general 1,4-linked, but small amounts of 1,3,4- and terminally linked galacturonic acid residues were also present. In the 4 M NaOH extracts xylose, glucose, and mannose, present as 1,4-linked residues, were detected in higher concentrations compared to the NaOH/EDTA and autoclave extractions.

4. Discussion

In this study an alkali (A) and a combined autoclave and alkali (B) extraction of sugar beet pulp were used to extract arabinose rich and ferulic acid rich pectic polysaccharides. The extraction methods were carried out repetitively; it was experienced that the results were reproducible. Both extraction methods included a repeated

H_2O extraction to remove residual water soluble and suspendable material. The low total sugar content of the first H_2O extraction confirmed that most of the water soluble polysaccharides had been removed during the sugar extraction process. The low amount of material released by the second H_2O extraction indicates that one H_2O extraction is sufficient to remove residual water soluble and suspendable material. No explanation could be found for the difference in glucose content between method A and B.

Several similarities were found between the autoclave and NaOH/EDTA extracts. The yield of the first NaOH/EDTA extraction was comparable to the yield of the extract B autoclave 1 and was also comparable to data reported in the literature obtained with mild alkali extraction [8]. The sugar compositions of the first autoclave and NaOH/EDTA extract were very similar, only the galacturonic acid content was somewhat lower in the latter. Rombouts and Thibault [8] found a lower arabinose content in their alkali extract (OHP), which is probably caused by the acid extraction which preceded the alkali extraction, causing hydrolysis of the arabinosyl linkages. Both the first autoclave and the first alkali extract contained high amounts of 1,4-linked galacturonic acid, which indicates that these extracts consisted mainly of 'smooth regions' of pectin. The presence of 1,2,4- and 1,2-linked rhamnose, 1,3,5-, 1,5-, and terminally linked arabinose and terminally linked and branched galactoses in all extracts is indicative for rhamnogalacturonan type I [24]. Compared to acid extracted sugar beet pectins [1,9] high amounts of arabinans were present in A NaOH/EDTA 1 and B autoclave 1, which may affect the functional properties [25]. The arabinosyl linkage compositions of the extracted pectins were similar to those found in acid and alkali extracted pectins [3] and in arabinans from other sources [24]. The average molecular weight of the alkali extract was higher than the molecular weight of the autoclave extract and was also somewhat higher compared to literature values for beet pulp pectins [1,8,26], although calibration of the column using pectin standards may have lead to an underestimation of the molecular weight of polysaccharides with high neutral sugar content. During the autoclave extraction apparently some breakdown occurs (pH 5.2), probably caused by β-elimination. An obvious difference between extract B autoclave 1 and A NaOH/EDTA 1 is the amount of methyl esters and acetyl and feruloyl groups. The amounts of methyl esters and acetyl groups in the autoclave extract were comparable to various other beet pectins reported in the literature [8,26,27]. Neither methyl esters nor acetyl groups were present in the first NaOH/EDTA extract, due to saponification. Ferulic acid has been found in the 'hairy regions' of sugar beet pectin and is known to be linked both to arabinose and galactose [3–7]. The amount of ferulic acid found in the first autoclave extract is comparable to values found in EDTA, ammonium oxalate [4], and acid extracts [8,9]. HPSEC was used to determine if the extracts were homogeneous according to molecular size. In B autoclave 1 three populations could be distinguished. Population I and II contained a relatively large amount of ferulic acid. This suggests that these populations contain predominantly 'hairy regions'. The third population contains only small amounts of ferulic acid and a high concentration of polysaccharides, which indicates the presence of 'smooth regions'. A lower amount of ferulic acid groups was found in the first NaOH/EDTA extract. Again, ferulic acid was predominantly present in the first two populations of the size exclusion chromatogram.

A substantial yield of about 5% was obtained for the second autoclave and

NaOH/EDTA extraction, showing that not all the extractable material had been released. The sugar compositions of these extracts were similar. Compared to the first extractions a significantly higher amount of neutral sugars was found, which indicates the presence of more 'hairy regions'. Also a significantly higher amount of ferulic acid was present as compared to extract B autoclave 1. These facts are in agreement with the observation that in both extracts the higher molecular weight populations, which contained the ferulic acid groups, predominated.

For both extraction method A and B residual arabinan rich polysaccharides were extracted using 4 M NaOH at 80 °C during 2 h. In agreement with the literature [28,29], high yields were obtained with 4 M NaOH (23.3% and 19.0% respectively for extraction method A and B). A single 4 M NaOH extraction proved to be sufficient to remove all the alkali soluble material. Only small differences were found in the composition of the resulting extracts A and B 4 M NaOH 1. Kobayashi et al. [28] found similar sugar compositions in their 1 M and 6 M NaOH extracts. The small amount of 1,4-linked galacturonic acid in A and B 4 M NaOH 1 indicates the absence of a large amount of 'smooth regions'. The sugar linkages of arabinose, galactose, rhamnose, and galacturonic acid show that arabinans are probably present as side chains of a RG-I type polysaccharide [24]. Xylose was mainly present as 1,4-linked xylan. The equimolar ratio of terminally linked xylose and 1,3,4-linked galacturonic acid in the 4 M NaOH extracts indicate the presence of xylogalacturonan. A similar structure was found as part of the backbone of 'hairy regions' isolated from apples [30].

It can be concluded that both extraction method A and B resulted in three arabinose rich pectin fractions. Increasing amounts of neutral sugars were observed for A EDTA/NaOH 1, A EDTA/NaOH 2, and A 4 M NaOH 1, respectively. The neutral sugar (linkage-) compositions of these extracts were very similar and indicated the presence of a rhamnogalacturonan with highly branched arabinans and some galactose attached to it. This in contrast to acid extracted pectins, which contain galactose as main neutral sugar since most of the arabinose has been removed by the acid conditions. For the extracts B autoclave 1, B autoclave 2, and B 4 M NaOH 1 also increasing amounts of neutral sugars were found. The composition of the extracts obtained in the subsequent extraction steps of method A and B were very similar, the only difference being the high amounts of methylesters and acetyl and feruloylgroups present in B autoclave 1 and 2. Especially the high amount of feruloyl groups is of interest, since it is possible to cross-link polysaccharides at the feruloyl groups, thus increasing the molecular weight.

Future research will include further elucidation of the structure of separate populations of the three most prominent extracts of both method A and B. Another topic of interest involves the oxidative cross-linking of the extracts containing high amounts of arabinose and ferulic acid, followed by physicochemical characterization of the material formed.

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