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Isolation of feruloylated arabinans and rhamnogalacturonans from sugar beet pulp and their gel forming ability by oxidative cross-linking

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Abstract

Rhamnogalacturonans and arabinans were purified from an autoclave extract of sugar beet pulp by size-exclusion and anion-exchange chromatography. Treatment with polygalacturonase and pectin methyl esterase was used to improve chromatographic separation of the rhamnogalacturonans from the homogalacturonans. Four populations were obtained: two rhamnogalacturonan populations with a high arabinose and ferulic acid content and with apparent molecular weights of 930 and 130 kDa, respectively, a feruloylated arabinan population and a (digested) homogalacturonan population with apparent molecular weights of 18 and 6 kDa, respectively.

Cross-linking of the high molecular weight rhamnogalacturonans with hydrogen peroxide and peroxidase gave an increase in viscosity and led to gel formation at concentrations <1.0%. The second rhamnogalacturonan population formed gels at concentrations as low as 0.75%. Cross-linking of the arabinan resulted in gel formation at a concentration of 4.0%. The properties of the gels are discussed in terms of the storage modulus (G') and tan δ.

Keywords: Sugar beet pulp; Arabinans; Rhamnogalacturonans; Cross-linking

1. Introduction

Sugar beet pectins contain high amounts of ferulic acid, in contrast to pectins obtained from other sources, e.g. citrus and apples (Rombouts & Thibault, 1986; Voragen, Pilnik, Thibault, Axelos & Renard, 1995). Treatment of sugar beet pectin with ammonium persulfate or hydrogen peroxide/peroxidase improves the viscosity and gelling properties of beet pectin; an effect apparently due to oxidative cross-linking of the ferulate monomers into dehydrodimers (Oosterveld, Grabber, Beldman, Ralph & Voragen, 1997; Oosterveld, Beldman, Schols & Voragen, 2000a; Thibault & Rombouts, 1986). Ferulic acid in beet pectin is located in the rhamnogalacturonan regions and is attached to the O-2 position of (1-5)-linked arabinose residues and to the O-6 positions of galactose residues in (1-4)-linked galactans (Colquhoun, Ralet, Thibault, Faulds & Williamson, 1994; Guillon & Thibault, 1988, 1989a,b; Ralet, Thibault, Faulds & Williamson, 1994). The traditional extraction method for pectin, using acid treatment, degrades the arabinan side-chains of the rhamnogalacturonans (Guillon & Thibault, 1990), and therefore leads to a loss of ferulic acid.

Recently the extraction of pectic polysaccharides from beet pulp by autoclaving was described. In the extract, high molecular weight rhamnogalacturonans to which ferulic acid is still attached were present. Besides rhamnogalacturonans, a separate population containing homogalacturonans and arabinans was found in this extract (Oosterveld, Beldman, Schols & Voragen, 1996; Oosterveld, Beldman & Voragen, 2000b). Oxidative cross-linking of the autoclave extracts with hydrogen peroxide/peroxidase gives an increase in viscosity and eventually leads to the formation of a gel at a concentration as low as 1.5%. It was shown that only rhamnogalacturonans and possibly low molecular weight arabinans were involved in cross-linking (Oosterveld et al., 2000a).

The current study deals with the isolation and characterization of the rhamnogalacturonan and arabinan populations present in the autoclave extract, in order to study the effect of oxidative cross-linking with hydrogen peroxide and peroxidase of these individual populations, as determined by their viscosity increase and by the characteristics of the gels formed.

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2. Experimental

2.1. Materials

Wet beet pulp (8.9% dry weight) was obtained from CSM Suiker bv (Breda, the Netherlands). Autoclave extraction of sugar beet pulp was performed as described previously (Oosterveld et al., 1997). Two subsequent autoclave treatments were performed. The extract obtained after the second autoclave treatment (Autoclave 2) was used in this study.

2.2. Analytical methods

The uronic acid content of the extract was determined by the automated *m*-hydroxy biphenyl assay (Thibault, 1979). The neutral sugar composition was determined after hydrolysis with 2 M trifluoroacetic acid (1 h, 121°C), conversion to alditol acetates and analysis by gas chromatography as described previously (Oosterveld et al., 1996). 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 (Rombouts & Thibault, 1986). The protein contents were determined according to the procedure of Sedmak and Grossberg (1977). BSA was used as a standard.

2.3. Enzyme treatment

The extract Autoclave 2 was treated with a combination of purified endo-polygalacturonase (PG) (Pasculli, Gereads, Voragen & Pilnik, 1991) and pectin methyl esterase (PE) (Baron, Rombouts, Drilleau & Pilnik, 1980) to depolymerize the homogalacturonan population present and to facilitate its separation from rhamnogalacturonans by size-exclusion chromatography. The extracts (5 mg/ml) were dissolved in 0.05 M sodium acetate buffer pH 5.0. The enzymes were added to obtain a final concentration of 1 μg protein/ml. Incubations were carried out at 30°C for 20 h. The reactions were stopped by heating at 100°C for 5 min.

2.4. Chromatography

High-performance size-exclusion chromatography (HPSEC) was performed on three Bio-Gel TSK columns in series (60XL-40XL-30XL) as described (Oosterveld et al., 2000b) 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). Apparent molecular weights and intrinsic viscosities were calculated using the light-scattering module of the Trisec software (Viscotek).

Preparative size-exclusion chromatography was performed on two columns (50 × 10 cm) of Sephacryl S 500 (Pharmacia) in series using a Biopilot system (Pharmacia). Sample (3 g in 75 ml) was eluted with 0.05 M NaOAc pH 5.0 at a flow rate of 37 ml/min. The separation was repeated four times and corresponding populations were pooled after analysis of the fractions (135 ml) for total neutral sugar (Tollier & Robin, 1979) and uronic acid (Thibault, 1979) contents using arabinose and galacturonic acid as standards, respectively. A correction was made for the response of uronic acids in the neutral sugar test. The presence of ferulic acid was monitored spectrophotometrically at 335 nm (Ralet et al., 1994). Pooled fractions were dialyzed against distilled water and freeze-dried.

Preparative anion-exchange chromatography was performed on a column (15 × 10 cm) of Source Q (Pharmacia) using a Biopilot system (Pharmacia). The sample (2.5 g in 75 ml) was applied onto the column at a flow rate of 15 ml/min (0.005 M NaOAc, pH 5.0). After 105 min the flow rate was increased to 80 ml/min and a linear gradient to 2 M NaOAc (pH 5.0) in 90 min was used to elute the polysaccharides. The elution with 2 M NaOAc was continued for another 15 min. The columns were washed with 0.5 M NaOH, followed by regeneration with 2 M NaOAc (pH 5.0) and equilibration with 0.005 M NaOAc (pH 5.0). The separation was repeated four times and corresponding fractions, identified by analysis of the fractions for total neutral sugar (Tollier & Robin, 1979) and uronic acid (Thibault, 1979), were pooled. Pooled fractions were dialyzed against distilled water and freeze-dried.

2.5. Oxidative cross-linking

Oxidative cross-linking of the purified polysaccharides with hydrogen peroxide/peroxidase was performed by adding 10 μl of horseradish peroxidase (0.5 mg/ml, Sigma) and 10 μl of hydrogen peroxide (0.5 M) to 1 ml of a solution of 0.25–4.0% of beet pectin in 0.1 M phosphate buffer (pH = 6.0, 25°C) (Oosterveld et al., 1997).

2.6. Rheological measurements

Relative viscosities of polysaccharide solutions (0.25–4.0% w/v) in 0.1 M phosphate buffer pH 6.0 were determined using Ubbelohde viscometers before and after oxidative cross-linking.

The formation of a gel network of the purified pools after cross-linking was investigated by small amplitude shear strain oscillatory testing. A VOR rheometer (Bohlin) in oscillatory mode was used to monitor the gel structure development as follows. Polysaccharide solutions (various concentrations) were cross-linked. Immediately after addition of the oxidant, the samples were placed in the geometry. A thin layer of soy oil was added to cover the bob and prevent evaporative losses throughout the measurements. All measurements were carried out at 25°C at a frequency of 1 Hz using a torsion bar of 20 g cm. Effort was made to ensure that the strain remained within the linear region of the material.

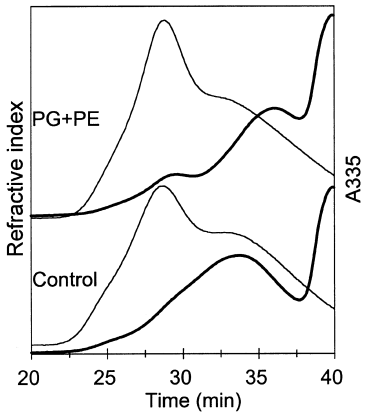

Fig. 1. High-performance size-exclusion chromatography of Autoclave 2 before and after treatment with PG + PE. Thick line—refractive index; thin line—A_{335}.

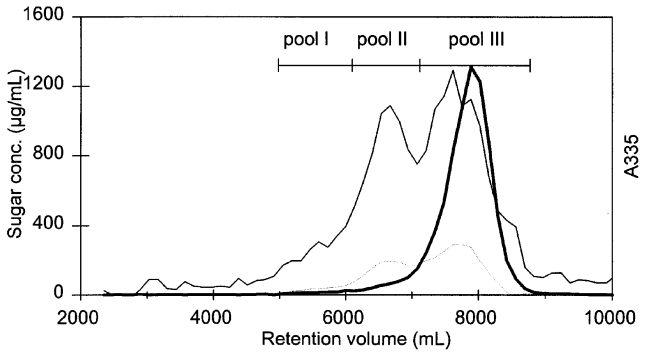

Fig. 2. Preparative size-exclusion chromatography (S 500) of Autoclave 2 after treatment with PG + PE. Thick line—uronic acid; dashed line—neutral sugars; thin line—A_{335}.

3. Results and discussion

To study the cross-linking process of the rhamnogalacturonans and arabinans, individual populations were purified from the Autoclave 2 extract by preparative size-exclusion chromatography and by preparative anion-exchange chromatography as described below.

3.1. Enzyme treatment

In order to facilitate the isolation of the rhamnogalacturonans and arabinans, the Autoclave 2 extract was treated with a combination of the enzymes endopolygalacturonase (PG) and pectin methyl esterase (PE) to depolymerize the homogalacturonan population. The elution patterns of the extract obtained by high-performance size-exclusion chromatography, before and after this enzyme treatment, are shown in Fig. 1. In the control three polysaccharide populations could be distinguished (23–27, 27–32, and 32–38 min), using UV and RI detection. The peak at 40 min was caused by the buffer. It has been shown that the two high molecular weight populations (23–27 and 27–32 min) consisted of rhamnogalacturonans and that the low molecular weight population (32–38 min) mainly consisted of homogalacturonan, but also contained arabinans (Oosterveld et al., 2000b). Treatment with PG and PE indeed resulted in a shift of the peak fraction of the low molecular weight population (from 33 to 37 min on RI), which was previously identified as a homogalacturonan (Oosterveld et al., 2000b). The UV signal at 335 nm, which indicated the presence of rhamnogalacturonans and arabinans with ferulic acid attached, did not change significantly. It can be concluded that these polysaccharides were not degraded by PG + PE.

3.2. Preparative size-exclusion chromatography

After treatment with PG + PE the extract Autoclave 2 was applied on a preparative S 500 size-exclusion column (Fig. 2). Based on the HPSEC results, the extract was separated into three pools. The sugar content was relatively low for pool I (~60%) in comparison with the other pools (~70%), probably because not all buffer salts were removed during dialysis. The sugar compositions of the pools were comparable to previous results obtained for the extract before treatment with PG + PE (Oosterveld et al., 2000b) and are shown in Table 1. All the degrees of feruloylation (DF; moles of feruloyl groups/100 mol arabinose + galactose residues) were somewhat lower than found in the original extract. No explanation could be found for this observation. Pool I (eluting at around 5500 ml) mainly consisted of arabinose. Also some galactose, galacturonic acid and rhamnose were present, in a ratio of 2.4:2:1, respectively. The DF and the arabinose content of this pool were relatively high and therefore also the absolute ferulic acid content. Pool II (eluting at 6600 ml) had a sugar composition comparable to pool I, although the galacturonic acid content was higher in this pool. This was probably due to some remaining homogalacturonan. From the sugar composition and the SEC elution pattern, it was concluded that both pool I and II consisted of rhamnogalacturonans with similar compositions, but with different apparent molecular weights. The composition of these rhamnogalacturonans is comparable to the high molecular weight fraction which was formed after cross-linking of the original extract Autoclave 2 with hydrogen peroxide/peroxidase (Oosterveld et al., 2000a). Furthermore, the sugar composition of the rhamnogalacturonans was comparable to that of RG-I from sycamore cells (Ishii, Thomas & Albersheim, 1989) except for the arabinose content, which is higher here. Pool III predominantly consisted of galacturonic acid. Nevertheless, this pool still contained a substantial amount of arabinose (27.6 mol%). Previous studies suggested that this might be due to the presence of low molecular weight arabinans (Oosterveld et al., 2000b). The DF of pool III was comparable to those of pool I and II, while the absolute ferulic acid content was relatively low.

The ferulic acid groups were spread relatively evenly

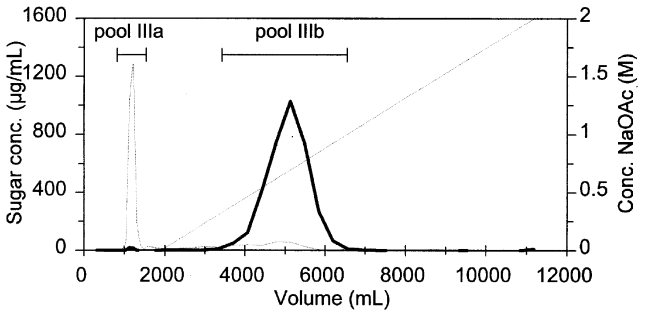

Fig. 3. Preparative anion-exchange chromatography (Source Q) of pool III obtained by S 500 size-exclusion chromatography. Thick line—uronic acid; dashed line—neutral sugars; dotted line—concentration NaOAc.

over the populations, since the DF of the various populations did not differ much.

3.3. Preparative anion-exchange chromatography

The arabinans, which elute together with the homogalacturonan fragments were separated from the homogalacturonans by anion-exchange chromatography (Fig. 3). Two populations were found, which were pooled in pool IIIa and pool IIIb. Pool IIIa contained 70% of the neutral sugars present in pool III (Table 1) and represented 15% of the polysaccharides. Arabinose was the predominant neutral sugar in pool IIIa. Furthermore, relatively high amounts of xylose, mannose and glucose were present in this pool, whereas the uronic acid and galactose contents were very low. Pool IIIb accounted for 85% of the polysaccharides of pool III and mainly consisted of galacturonic acid and low amounts of arabinose, galactose, and rhamnose.

3.4. High-performance size-exclusion chromatography

The molecular size distributions of the purified pools from Autoclave 2 as determined by HPSEC are shown in Fig. 4. The peak eluting at >37.5 min was caused by the buffer, and was not included in the calculation of the apparent molecular weights. The ratio UV: RI was similar for both pool I (27 min), and II (30 min). After separation on Source Q anion-exchange chromatography almost all A_{335} response which was originally present in pool III was found in the arabinan pool IIIa.

Table 2 shows some physico-chemical characteristics of the pools e.g. the apparent molecular weight and intrinsic viscosity. The apparent molecular weight of pool I was somewhat lower than we found for the pool I of the Autoclave 2 extract before treatment with PG + PE as determined by analytical scale separations of the whole extract (Oosterveld et al., 2000b). This might be a result of the enzyme treatment or the pooling procedure of the population. An apparent molecular weight of 930 kDa for pool I implies an average degree of polymerization (DP) of ~6000. Since the pool contains 80 mol% of arabinose and galactose, and 1 of every 100 arabinose and galactose residues carry a ferulic acid group, it can be calculated that on average every rhamnogalacturonan molecule carries 48 ferulic acid residues. The apparent molecular weight of pool II was comparable to that of a similar pool present in the extract before treatment with PG + PE (Oosterveld et al., 2000b). It can be calculated that its average DP was approximately 900, whereas every molecule carries on average 6 feruloyl groups. The intrinsic viscosity was low for pool I and II, especially in relation to their high apparent molecular weights. This is probably caused by the high

Table 1
Composition of Autoclave 2 and of the pools obtained by S 500 size-exclusion chromatography and by anion-exchange chromatography (Source Q) (nd Not determined)

	Autoclave 2[a]	S 500			Source Q	
		Pool I	Pool II	Pool III	Pool IIIa	Pool IIIb
Rha[b]	3.6	5.5	4.3	1.2	0	1.4
Ara[b]	68.9	68.9	61.1	27.6	82.6	8.8
Xyl[b]	0.0	0.7	0.3	0.4	4.7	0.6
Man[b]	0.4	0.5	0.6	2.8	3.7	1.8
Gal[b]	6.6	12.5	9.7	3.7	1.4	3.3
Glc[b]	0.9	0.9	0.5	1.4	4.7	0.6
Ua[b]	27.7	10.9	23.4	62.9	2.8	83.5
DA[c]	52	nd	nd	nd	nd	nd
DM[c]	60	nd	nd	nd	nd	nd
DF[d]	1.3	1.0	1.0	0.7	0.8	1.1
Sugar content[e]	88.3	60.1	71.5	73.1	95.3	57.9
Protein[e]	0.4	0.4	0.2	0.1	0.2	0.1

[a] From (Oosterveld et al., 1996).
[b] Expressed as mol%.
[c] Expressed as moles acetyl/methyl per 100 mol of galacturonic acid.
[d] Expressed as moles ferulic acid per 100 mol of galactose + arabinose.
[e] Weight percentage.

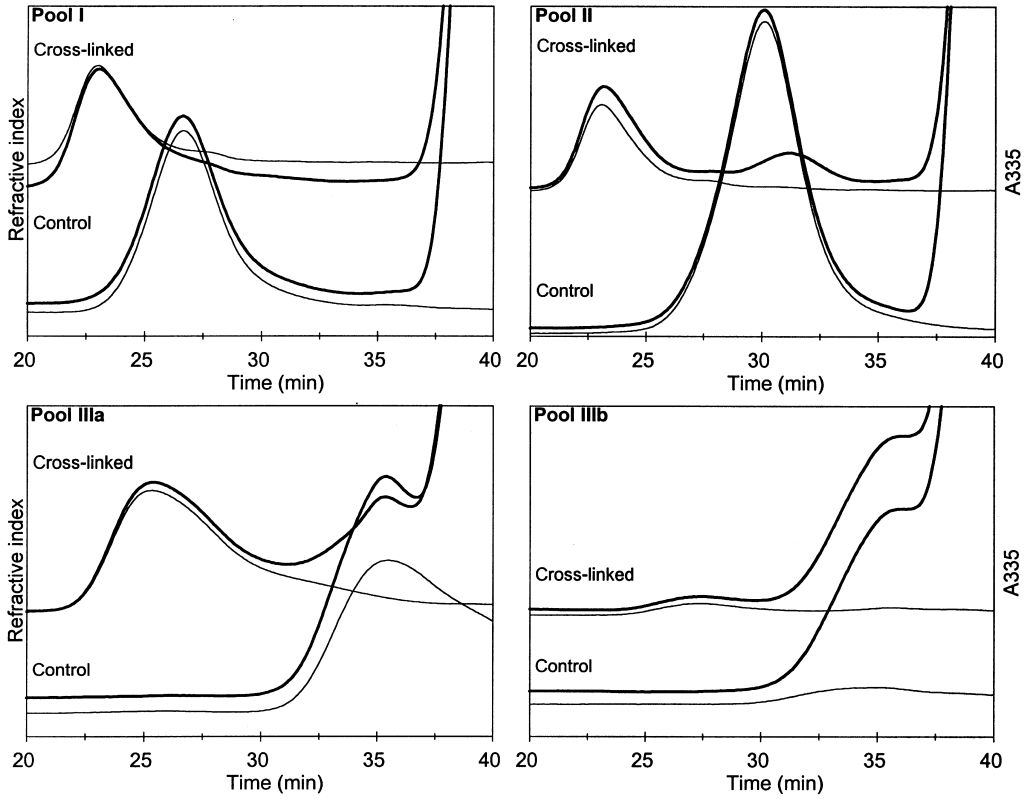

Fig. 4. High-performance size-exclusion chromatography of the pools purified from Autoclave 2 (0.5% w/v) before and after oxidative cross-linking with hydrogen peroxide/peroxidase. Thick line—refractive index; thin line—A_{335}.

degree of branching of these polysaccharides. Pool IIIa had an apparent molecular weight of 18 kDa, which shows that it consists of approximately 120 arabinose residues. The apparent molecular weight and intrinsic viscosity of pool IIIb were much lower than of the homogalacturonan population present in the original extract (Oosterveld et al., 2000b), due to the degradation of the homogalacturonans with PG + PE. For this pool an average DP of 40 can be calculated.

3.5. High-performance size-exclusion chromatography after cross-linking

After cross-linking the pools at a concentration of 0.5% with hydrogen peroxide/peroxidase, a shift towards higher molecular weight for part of the material was seen as determined by HPSEC (Fig. 4). The peak at 27 min in pool I shifted entirely to the void. A large part of the material in pool II shifted towards 23 min. Approximately 25% of this material did not shift to a higher molecular weight. This part did not contain ferulic acid and consisted probably of homogalacturonans. Most of the arabinan material, which was collected in pool IIIa, shifted towards higher molecular weight (25 min). The homogalacturonan population, which was collected in pool IIIb, contained a minute amount of material, which was able to cross-link, indicating the presence of some low molecular weight rhamnogalacturonans. It could be shown that for all pools the ferulic acid containing material shifted quantitatively towards higher molecular weight. So, it can be concluded that in the isolated fractions almost all ferulic acid containing material participates in the cross-linking reaction. For the whole extract, however, it was previously shown that not all ferulic acid participates in the cross-linking reaction (Oosterveld et al., 1997; Oosterveld et al., 2000a).

Table 2
Apparent molecular weight (M_w) and intrinsic viscosity ($[\eta]_w$) of the S 500 and Source Q pools (0.5% w/v) before and after cross-linking with hydrogen peroxide/peroxidase

Sample	M_w (kDa)		$[\eta]_w$ (dl/g)	
	Control	Cross-linked	Control	Cross-linked
Pool I	930	7300	0.66	0.99
Pool II	130	8800	0.33	0.56
Pool IIIa	18	1900	0.19	0.24
Pool IIIb	6	17	0.06	0.08

The physico-chemical properties of the pools after cross-linking are also presented in Table 2. The largest relative increase in apparent molecular weight was found for pool IIIa, which showed more than a 100-fold increase. The apparent molecular weight of pool II after cross-linking was higher than that of pool I, which

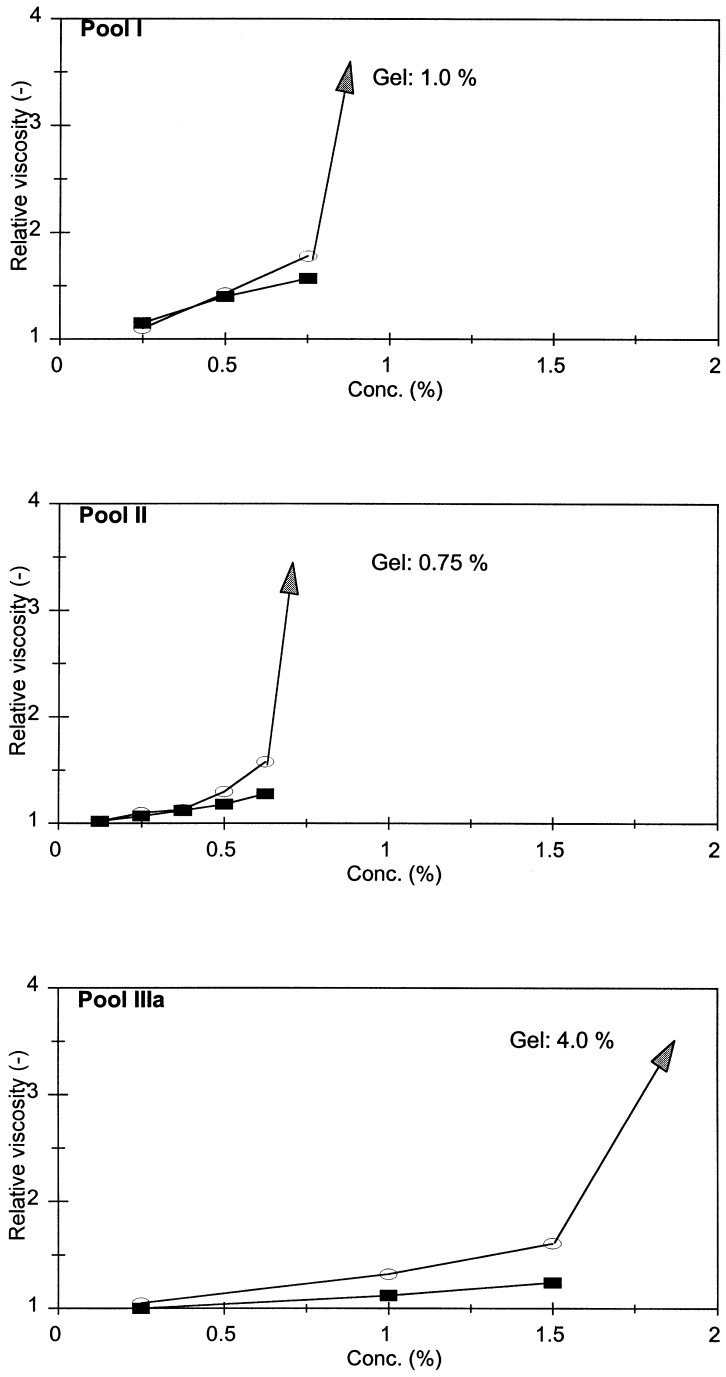

Fig. 5. Relative viscosity versus concentration of the pools purified from Autoclave 2 before (■) and after (○) oxidative cross-linking with hydrogen peroxide/peroxidase.

confirms the results of the gelation experiments, in which it was found that pool II formed a gel at the lowest concentration (see further; Fig. 5). The largest relative increase in intrinsic viscosity was seen for pool I and II and a much smaller relative increase in intrinsic viscosity was seen for pool IIIa and pool IIIb. These results show that the relative increase in intrinsic viscosity is higher for larger molecules.

3.6. Effect of concentration of the pools on viscosity after cross-linking

The pools I, II and IIIa were cross-linked at various concentrations to determine the effect on relative viscosity and the concentration at which gel formation occurs (Fig. 5). Pool IIIb was omitted because of its low ferulic acid content. Hydrogen peroxide and peroxidase were used as cross-linking agents,

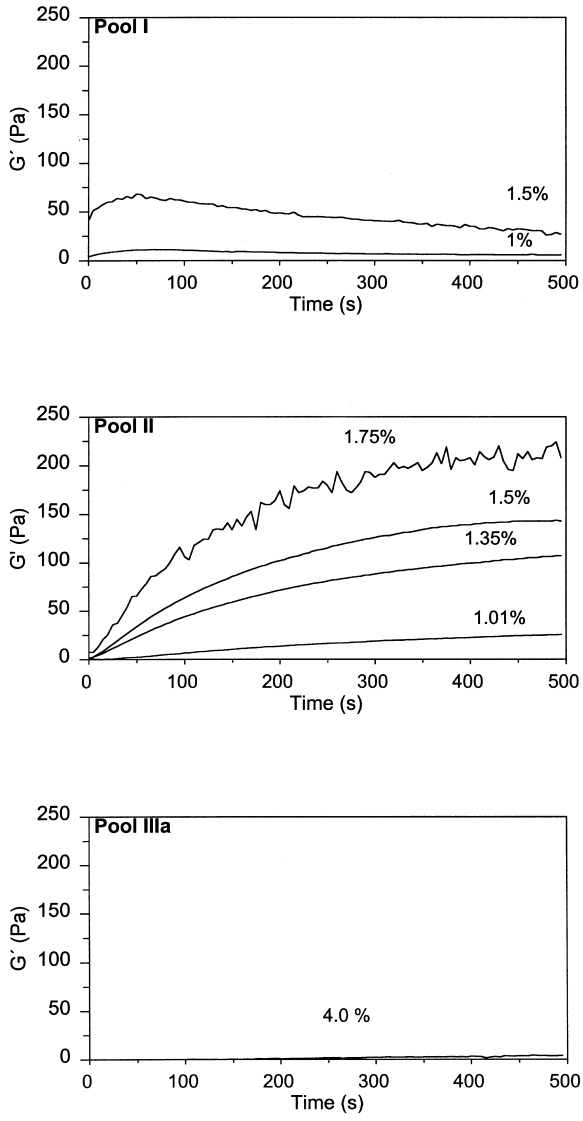

Fig. 6. Storage modulus (G') versus time of gels made by oxidative cross-linking with hydrogen peroxide/peroxidase of the pools purified from Autoclave 2.

since previous results showed that this combination was more effective than ammonium persulfate (Oosterveld et al., 2000a). Non cross-linked samples were used as controls. For these controls the largest increase in relative viscosity with increasing concentration was seen for pool I and the smallest increase for pool IIIa. This effect can be related to the coil overlap concentration, which can be estimated from the intrinsic viscosity. Pool I had the lowest estimated coil overlap concentration (1.5%), whereas those of pool II and pool IIIa were much higher (3.0 and 5.3%).

An increase in relative viscosity after oxidative cross-linking was seen in all pools. Pool II formed a gel at the lowest concentration (0.75%), although its apparent molecular weight was much lower than from pool I, which formed a gel at 1.0%. This may be partly due to the lower polysaccharide content of pool I in comparison with pool II.

Table 3
Storage modulus (G'), loss modulus (G'') and tan δ of the S 500 and Source Q pools after oxidative cross-linking with hydrogen peroxide/peroxidase (500 s)

Sample	Concentration (%)	G' (Pa)	G'' (Pa)	tan δ (–)
Pool I	1.00	11	0.1	0.009
	1.50	68	1.2	0.018
Pool II	1.00	26	0.5	0.019
	1.35	107	1.0	0.009
	1.50	143	2.0	0.014
	1.75	208	17.5	0.084
Pool IIIa	4.00	4	0.3	0.075

Surprisingly, considering its low apparent molecular weight and the fact that on average only one feruloyl group is present per arabinan molecule, pool IIIa was able to form a gel at a concentration of 4.0%. These are the first results that show that rhamnogalacturonans and arabinans are able to form gels by cross-linking reactions. Gel formation of non cross-linked arabinans, based on physical entanglements, was reported previously by McCleary et al. (Cooper, McCleary, Morris, Richardson, Marrs & Hart, 1992; McCleary, Cooper & Williams, 1989). The arabinans in that study were linearized with arabinofuranosidase and had an intrinsic viscosity of 0.28 dl/g, which is comparable to the value we found (0.20 dl/g). Concentrations from 10 to 20% w/v were needed to form gels from these linear arabinans, whereas we obtained a gel at a concentration between 1.5 and 4%. Furthermore, a setting time of $>$15 h found for the linear arabinans is considerably longer than the time needed to cross-link the arabinans in our system ($<$500 s). The behavior of the linear arabinan gels was typical for disordered polysaccharide chains interacting solely by physical entanglement, whereas the cross-linked extracts mainly interact via covalent links (Cooper et al., 1992).

3.7. Gelation

The formation of the gel network of the purified pools after cross-linking was investigated by small amplitude shear strain oscillatory testing. Fig. 6 shows the development of the storage modulus (G'), which is indicative for the amount of elastically effective cross-links formed and for the stiffness of the gel, at various concentrations in time. Table 3 summarizes the rheological parameters of the networks formed after hydrogen peroxide/peroxidase treatment for 500 s. A rapid increase in G' to its maximum value was seen for pool I at both concentrations investigated. However, after a large initial increase in G' for pool I during the first 10–20 s, a subsequent decrease was observed, even at low amplitudes. Possibly the settings of the apparatus were not optimal. Due to insufficient amounts of sample we could not fully optimize this measurement. The maximum value of G' for pool II increased rapidly with increasing concentration. The G' of pool IIIa increased to a relatively

low value even at high concentration (4%), indicating that the amount of cross-links in the gel was relatively low.

Tan δ ($= G''/G'$; G'' is indicative for relaxation of cross-links) is also a good parameter to describe the nature of the gel. A low value for tan δ (<0.1) shows the presence of an elastic system, whereas high values (>1) imply a more liquid-like character of the network (Ross-Murphy, 1984). The very small values of tan δ for Pool I and II show that elastic networks were formed, whereas the nature of the network of pool IIIa is more viscous than of most other pools (see Table 3). Only pool II gave a relatively high value for tan δ at a concentration of 1.75%. No explanation could be found for this observation. The low values of tan δ may be caused by the covalent nature of the cross-links.

3.8. Outlook for applications of cross-linked polysaccharides

Rhamnogalacturonans and arabinans have two advantages over acid extracted pectins from sugar beet pulp, namely the good solubility of the samples before cross-linking and their low viscosity prior to cross-linking, which makes them easier to handle in industrial applications, e.g. for in situ gel formation. Oxidative cross-linking of these polysaccharides can be an addition to the known applications for polysaccharides from sugar beet pulp, and may therefore play a role in the valorization of the pulp.

4. Concluding remarks

It was shown that both rhamnogalacturonan populations and the arabinan population, obtained from a sugar beet pulp extract by autoclave extraction, take part in the oxidative cross-linking reaction. Cross-linking of the rhamnogalacturonan population with an apparent molecular weight of 930 kDa resulted in an increase of the viscosity at low concentrations and in gel formation at a concentration of 1.0%. Although the second rhamnogalacturonan population isolated had a lower apparent molecular weight (130 kDa), it formed gels at concentrations as low as 0.75%. It can be concluded that this population is the most suitable population to use as a gelling agent. Since it was also present in higher amounts than pool I and IIIa in the original autoclave extract this population probably is the most important for cross-linking of the whole extract. The gels obtained from both types of rhamnogalacturonans were relatively stiff networks in terms of G'. The gels were highly elastic as determined by tan δ, which can be explained by the covalent nature of the bonds formed. These results show that gels can be formed after oxidative cross-linking of rhamnogalacturonans, a feature that has not been described before. The arabinan fraction formed a gel as well after oxidative cross-linking with hydrogen peroxide/peroxidase at a considerably lower concentration (4% w/v) than the gelling concentration for linear arabinan (10–20% w/v) (McCleary et al., 1989; Cooper et al., 1992).

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