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Oxidative cross-linking of pectic polysaccharides from sugar beet pulp

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Abstract

Oxidative cross-linking of three beet pectin extracts with hydrogen peroxide/peroxidase resulted in an increase in viscosity at low concentrations and in the formation of a gel at higher concentrations. Gels were formed using concentrations of 1.5% for an autoclave preparation and one obtained by an acid extraction and of 3% for a second autoclaved extract. It was shown that in the autoclave extracts only rhamnogalacturonans and possibly the arabinans participated in the cross-linking reaction. Cross-linking of the autoclave extracts with ammonium persulfate resulted in a decrease in reduced viscosity and molecular weight, although ferulic acid dehydrodimers were formed. Treatment of the acid extracted pectin with ammonium persulfate gave a slow increase in viscosity and the formation of a high-molecular-weight population was observed. For both oxidative systems, the 8-5 dehydrodimer was predominant after cross-linking.

Keywords: Sugar beet pulp; Pectin; Rhamnogalacturonan; Viscosity; Ferulic acid dehydrodimers; Autoclave extraction; Oxidative cross-linking; Gel formation

1. Introduction

The presence of ferulic acid in sugar beet pectin allows gel formation through oxidative cross-linking of the ferulic acid groups [1] as an alternative for gel formation in an acid-sugar system, or by calcium complexation. Of several oxidising agents, only ammonium persulfate and hydrogen peroxide/peroxidase have been shown to be able to initiate oxidative cross-linking of beet pectins. This reaction increased the molecular weight and viscosity of beet pectins, and eventually a gel was formed [2]. Previous experiments revealed that treatment of an acid extracted sugar beet pectin with ammonium persulfate results in a gradual increase in viscosity, whereas the combination of hydrogen peroxide/peroxidase leads to an instantaneous increase in viscosity [2].

Recently, we described that sequential extraction of pectins from sugar beet pulp by autoclaving yields extracts containing two populations consisting of feruloylated rhamnogalacturonans with highly branched arabinan side-chains (1300 and 120 kDa), a population consisting of homogalacturonans (21 kDa), as well as a population containing highly feruloylated arabinans (10–15 kDa) [3,4]. The first of two sequential extracts (Autoclave 1) contained mainly homogalacturonans, whereas the second (Autoclave 2) consisted predominantly of rhamnogalacturonans [3,4].

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The current study deals with the ability of oxidative cross-linking to increase viscosity and to form gels of the autoclave extracts using hydrogen peroxide/peroxidase or ammonium persulfate. The reactivity of these autoclave extracts is compared with that of acid extracted beet pectin.

2. Experimental

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 [3]. The acid extracted pectin (HP) was a gift from Dr J.-F. Thibault from INRA in Nantes (France) [2]. It had a DF of 5.3 and contained 0.8% ferulic acid and 83.0% polysaccharides (77.4 mol% GalA, 15.2 mol% Gal, 3.8 mol% Rha and 2.5 mol% Ara).

Methods.—Ferulic acid and its dimers were analyzed after saponification and silylation, as described by Ralph et al. [5]. High-performance size-exclusion chromatography (HPSEC) with RI, UV, viscosity, and light-scattering detection was performed as described previously [4]. The intrinsic viscosities [6] were calculated based on the polymeric material. Preparative size-exclusion chromatography was performed on a column (75×2.6 cm) of Sephacryl S 500 (Pharmacia) using a Hiload system (Pharmacia). Samples (0.3 g) were eluted with 0.05 M NaOAc (pH 5.0) at a flow rate of 2.5 mL min^{-1}. Fractions (2.5 mL) were assayed for total neutral sugar [7] and uronic acid [8] content using Ara and GalA as standards, respectively. The presence of ferulic acid was monitored spectrophotometrically at 335 nm [9].

Oxidative cross-linking of the extracts with hydrogen peroxide/peroxidase was performed by adding 10 μL of horseradish peroxidase (0.5 mg mL^{-1}, Sigma) and 10 μL of hydrogen peroxide (0.5 M) to 1 mL of 0.1 M phosphate buffer (pH 6.0, 25 °C) containing various concentrations of beet pectin. Cross-linking with persulfate was performed by adding 10 μL of an ammonium persulfate solution (1 M) to 1 mL of a solution of pectin with various concentrations (25 °C) in distilled water. The relative viscosity of polysaccharide solutions (0.25–4.0% w/v) in 0.1 M phosphate buffer (pH 6.0) before and after oxidative cross-linking was measured using Ubbelohde viscometers [6].

The formation of a gel network of the purified pools after cross-linking was investigated by small amplitude shear strain oscillatory testing using a Bohlin VOR rheometer in oscillatory mode at a frequency of 1 Hz using a torsion bar of 20 g cm (25 °C). Polysaccharide solutions were cross-linked by the addition of the oxidant in the geometry and were measured immediately. A thin layer of soy oil was added to prevent evaporative losses. It was ensured that the strain remained within the linear region of the material.

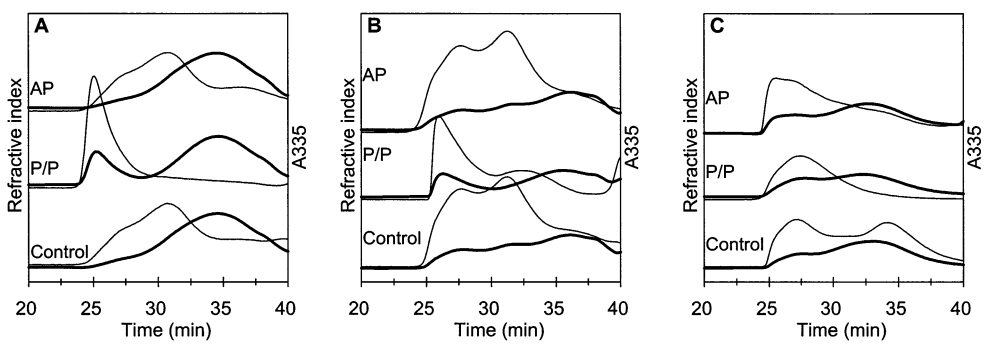

Fig. 1. High-performance size-exclusion chromatography elution patterns of Autoclave 1 (A), Autoclave 2 (B), and HP (C) before and after oxidative cross-linking with hydrogen peroxide/peroxidase (P/P) or ammonium persulfate (AP) for 6 h. Thin line: UV (335 nm), thick line: RI.

Table 1
Apparent molecular weights (M_w), intrinsic viscosities ($[\eta]_w$), and radii of gyration (Rg_w) of the autoclave extracts (0.5% w/v) before and after oxidative cross-linking (6 h) with hydrogen peroxide/peroxidase (P/P) or ammonium persulfate (AP)

Sample	M_w (kDa)	$[\eta]_w$ (dL g^{-1})	Rg_w (nm)
Auto 1	77	0.54	9.9
Auto 1 P/P	1730	0.58	25.0
Auto 1 AP	56	0.58	9.4
Auto 2	295	0.43	13.7
Auto 2 P/P	12250	0.54	54.6
Auto 2 AP	245	0.53	14.1
HP	247	0.96	18.6
HP P/P	2692	1.32	45.9
HP AP	752	1.4	28.4

3. Results and discussion

Molecular size distribution.—The molecular size distribution and ferulic acid distribution of the extracts showed three populations for both autoclave extracts (Fig. 1). In previous research [4] it was found that the two high molecular weight populations (26 and 31 min) contain the rhamnogalacturonans, while the low molecular weight population (35 min) contains both homogalacturonans and neutral arabinans. In contrast to the autoclave extracts, the HP extract contained only two populations; a high-molecular-weight population with a relatively high ferulic acid content and a low-molecular-weight population with a lower ferulic acid content.

Physico-chemical properties of the extracts before and after cross-linking showed that the apparent molecular weight of Autoclave 1 was much lower than that of Autoclave 2 (Table 1). This was caused by the presence of a larger amount of low-molecular-weight homogalacturonans in Autoclave 1 (Fig. 1). The intrinsic viscosities of both autoclave extracts were very low in comparison with pectins obtained by other extraction methods, as described in the literature (1.1–2.3 dL g^{-1}) [10,11]. The current data were confirmed by a series of viscosity measurements using Ubbelohde viscometers (data not shown). The average radius of gyration of Autoclave 2 was higher than that of Autoclave 1 as a result of the presence of higher quantities of high-molecular-weight rhamnogalacturonans in Autoclave 2. The apparent molecular weight of the HP extract was 247 kDa. The intrinsic viscosity of HP (0.96 dL g^{-1}) was higher than that of the autoclave extracts and was comparable with that of acid extracted beet pectins, as described in the literature [10]. Furthermore, the radius of gyration of HP was much higher than that of Autoclave 2, although the apparent molecular weights were similar. An explanation for this is the high degree of branching in the rhamnogalacturonans in Autoclave 2, which results in a more compact molecule.

Oxidative coupling of the extracts.—After cross-linking the extracts (0.5%) with hydrogen peroxide/peroxidase, a high-molecular-weight population was formed (Fig. 1). This population accounted for approximately 25% of the total RI response for Autoclave 1 and for approximately 45% for both Autoclave 2 and HP. A shift of the UV signal towards higher molecular weights was seen for both autoclave extracts. However, a part of the UV signal did not shift during cross-linking. The total UV response decreased after treatment with hydrogen peroxide/peroxidase, which was in agreement with the amount of ferulates recovered (see below). The apparent molecular weight increased 22-fold for Autoclave 1 and 44-fold for Autoclave 2 (Table 1). Only a small shift towards higher molecular size was found for the HP extract. The total UV response decreased significantly. The apparent molecular weight still increased 11-fold. Remarkably, only a small increase in intrinsic viscosities of the autoclave extracts was observed after cross-linking at this concentration, whereas the intrinsic viscosity of HP increased considerably (38%). The high linearity, as indicated by the high intrinsic viscosity, probably causes this effect.

Treatment of the autoclave extracts with ammonium persulfate did not result in a shift towards higher molecular weights within the timespan (20 h) of the experiment (Fig. 1(A) and (B)). The apparent molecular weights of the extracts slightly decreased (Table 1), whereas only small changes in the intrinsic viscosities were observed. In the HP extract cross-linking did occur and a high-molecular-weight population was formed within 2 h (Fig. 1(C)). The apparent molecular weight increased to 752 kDa. Although this increase

was lower than after cross-linking with hydrogen peroxide/peroxidase, the intrinsic viscosity increased to a similar value.

Effect of cross-linking on viscosity in time.— In order to obtain information about the speed of cross-linking, the reactions with hydrogen peroxide/peroxidase and ammonium persulfate were followed in time (Fig. 2). Treatment of a 0.75% solution of the extracts with hydrogen peroxide/peroxidase for 2 h indeed resulted in a fast increase in reduced viscosity [12] for all extracts. Within approximately 10 min the reaction was complete and the reduced viscosity remained stable for the duration of the experiment. The smallest increase in reduced viscosity was found for Autoclave 1 and HP (both to 1.3 times the initial value), whereas the reduced viscosity of Autoclave 2 increased to twice the initial value.

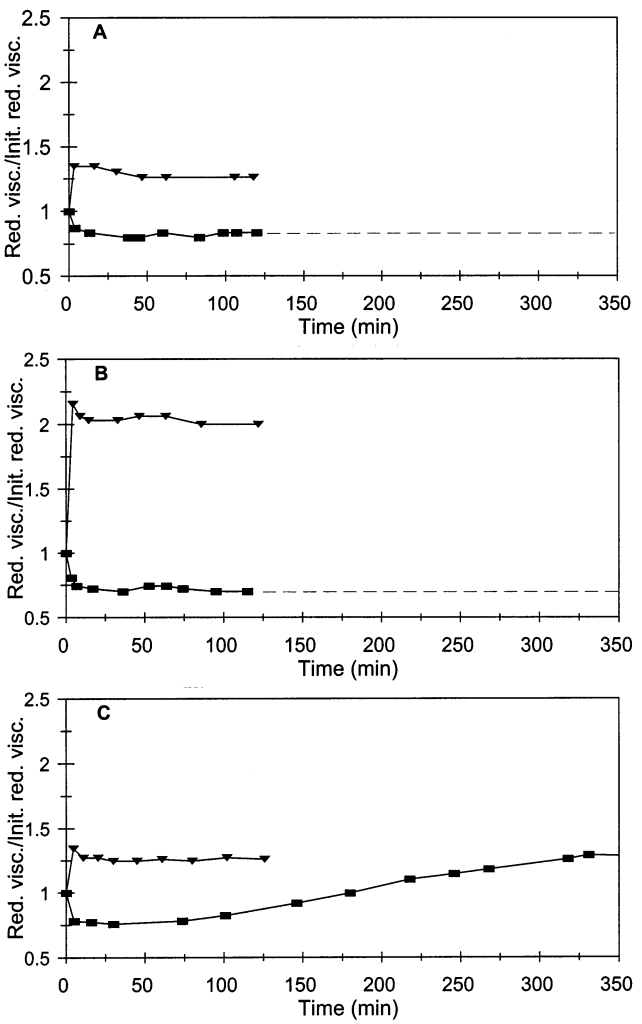

Fig. 2. Changes with time of the ratio of reduced viscosity to initial reduced viscosity for sugar beet pectins (0.75% w/v, 25 °C) after adding hydrogen peroxide/peroxidase (▼) or with ammonium persulfate (■). (A) Autoclave 1; (B) Autoclave 2; (C) HP.

Addition of ammonium persulfate resulted in a rapid decrease in reduced viscosity in Autoclave 1 and 2. Even after 20 h no increase was observed. This is somewhat unexpected since after oxidative cross-linking of the extract at a comparable concentration (0.5%) a small increase in intrinsic viscosity was observed. However, in our study the intrinsic viscosity was solely determined based on the polymeric material after separation on HPSEC, whereas the reduced viscosity was a measure of the total reaction mixture and therefore also included the low molecular weight material. Therefore, a possible explanation might be that both cross-linking and degradation of the polysaccharide occur at the same time. In the HP extract, the reduced viscosity also initially decreased, but this was followed by a slow increase to a maximum of 1.3 times the initial value after 5.5 h. After 40 h, however, the reduced viscosity had decreased again to 70% of the initial value (not shown). We showed that cross-links (dehydrodimers) were formed after 6 h. Since a decrease of the reduced viscosity was also observed, this also implies that some degradation of the polysaccharide must have occurred. These findings are in agreement with data found by Thibault and Rombouts [2], who found an increase in reduced viscosity for an extract similar to the HP extract, followed by a slow decrease. They also found a decrease in reduced viscosity of 25% for (ferulate free) apple pectin upon this treatment, which indicates that persulfate indeed degrades the pectin molecule [2]. Furthermore, the high Ara content of the autoclave extracts may be a reason for the poor cross-linking properties, since Guillon and Thibault [13,14] also found that cross-linking of sugar beet pectins with a high Ara content using ammonium persulfate did not result in an increased viscosity, unless the arabinan side-chains had been removed.

Ferulic acid dehydrodimer composition.— Table 2 shows the ferulate composition of Autoclave 2 and HP. In Autoclave 2 [15] and HP, 9 and 12% of the ferulates were present as dehydrodimers, respectively. This is considerably lower than the values reported for sugar

Table 2
Ferulate composition of the extracts HP and Autoclave 2 (1.0% w/v) before and after cross-linking with hydrogen peroxide/peroxidase or ammonium persulfate for 6 h (mg g^{-1})

Sample (treatment)	Monomer	8-8	8-5	8-O-4	5-5	Total diferulates	Total ferulates
HP (control)	7.58	0.24	0.14	0.50	0.12	1.00	8.59
HP (peroxide)	0.27	0.41	1.90	0.59	0.00	2.90	3.18
HP (persulfate)	1.92	0.50	2.76	1.21	0.41	4.88	6.79
Auto 2 (control) [a]	14.82	0.38	0.30	0.53	0.23	1.43	16.26
Auto 2 (peroxide) [a]	3.30	0.29	3.38	2.34	1.02	7.03	10.33
Auto 2 (persulfate)	12.75	0.46	0.88	0.85	0.27	2.46	15.21

[a] Data from Ref. [15].

beet cell walls (22%) [16] or for other species, e.g. (suspension cultured) corn, cocksfoot, and switchgrass (15–71%) [5,17]. Apparently, material with a relatively low amount of ferulate cross-links is more easily released during autoclaving than material with a high amount of cross-links. 8-O-4 dehydrodimers were predominantly present in both extracts. 4-O-5 dehydrodimers were not detected. The predominance of the 8-O-4 coupled dehydrodimer over other types was in contrast with results found for most grasses in which the 5-5 dehydrodimer prevailed [5].

Cross-linking of Autoclave 2 and HP using hydrogen peroxide/peroxidase resulted in a large decrease of the ferulic acid content and in an increase in the amount of ferulic acid dimers present (Table 2). A loss of $\sim 60\%$ of total ferulates was found for the HP extract, indicating that other oxidative reactions might have occurred or that some ferulates had been lost during analysis. In the Auto 2 extract 36% of the ferulates could not be recovered. The largest increase was found for the 8-5 and 8-O-4 dimers for both extracts.

Ammonium persulfate cross-linking led to a larger decrease of ferulic acid in HP than in Autoclave 2. This was accompanied by a larger increase in dehydrodimers in the HP extract. The loss of total ferulates was smaller than occurred with the reactions with hydrogen peroxide/peroxidase, namely 21% for HP and 6% for Autoclave 2. Using ammonium persulfate the largest increase was also seen for the 8-O-4 and 8-5 dehydrodimers. Since the 8-8 and 5-5 dehydrodimers increased to a limited extent, it can be concluded that these dimers have limited importance in the cross-linking reaction, either with hydrogen peroxide or with ammonium persulfate, as opposed to what has been suggested in previous studies on sugar beet pectins [18] and arabinoxylans [19]. Our results are in agreement with Grabber et al. [17] who showed that oxidative cross-linking of ferulates in maize with hydrogen peroxide/peroxidase is a radical reaction, and that several active sites (C-5, C-8, and O-4) are present on the ferulic acid radicals, resulting in the formation of several reaction products. The abundance of the 8-O-4 and 8-5 dimers is probably caused by the spatial arrangement of the ferulic acid groups on the pectin. Since the reactions with hydrogen peroxide/peroxidase and ammonium persulfate resulted in the formation of the same types of dehydrodimers in similar ratios for both HP and Autoclave 2, it is assumed that the reaction mechanism was similar, although a relatively high amount of ferulates was not analyzed after cross-linking.

An increase in the reduced and intrinsic viscosity was observed, together with the formation of dehydrodimers for all the samples, which were analyzed for their dehydrodimer composition, except for Autoclave 2 after treatment with ammonium persulfate, which showed a slight decrease in reduced viscosity. It is possible that this could be related to the location of the ferulic acid, which is restricted to the ends of the relatively linear pectin chain in the acid extracted beet pectin [20] and distributed throughout the branched rhamnogalacturonan molecule in the extract obtained by autoclaving. Furthermore, it might be caused by a difference in the initial release of free radicals, since the reaction with hydrogen

peroxide/peroxidase occurs much more rapidly than the reaction with ammonium persulfate. Also, the high amount of Ara may play a role, as was also suggested by Guillon and Thibault [13,14].

Since the treatment of the autoclave extracts with hydrogen peroxide/peroxidase resulted in the largest increase in viscosity and in gel formation, further experiments were performed with hydrogen peroxide/peroxidase.

Preparative size-exclusion chromatography.—The cross-linked Autoclave 2 extract was applied onto a Sephacryl S 500 size-exclusion column (Fig. 3, Table 3). Two populations could be distinguished. The first is a high-molecular-weight population, which is formed by cross-linking, is rich in ferulic acid, and has a composition comparable to that of the 'hairy fragments' from sugar beet pectin [21] (pool I). The second population is a low-molecular-weight population consisting mainly of homogalacturonans (pool II). These findings show that for the autoclave extracts only arabinan/galactan side-chains of the rhamnogalacturonans and possibly (free) arabinans participate in cross-linking and that very little homogalacturonan is linked to these rhamnogalacturonans. This in contrast to the cross-linked fraction of acid extracted beet pectins, which contained neutral sugars and GalA in a ratio of approximately 1:2, suggesting that the rhamnogalacturonans are still linked to the homogalacturonans [2].

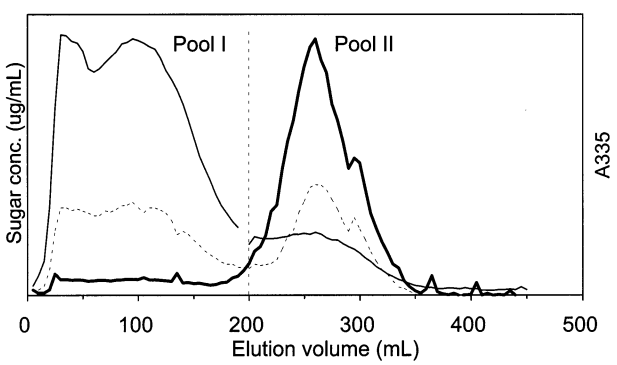

Fig. 3. Size-exclusion chromatography (S 500) of Autoclave 2 (0.5% w/v) after cross-linking with hydrogen peroxide/peroxidase. Thick line: GalA, thin line: UV (335 nm), dashed line: neutral sugars.

Table 3
Sugar composition of the pools obtained by preparative size-exclusion chromatography (S 500) of Autoclave 2 (0.5% w/v) after oxidative cross-linking with hydrogen peroxide/peroxidase

	Pool I	Pool II
Rha [a]	2.7	1.0
Ara	76.2	26.9
Xyl	0.5	0.4
Man	0.7	1.9
Gal	10.8	3.7
Glc	2.4	1.7
GalA	7.0	64.5
Relative weight [b]	36.7	63.3

[a] Expressed as molar percentage.
[b] Expressed as weight percentage of the polysaccharides recovered.

Influence of the concentration of the extracts on the cross-linking reaction.—The influence of the concentration of the extracts on the relative viscosity of the extracts before and after cross-linking with hydrogen peroxide/peroxidase is presented in Fig. 4. At higher concentrations some overestimation of the relative viscosities may have occurred due to non-Newtonian behaviour in the Ubbelohde viscometers [22]. Of the controls the largest increase in relative viscosity with increasing concentration was found for HP, due to its higher intrinsic viscosity and radius of gyration.

Upon oxidative cross-linking with hydrogen peroxide/peroxidase a clear increase in the relative viscosity compared with the control was observed at a concentration of 1.5% for Autoclave 1, at 0.75% for Autoclave 2 and at 1% for HP. Gels were formed at concentrations between 1.0 and 1.5% for Autoclave 2 and the HP extract. Autoclave 2 seems to form a gel at a somewhat lower concentration than HP. Autoclave 1 formed a gel at a concentration between 2 and 4%. Therefore, it can be concluded that although HP has the highest intrinsic viscosity, Autoclave 2 showed the largest increase in relative viscosity and formed a gel at the lowest concentration, which is probably caused by the high ferulic acid content of Autoclave 2.

Gelation.—The formation of a gel network of the sugar beet pectins was investigated by small amplitude shear strain oscillatory testing. Fig. 5 shows the development of the

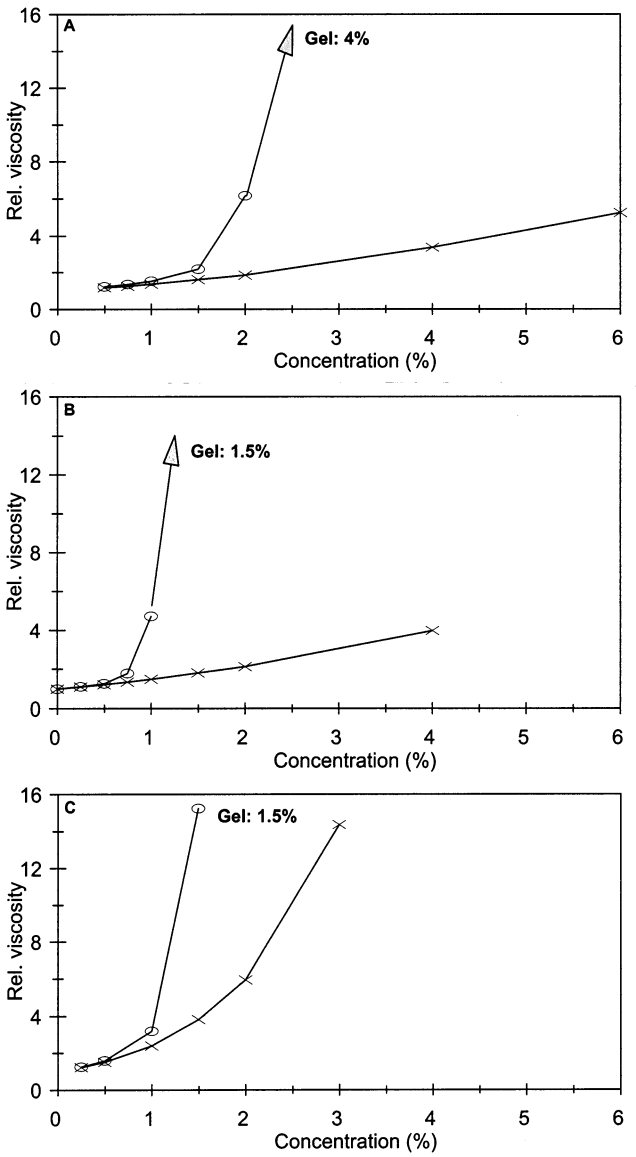

Fig. 4. Increase in relative viscosity with increasing concentration of sugar beet pectins before (x) and after cross-linking (○) with hydrogen peroxide/peroxidase (15 min, 25 °C). (A) Autoclave 1; (B) Autoclave 2; (C) HP.

storage modulus (G'), which is indicative of the amount of elastically effective cross-links formed and of the stiffness of the gel at various concentrations of the extracts in time. G' rapidly increased in time for all concentrations (Fig. 5). The G' and also the initial speed of gel formation increased with increasing concentration. A comparison of all samples could only be made at a concentration of 3.0%. The highest values for G' at this concentration were found for autoclave 2 and to a lesser extent for HP. A much lower value was found for Autoclave 1. Comparing Autoclave 2 and HP, we found a higher G' value for Autoclave 2 at a concentration of 3%, whereas the HP extract had a higher increase in intrinsic viscosity after cross-linking at 0.5%. This is probably related to the higher radius of gyration of HP. This increases the probability of intermolecular cross-linking at concentrations lower than the coil overlap concentration ($C^* \approx 1\%$ for HP and 2% for Autoclave 2). At concentrations higher than the coil overlap concentration, the amount of ferulic acid and the placement are more important. Since Autoclave 2 has a higher ferulic acid content, and the ferulic acid is linked to flexible side-chains along the whole molecule, whereas most of the ferulic acid in the acid extracted pectin is linked to the relatively short galactan side-chains, the amount of cross-links possible per molecule is higher in Autoclave 2 and more

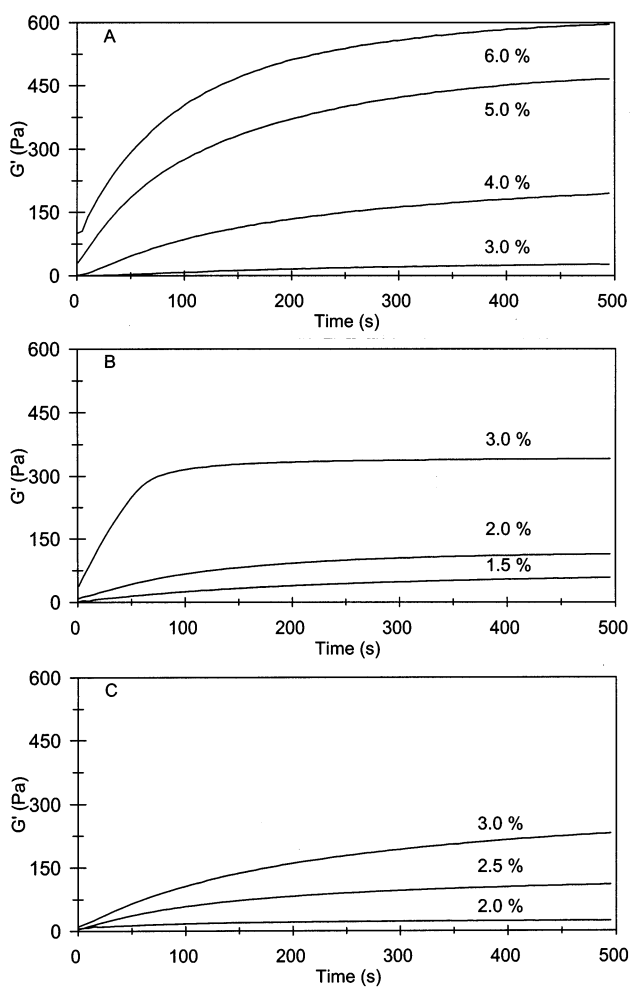

Fig. 5. Storage (G') moduli vs. time for sugar beet pectins at various concentrations treated with hydrogen peroxide/peroxidase (25 °C). (A) Autoclave 1; (B) Autoclave 2; (C) HP.

Table 4
Storage (G') and loss (G'') moduli and tan δ for gels from sugar beet pectin after oxidative cross-linking with hydrogen peroxide/peroxidase after 500 s

Sample	Concentration (%)	G' (Pa)	G'' (Pa)	tan δ (−)
Autoclave 1	3.0	27	1.5	0.055
	4.0	195	8.4	0.043
	5.0	466	11.9	0.026
	6.0	596	15.5	0.026
Autoclave 2	1.5	58	0.8	0.014
	2.0	114	0.4	0.003
	3.0	341	2.2	0.006
HP	2.0	23	0.6	0.025
	2.5	110	0.2	0.001
	3.0	230	0.3	0.001

molecules are linked together. This results in a more rigid gel at higher concentrations for Autoclave 2. The values for tan δ ($= G''/G'$; where $G'' =$ indicative for the relaxation of cross-links in the gel) are shown in Table 4. A low value for tan δ (< 0.1) is indicative of an elastic system, whereas high values (> 1) imply a more liquid-like character of the network [23]. The very low values of tan δ for HP and Autoclave 2 shows the formation of elastic networks, especially at higher concentrations, whereas the nature of the Autoclave 1 network is somewhat more liquid-like (see Table 4). The low values of tan δ, indicative of the absence of facile relaxation of cross-links, is probably caused by the covalent nature of the cross-links, which do not easily allow relaxation.

4. Concluding remarks

Despite large structural differences, it was shown that pectins obtained by acid extraction and autoclaving could form a gel after oxidative cross-linking using polysaccharide concentrations as low as 1.5%. A rhamnogalacturonan gel with only 7% of GalA in the cross-linked population was formed with the Autoclave 2 extract, whereas it was shown that a gel with more than 60% of GalA in the cross-linked fraction was formed with an extract similar to HP [2]. The properties of the gels, as indicated by tan δ, were in a similar range for both autoclave 2 and HP.

It was found that hydrogen peroxide/peroxidase is a better cross-linking agent than ammonium persulfate for the pectins described, since addition of the latter led to degradation of the pectin to some extent. Nevertheless, the reduced viscosity increased for HP after addition of persulfate, followed by a gradual decrease upon standing. The reduced viscosity of the autoclave extracts did not increase after addition of persulfate.

The total amount of dehydrodimers was lower for the ammonium persulfate cross-linked material. For both oxidative systems, the 8-5 dehydrodimer was predominant after cross-linking.

The low intrinsic viscosity and good solubility of the extracts obtained by autoclaving will facilitate the handling (e.g., pumping) of the extracts in industrial applications. This makes them an interesting substrate for in situ gel formation. Future research will focus on the purification and oxidative cross-linking of the rhamnogalacturonans and arabinans from the Autoclave 2 extract.

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References

[1] F.M. Rombouts, J.F. Thibault, in M.L. Fishman, J.J. Jen (Eds.), *Chemistry and Function of Pectins*, *ACS Symp. Ser. 310*, American Chemical Society, Washington, DC, 1986, pp. 49–60.

[2] J.F. Thibault, F.M. Rombouts, *Carbohydr. Res.*, 154 (1986) 205–215.
[3] A. Oosterveld, G. Beldman, H.A. Schols, A.G.J. Voragen, *Carbohydr. Res.*, 288 (1996) 143–153.
[4] A. Oosterveld, G. Beldman, H.A. Schols, A.G.J. Voragen, *Carbohydr. Res.* this issue.
[5] J. Ralph, S. Quideau, J.H. Grabber, R.D. Hatfield, *J. Chem. Soc. Perkin Trans. 1*, (1994) 3485–3498.
[6] E. Dickinson, *An Introduction to Food Colloids*, Oxford University Press, Oxford, 1992, pp. 51–78.
[7] M. Tollier, J. Robin, *Ann. Technol. Agric.*, 28 (1979) 1–15.
[8] J.F. Thibault, *Lebensm.-Wiss. Technol.*, 21 (1979) 247–251.
[9] M.-C. Ralet, J.-F. Thibault, C.B. Faulds, G. Williamson, *Carbohydr. Res.*, 263 (1994) 227–241.
[10] N. Arslan, *J. Food Sci. Technol.*, 32 (1995) 381–385.
[11] M.L. Fishman, D.T. Gillespie, S.M. Sondey, Y.S. El-Atawy, *Carbohydr. Res.*, 215 (1991) 91–104.
[12] J.-F. Thibault, *Carbohydr. Polym.*, 8 (1988) 209–223.
[13] F. Guillon, J.F. Thibault, *Carbohydr. Polym.*, 12 (1990) 353–374.
[14] F. Guillon, J.F. Thibault, *Food Hydrocoll.*, 1 (1987) 547–549.
[15] A. Oosterveld, J.H. Grabber, G. Beldman, J. Ralph, A.G.J. Voragen, *Carbohydr. Res.*, 300 (1997) 179–181.
[16] K.W. Waldron, A. Ng, M.L. Parker, A.J. Parr, *J. Sci. Food Agric.*, 74 (1997) 221–228.
[17] J.H. Grabber, R.D. Hatfield, J. Ralph, J. Zon, N. Amrhein, *Phytochemistry*, 40 (1995) 1077–1082.
[18] J.F. Thibault, C. Garreau, D. Durand, *Carbohydr. Res.*, 163 (1987) 15–27.
[19] T. Geismann, H. Neukom, *Lebensm.-Wiss. Technol.*, 6 (1973) 59–62.
[20] A. Oosterveld, Pectic substances from sugar beet pulp: structural features, enzymatic modification, and gel formation, PhD thesis, Wageningen University, Wageningen, 1997, pp. 91–106 (Chapter 7).
[21] F. Guillon, J.-F. Thibault, *Lebensm.-Wiss. Technol.*, 21 (1988) 198–205.
[22] M.A.V. Axelos, J. Lefebvre, C.-G. Qiu, M.A. Rao, in R.H. Walter (Ed.), *The Chemistry and Technology of Pectin*, Academic Press, San Diego, CA, 1991, pp. 227–250.
[23] S.B. Ross-Murphy, in H.W.-S. Chan (Ed.), *Biophysical Methods in Food Research*, Blackwell, Oxford, 1984, pp. 138–199.