ELSEVIER

Carbohydrate Polymers 48 (2002) 73–81

Carbohydrate Polymers

www.elsevier.com/locate/carbpol

# Enzymatic modification of pectic polysaccharides obtained from sugar beet pulp

Alexander Oosterveld, Gerrit Beldman, Alphons G.J. Voragen*

*Laboratory of Food Chemistry, Department of Agrotechnology and Food Science, Wageningen University, P.O. Box 8129, 6700 EV Wageningen, The Netherlands*

Received 29 September 2000; revised 18 December 2000; accepted 14 March 2001

**Abstract**

Rhamnogalacturonans and arabinans, purified from an autoclave extract of sugar beet pulp, as well as an acid extracted beet pectin (ABP) were treated with enzymes in order to modify their physico-chemical properties. The enzymes used were arabinofuranosidase B (AF), endo-arabinanase plus arabinofuranosidase (EA + AF), rhamnogalacturonase plus rhamnogalacturonan acetyl esterase (RGase + RGAE), and polygalacturonase plus pectin methyl esterase (PG + PE). During the enzyme treatments the intrinsic viscosity ($[\eta]_w$), apparent molecular weight ($M_w$), and radius of gyration ($Rg_w$) of the polysaccharides were monitored.

We found in most cases that treatment with glycanases decreased the $[\eta]_w$ of the polysaccharides investigated. However, results showed that degradation of the rhamnogalacturonan backbone of a beet pectin obtained by acid extraction had little influence on the $[\eta]_w$ which led to the conclusion that the rhamnogalacturonans present in this type of pectin are mainly located at the extremities of the pectin molecules. Some evidence was found that the rhamnogalacturonans obtained by autoclaving are linked through the arabinose side-chains, probably by diferulic acid cross-links. Removal of the side-chains of the arabinans present in the rhamnogalacturonans of ABPs with enzymes also showed that the arabinan side-chains contribute relatively little to the $[\eta]_w$.

In our experiments we found that modification with glycanases can be used to change the structural characteristics of pectic polysaccharides without a significant loss of viscosity. In this way glycanases can be used for the removal of structural elements of pectic polysaccharides, which limit their physico-chemical properties. 

*Keywords*: Sugar beet pulp; Pectins; Rhamnogalacturonans; Enzymatic modification

## 1. Introduction

The structural characteristics of pectic polysaccharides from sugar beet have been the subject of many studies (Colquhoun, Ralet, Thibault, Faulds & Williamson, 1994; Guillon & Thibault, 1988, 1989a, 1989b; Oosterveld, Beldman, Schols & Voragen, 1996, 2000a; Ralet, Thibault, Faulds & Williamson, 1994; Rombouts & Thibault, 1986; Thibault, Guillon & Rombouts, 1991; Thibault, Renard, Axelos, Roger & Crépeau, 1988). Also several studies have been attended towards the physico-chemical properties of these polysaccharides (Faulds & Williamson, 1990; Fishman, Gillespie, Sondey & El-Atawy, 1991; Hwang & Kokini, 1991; Matthew, Howson, Keenan & Bolton, 1990; Thibault, 1986). However, relatively little is known about the relationship between their structure and physico-chemical properties.

Due to their specificity, enzymes are appropriate tools to modify the structure of pectins. In this way information can be obtained about the relation between chemical structure and physical characteristics. The knowledge acquired can be used to produce tailor made polysaccharides with specific (physico-chemical) properties.

Several studies have been carried out, addressing the enzymatic modification of beet pectins. Some of these studies focused on the enzymatic removal of acetyl groups from acid extracted sugar beet pectins. As a result the gelling properties of these pectins improved (Faulds and Williamson, 1990; Matthew et al., 1990; Oosterveld, Beldman, Searle-van Leeuwen & Voragen, 2000b; Pippen, McCready & Owens, 1950). McCleary et al. showed that arabinans extracted from sugar beet pulp could be used e.g. as fat replacer after debranching with the enzyme arabino-furanosidase (Cooper, McCleary, Morris, Richardson, Marrs & Hart, 1992; McCleary, Cooper & Williams, 1989). An improvement of the gel formation of pectins from sugar beet pulp through oxidative cross-linking with ammonium persulfate after pretreatment with the

* Corresponding author. Tel.: +31-317-48-3209; fax: + 31-317-48-4893.

*E-mail address:* fons.voragen@chem.fdsci.wag-ur.nl (A.G.J. Voragen).

0144-8617/02/$ - see front matter 
PII: S0144-8617(01)00216-8

same enzyme was found by Guillon and Thibault (1987, 1990)).

The current study deals with the elucidation of the chemical structure and the modification of the physicochemical properties of three types of pectic polysaccharides obtained from sugar beet pulp by using specific enzymes. The polysaccharides used were rhamnogalacturonans and arabinans obtained from beet pulp by autoclaving (Oosterveld et al., 1996, 2000a), and a commercially available acid extracted pectin, mainly consisting of homogalacturonans.

## 2. Experimental

### 2.1. Materials

Wet beet pulp (8.9 % dry weight) was obtained from CSM Suiker bv (Breda, the Netherlands). Pectins were extracted by autoclave treatment as described previously (Oosterveld et al., 1996). For the experiments described in this manuscript the extract named Autoclave 2 was used. The acid extracted pectin obtained from sugar beet pulp (ABP) was a gift from the Copenhagen Pectin Factory Ltd.

### 2.2. Analytical methods

The uronic acid contents of the extracts were 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) as described previously (Oosterveld et al., 1996). The degrees of methylation (DM) and acetylation (DA) were determined 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, using a molar extinction coefficient of 31,600 (Rombouts & Thibault, 1986).

### 2.3. 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., 2000a) 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, intrinsic viscosities and radii of gyration were calculated using the light-scattering module of the Trisec software (Viscotek).

Preparative size-exclusion chromatography was performed as described previously (Oosterveld et al., 2000a).

Preparative anion-exchange chromatography was performed on a column (15 × 10 cm) of Source Q (Pharmacia) using a Biopilot system (Pharmacia). The sample (~0.4 g) was applied onto the column at a flow rate of 15 ml/min (0.005 M NaOAc, pH 5.0). After 1575 ml the flow rate was increased to 80 ml/min and a linear gradient to 2 M NaOAc (pH 5.0) was applied for 7200 ml to elute the polysaccharides. Subsequently, the elution was proceeded using elution with 1200 ml 2 M NaOAc. 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). Fractions (90 or 356 ml) were assayed for total neutral sugar (Tollier & Robin, 1979) and uronic acid (Thibault, 1979) content using arabinose and galacturonic acid as standards. A correction was made for the response of uronic acids in the neutral sugar test. Pooled fractions were dialyzed against distilled water (estimated molecular weight cut-off for polysaccharides ~2000 Da) and freeze-dried.

### 2.4. Enzymatic modification

The pools obtained by DEAE anion-exchange chromatography and preparative size-exclusion chromatography were treated with the following purified enzymes: arabinofuranosidase B (AF) (Rombouts, Voragen, Searle-van Leeuwen, Gereads, Schols & Pilnik, 1988), AF plus endo-arabinanase (EA) (Rombouts et al., 1988), endo-polygalacturonase (PG) (Pasculli, Gereads, Voragen & Pilnik, 1991) combined with pectin methyl esterase (PE) (Baron, Rombouts, Drilleau & Pilnik, 1980), and rhamnogalacturonase (RGase) (Schols, Gereads, Searle-van Leeuwen, Kormelink & Voragen, 1990) in combination with rhamnogalacturonan acetyl esterase (RGAE) (Searle-van Leeuwen, van de Broek, Schols, Beldman & Voragen, 1992).

All pools were dissolved in 0.04 M sodium acetate buffer containing 0.01% of $NaN_3$ (pH 5.0) to a final concentration of 5 mg/ml. Enzymes were added to a final concentration of 1 μg of protein/ml. Incubations were carried out at 20°C for 42 h. The digests were analyzed at several stages of the incubation by HPSEC.

## 3. Results and discussion

### 3.1. Composition of the substrates

#### 3.1.1. Pectins from an autoclave extract

Two rhamnogalacturonan fractions and an arabinan fraction were purified from an autoclave extract of sugar beet pulp by preparative size-exclusion chromatography and characterized as described previously (Table 1) (Oosterveld et al., 2000a). Additionally the degree of feruloylation (DF) of these substrates was determined, as well as the apparent molecular weight ($M_w$), intrinsic viscosity ($[\eta]_w$), and radius of gyration ($Rg_w$) (Table 2).

Three fractions are distinguished (see also Table 1). Pool I mainly contains rhamnogalacturonans with a very high $M_w$

Table 1
Sugar compositions (mol%) of the pools of the extracts obtained by autoclaving and of the acid extract

| | ABP[a] | Autoclave | | | | |
|---|---|---|---|---|---|---|
| | | Pool I[b] | Pool II[b] | Pool III[b] | Pool IIIA | Pool IIIB |
| Rha[c] | 5.4 | 6.8 | 5.3 | 1.5 | 0.1 | 3.8 |
| Ara[c] | 7.1 | 58.8 | 51.5 | 36.9 | 91.0 | 19.3 |
| Xyl[c] | 1.2 | 0.8 | 0.5 | 0.4 | 0.1 | 1.0 |
| Man[c] | 2.0 | 0.0 | 0.7 | 1.9 | 2.2 | 3.3 |
| Gal[c] | 11.2 | 13.2 | 9.9 | 3.2 | 0.9 | 7.7 |
| Glc[c] | 0.4 | 0.6 | 0.4 | 1.3 | 2.5 | 2.9 |
| UA[c] | 72.9 | 19.9 | 31.8 | 54.9 | 3.2 | 62.0 |
| DA[d] | 15.3 | 51.1 | 56.3 | 25.8 | nd | 25.6 |
| DM[d] | 57.7 | 28.8 | 33.4 | 56.0 | nd | 60.5 |
| DF[e] | 1.2 | 1.7 | 1.4 | 0.9 | 1.0 | 0.8 |

[a] Molar sugar composition from (Oosterveld et al., 2000b).
[b] Molar sugar composition from (Oosterveld et al., 2000a). nd not determined.
[c] Expressed as mol%.
[d] Expressed as moles acetyl or methyl per 100 moles of galacturonic acid.
[e] Expressed as moles ferulic acid per 100 moles of galactose + arabinose.

(1020 kDa, Table 2). The pool is highly acetylated and feruloylated. The DM is relatively low. The $[\eta]_w$ of this pool was relatively low for a molecule with such a high $M_w$, what can be explained by the high degree of branching.

In pool II rhamnogalacturonans with an apparent molecular weight of 225 kDa and with a relatively low intrinsic viscosity are predominantly present. Except for the galacturonic acid content, its composition is similar to that of pool I. The DF is somewhat lower than that of pool I.

Table 2
Apparent molecular weight ($M_w$, kDa), intrinsic viscosity ($[\eta]_w$, dl/g), and radius of gyration ($Rg_w$, nm) of ABP and pools obtained from the autoclave extract, before and after treatment with various enzyme combinations for 42 h (nd not determined)

| | | ABP | Autoclave | | |
|---|---|---|---|---|---|
| | | | Pool I | Pool II | Pool IIIA |
| Blank | $M_w$ | 271 | 1020 | 225 | 23 |
| | $[\eta]_w$ | 4.54 | 0.93 | 0.66 | 0.20 |
| | $Rg_w$ | 33.1 | 30.8 | 15.6 | 5.2 |
| Rgase + RGAE | $M_w$ | 112 | 157 | 60 | 18 |
| | $[\eta]_w$ | 4.01 | 0.52 | 0.45 | 0.18 |
| | $Rg_w$ | 22.7 | 12.7 | 9.2 | 4.8 |
| AF | $M_w$ | nd | 712 | 131 | 9 |
| | $[\eta]_w$ | nd | 0.99 | 0.67 | 0.18 |
| | $Rg_w$ | nd | 28.4 | 14.2 | 3.8 |
| EA + AF | $M_w$ | 207 | 99 | 126 | 4 |
| | $[\eta]_w$ | 4.24 | 0.79 | 0.42 | 0.11 |
| | $Rg_w$ | 29.2 | 14.0 | 12.2 | 2.3 |
| PG + PE | $M_w$ | 5 | 735 | 156 | nd |
| | $[\eta]_w$ | 0.12 | 0.83 | 0.46 | nd |
| | $Rg_w$ | 2.5 | 26.4 | 12.3 | nd |

Pool III is a mixture of homogalacturonans and arabinans with low $M_w$ as determined by light-scattering (~20 kDa). This fraction is highly methylated, while the DA and DF are low. The arabinans and homogalacturonans present in pool III were separated by Source Q anion-exchange chromatography (Fig. 1). Pool IIIA consisted almost exclusively of arabinans, with a high ferulic acid content. The composition of this arabinan population was similar to the neutral fraction of the autoclave extract as obtained after DEAE anion-chromatography (Oosterveld et al., 2000a). Most of the galacturonic acid eluted in pool IIIB, together with 38 mol% of neutral sugars. This fraction had a high DM, whereas the DA was lower than in pool I and pool II.

### 3.1.2. Acid extracted sugar beet pectin (ABP)

The ABP consist of 72.9 mol% of galacturonic acid and galactose was the predominant neutral sugar (Oosterveld, et al., 2000b). Substantial amounts of both methyl esters and acetyl groups were present. The DF was 1.2, but since this is calculated based on the amount of galactose plus arabinose, the absolute amount of ferulic acid was relatively low. The composition of ABP was comparable to other ABPs described in the literature (Michel, Thibault, Mercier, Heitz & Pouillaude, 1985; Phatak, Chang & Brown, 1988).

### 3.2. Effect of enzymatic modification

Pool I, II, and IIIA of the autoclave extract, as well as ABP, were subjected to the following combinations of enzymes: AF; RGase + RGAE, AF + EA, and PG + PE. During enzymatic treatment for 42 h, changes in the apparent molecular weight ($M_w$), intrinsic viscosity ($[\eta]_w$), and radius of gyration ($Rg_w$) were monitored in time, as shown in Figs. 2–5. The values of these parameters after 42 h are shown in Table 2. The homogalacturonan pool IIIB was not subjected to enzymatic modification, because of its low $M_w$, which indicates that this pool was severely degraded during autoclave treatment. ABP was used as an example of a beet pectin with relatively intact homogalacturonan regions.

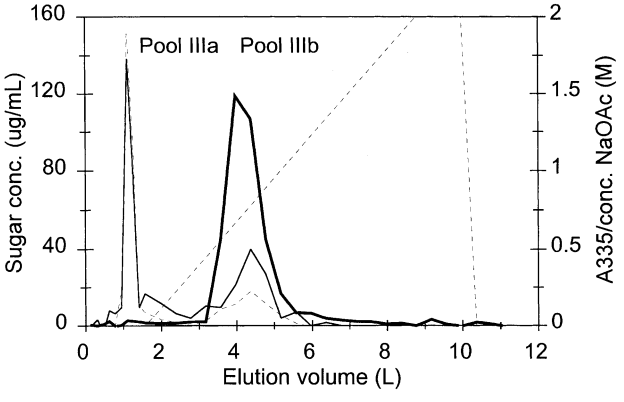


Fig. 1. Preparative anion-exchange chromatography (Source Q) of pool III obtained by S 500 size-exclusion chromatography. Thick line: uronic acid; dashed line: neutral sugars; thin line: $A_{335}$; dotted line: concentration NaOAc.

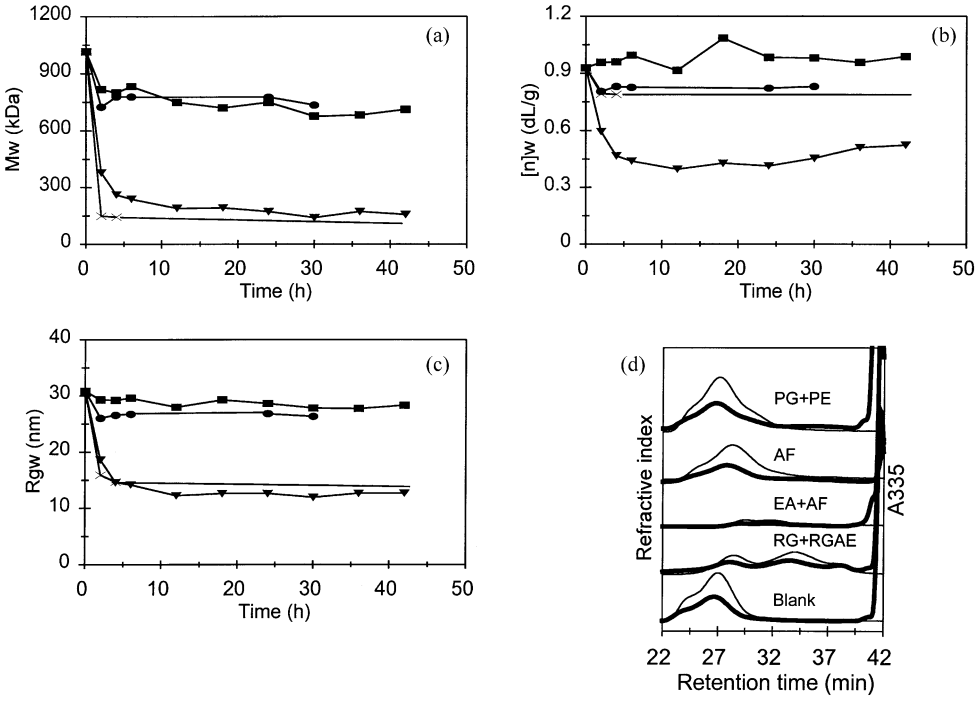


Fig. 2. Effect of enzymatic modification of pool I of the autoclave extract on $M_w$ (A), $[\eta]_w$ (B), and $Rg_w$ (C) in time, and on the HPSEC elution pattern (after 42 h; D). ■: AF; ×: EA + AF; ▼: RGase + RGAE; ●: PG + PE. (D) Thick line: refractive index; thin line $A_{335}$.

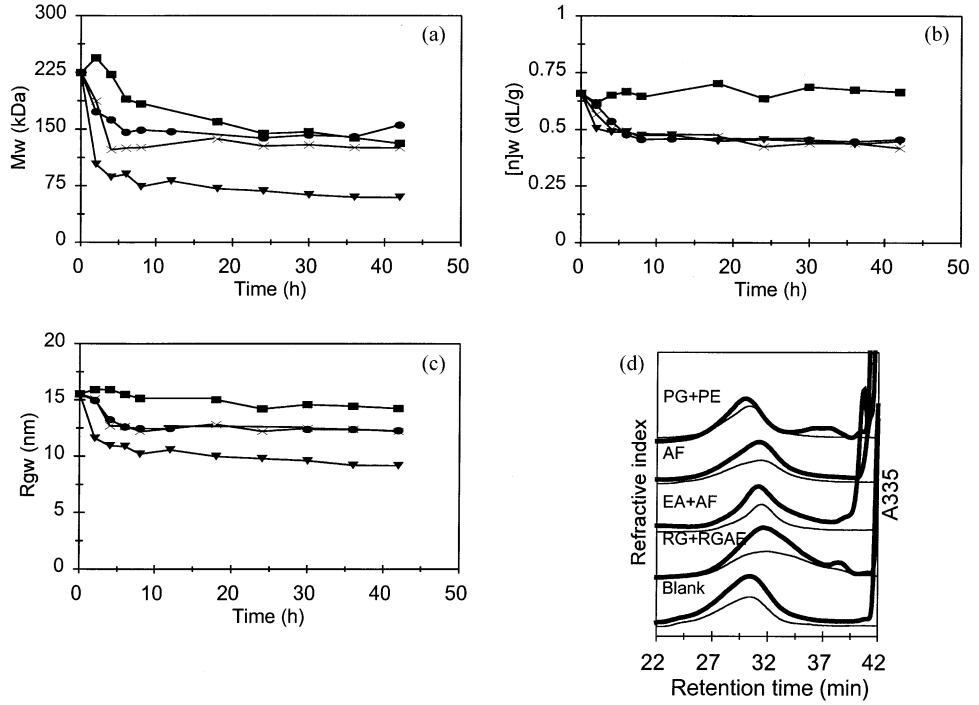


Fig. 3. Effect of enzymatic modification of pool II of the autoclave extract on $M_w$ (A), $[\eta]_w$ (B), and $Rg_w$ (C) in time, and on the HPSEC elution pattern (after 42 h; D). ■: AF; ×: EA + AF; ▼: RGase + RGAE; ●: PG + PE. (D) Thick line: refractive index; thin line $A_{335}$.

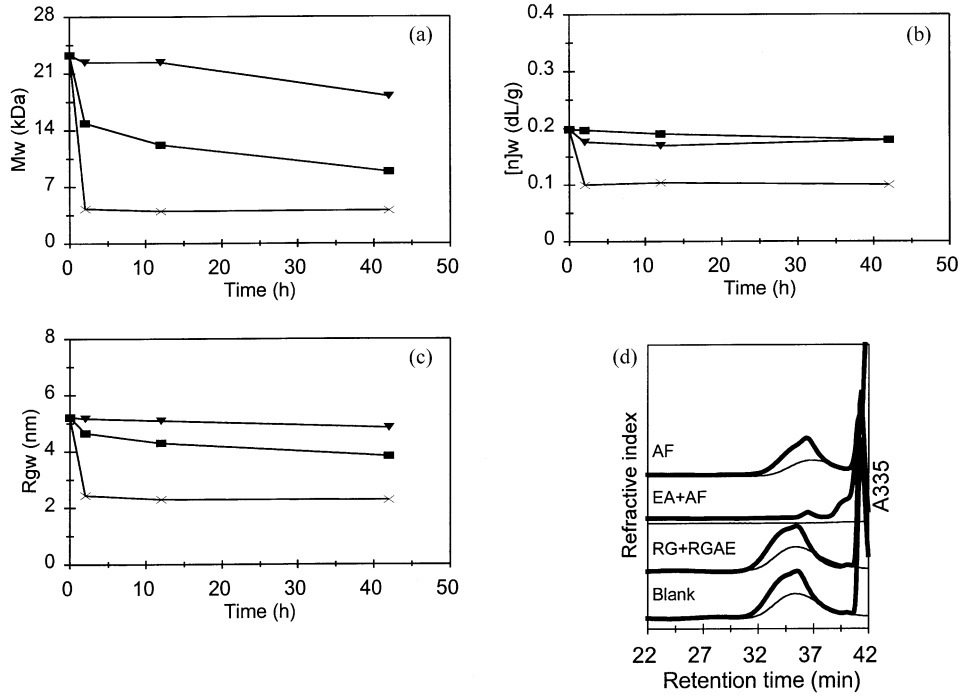


Fig. 4. Effect of enzymatic modification of pool IIIa of the autoclave extract on $M_w$ (A), $[\eta]_w$ (B), and $Rg_w$ (C) in time, and on the HPSEC elution pattern (after 42 h; D). ■: AF; ×: EA + AF; ▼: RGase + RGAE. (D) Thick line: refractive index; thin line $A_{335}$.

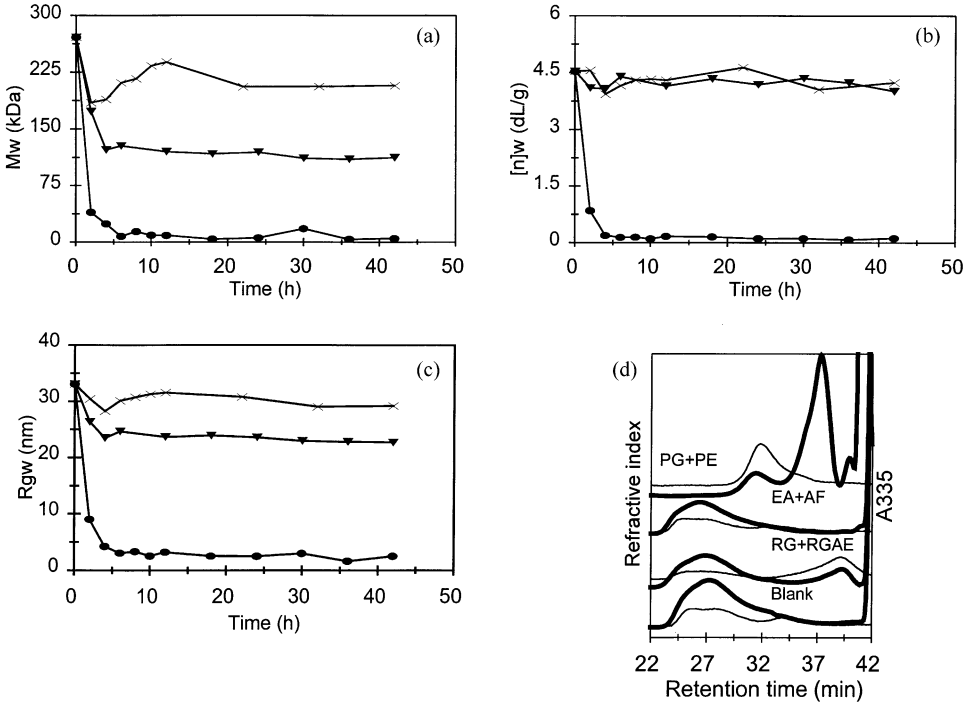


Fig. 5. Effect of enzymatic modification of ABP on $M_w$ (A), $[\eta]_w$ (B), and $Rg_w$ (C) in time, and on the HPSEC elution pattern (after 42 h; D). ×: EA + AF; ▼: RGase + RGAE; ●: PG + PE. (D) Thick line: refractive index; thin line $A_{335}$.

### 3.3. Effect of enzymatic modification on pool I of the autoclave extract

The changes in $M_w$, $[\eta]_w$, and $Rg_w$ during enzyme treatment of pool I of the autoclave extract are shown in Fig. 2a–c, while the HPSEC elution patterns before and after enzymatic treatment are shown in Fig. 2d.

Treatment of pool I of the autoclave extract with RGase + RGAE caused a rapid decrease of the $M_w$ from 1020 to 157 kDa. Moreover, the $[\eta]_w$ and the $Rg_w$ decreased approximately by a factor 2 and an additional population with a high ferulic acid content appeared upon HPSEC (34 min). The $M_w$ of this new pool was approximately 50 kDa, whereas its $[\eta]_w$ was approximately 0.37 dl/g. Since RGase is known to degrade the more linear rhamnogalacturonan backbone only substituted with single unit galactose residues, the material which remains is assumed to consist of the rhamnogalacturonan subunit highly branched with arabinose.

The addition of AF to pool I reduced the $M_w$ to 712 kDa. Interestingly, this resulted in a small increase of the $[\eta]_w$, whereas the $Rg_w$ decreased slightly. As a consequence of the lower $Rg_w$, this pool eluated somewhat later upon HPSEC. Apparently a significant part of the arabinose can be removed from this pectin, without a loss (and in fact a small increase) of the $[\eta]_w$. AF is known to be active both on the terminal arabinofuranosyl residues in side-chains and on the non-reducing end of the arabinan backbone. Since the arabinan side-chains are present in much higher amounts than the non-reducing ends, AF degrades the side-chains in the arabinans faster than the backbone (Voragen, Rombouts, Searle-van Leeuwen, Schols & Pilnik, 1987). Because only part of the arabinose had been removed, considering the $M_w$ after treatment with AF, it is assumed that mainly the side-chains of the arabinans had been removed and that these contribute little to the intrinsic viscosity of the rhamnogalacturonans. In the HPSEC chromatogram, the ratio $A_{335}$: RI increased after treatment with AF, which indicates that feruloylated arabinose is not removed, or with less preference than the non-feruloylated arabinose. Apparently, AF is hindered by the ferulic acid residues. Another possible explanation is the observation that the galactose residues in sugar beet pulp have a higher degree of feruloylation than the arabinose residues (Ralet et al., 1994).

Hydrolysis of the arabinan side-chains with EA + AF showed a decrease in $M_w$ to 99 kDa. The $[\eta]_w$ of this population remained relatively high, apparently due to the fact that the backbone of the rhamnogalacturonan was not degraded by EA + AF. Since 58.8 mol% of this pool consisted of arabinose, which was present as side-chain of the rhamnogalacturonan, and since mono- and oligomers were not included in the calculation of the $M_w$, a maximum decrease in $M_w$ of approximately 60% was expected. Therefore, the resulting low $M_w$ after treatment with EA + AF is striking and might be ascribed to the degradation of the backbone of arabinans that participate in cross-links between two different rhamnogalacturonan molecules in the original material. Diferulic acid bridges are present in the autoclave extract (Oosterveld, Grabber, Beldman, Ralph & Voragen, 1997), and are able to link multiple molecules together. ~10% of the ferulates present in the original autoclave extract is present as dimer (Oosterveld et al., 1997). Since the $M_w$ of pool I is ~1000 kDa, a degree of polymerization can be estimated of 6700. About 70 mol% of the sugars is made up by arabinose and galactose and the ferulic acid content was 1.7 molecule per 100 molecules of arabinose and galactose. From this it can be calculated that on average every molecule contains four diferulates, which shows that cross-linking might occur to some extent. The presence of ferulic acid cross-links is supported by our findings that the molecular weight of this population dropped to 50% of its initial value after saponification (Oosterveld et al., 2000a).

It can be concluded that extensive degradation of the arabinan side-chains with EA + AF has little influence on the $[\eta]_w$ of a rhamnogalacturonan. These findings are in agreement with the results of Hwang and Kokini, who investigated the contribution of side-chains to the rheological properties of apple pectins with different neutral sugar contents (Hwang & Kokini, 1991), although the neutral sugar contents were relatively low in all the pectins investigated. They found that the degree of branching had little effect on the intrinsic viscosity, which is determined at low concentrations, but that the side-chains of pectins were significantly involved in entanglements of the pectin molecules in concentrated solutions.

PG + PE caused a small decrease in $M_w$, $[\eta]_w$, as well as in $Rg_w$. Also, a small shift in hydrodynamic volume was observed upon HPSEC. This limited decrease in $M_w$ shows that this enzyme combination was only slightly active on this pool, showing that no homogalacturonan regions are present internally in this rhamnogalacturonan population. Addition of pectin acetyl esterase, which is known to increase the activity of PE (Oosterveld et al., 2000b) and therefore indirectly increases the activity of PG, besides PG + PE to the parental extract did not increase the degradation of this rhamnogalacturonan population (unpublished data), but only increased the degradation of the separately present homogalacturonan populations (pool III). These results indicate that pieces of homogalacturonan are possibly located at the extremities of the rhamnogalacturonan molecules or that some homogalacturonans are present as a separate population. Since PG + PE are active on the 'smooth' regions and not on the 'hairy' regions, the small decrease in $M_w$ indicates that the hairy regions of sugar beet pectin may have a very high $M_w$.

### 3.4. Effect of enzymatic modification on pool II of the autoclave extract

Pool II of the autoclave extract, a highly branched

rhamnogalacturonan with a lower $M_w$ than pool I, was incubated with the same enzyme combinations as pool I (Fig. 3). The results were rather similar to the results found for pool I. Hydrolysis with the enzymes RGase + RGAE caused a large decrease in $M_w$, $[\eta]_w$, and $Rg_w$. Degradation of the arabinan side-chains of pool II with AF decreased the $M_w$, after a small initial increase in $M_w$. We assumed that this small increase was due to inaccuracies in the measurements, and that is was not significant. The $[\eta]_w$ and the $Rg_w$ of pool II changed relatively little after degradation with AF. Hydrolysis of the arabinan side-chains of pool II by EA + AF led to a relatively large decrease in $M_w$. Although the $M_w$ decreased to a value similar to the value found after treatment with AF, the $[\eta]_w$ and $Rg_w$ decreased to lower values. Hydrolysis of homogalacturonan regions in pool II by PG + PE led to a relatively large decrease in $M_w$. If possible homogalacturonans stretches are only located at the extremities of the molecule, as was suggested for pool I, this decrease seems rather large. Possibly some high $M_w$ homogalacturonans eluted together with the rhamnogalacturonans in small amounts, as was also indicated by the higher galacturonic acid content in this pool as compared with pool I. The formation of an additional peak with no visible $A_{335}$ absorption at 37 min upon HPSEC after degradation with PG + PE is in agreement with this assumption. The $[\eta]_w$ and $Rg_w$ also decreased to relatively low values, which shows that this homogalacturonan fraction has a relatively large influence on these parameters.

### 3.5. Effect of enzymatic modification on pool IIIA of the autoclave extract

The arabinan fraction pool IIIa of the autoclave extract was also treated with the enzyme combinations mentioned above (Fig. 4).

Modification by RGase + RGAE resulted only in a small change in $M_w$. This is in agreement with the assumption that some arabinans are still linked to a small piece of rhamnogalacturonan backbone, too small to bind to the anion-exchange column, as was previously shown by the methylation analysis of the arabinans (Oosterveld et al., 2000a). The $[\eta]_w$ did not change, since this is determined by the longest backbone of the fragment, in this case probably the arabinan backbone. The HPSEC diagram hardly changed as compared with the blank.

Hydrolysis of the arabinans with AF decreased the $M_w$ from 23 to 9 kDa. From these results a degree of polymerization of the arabinans can be calculated of 130–170 residues before and 60–70 after modification with AF. However, the $[\eta]_w$ did not change. This can be explained by the fact that AF predominantly removes the side-chains from the arabinans, having little effect on the length of the backbone, which determines the $[\eta]_w$ to a large extent. So, probably the backbone has a length of 60–70 residues. This would implicate that more than 45–65% of the arabinose residues are present as single unit or oligomeric side group of the arabinan main-chain. Also, methylation analysis showed that approximately 35% is present as terminal residues indicating that on average the side-chains of the arabinans are relatively short (Oosterveld et al., 2000a). Cooper et al. (1992) described the production of linear arabinans with AF. They estimated a length of 50–80 residues after linearization, which is in good agreement with the value described in this paper.

Incubation of the arabinans with the enzymes EA + AF led to a decrease in both $M_w$ and $[\eta]_w$ of the arabinan population. In the HPSEC elution pattern only a very small population remained. It is speculated that this population consists of glucomannans, which represent less than 5 mol% of the sugars initially present in the arabinan fraction (Table 1). The $M_w$ of this fraction was 4 kDa.

The effect of treatment of the arabinans with PG + PE was not determined, since the galacturonic acid content of the arabinans was very low.

### 3.6. Effect of enzymatic modification on an ABP

Besides rhamnogalacturonans and arabinans obtained from an autoclave extract from beet pulp, also an acid extracted pectin from sugar beet pulp (ABP) was incubated with the enzyme combinations RGase + RGAE, PG + PE, and EA + AF (see Fig. 5). ABP consists primarily of homogalacturonans. As the arabinose content of this pectin is low (Table 1), and the effect of arabinan degrading enzymes on the physico-chemical properties was expected to be negligible, treatment with AF was omitted.

Treatment of ABP with RGase + RGAE decreased the $M_w$ from 271 to 112 kDa, due to hydrolysis of the rhamnogalacturonan backbone present in the hairy regions of ABP. Surprisingly, the $[\eta]_w$ hardly changed. Most of the material eluted at the same retention time upon HPSEC. However, a low $M_w$ population with a high $A_{335}$ absorption appeared at 38 min in low quantities. It made us conclude that the hairy regions in this pectin are located at the extremities of the molecules. It is also possible that a pectin population is degraded by this enzyme, which is present in small amounts and with a very high $M_w$. Berth, Dautzenberg & Rother (1994) described a pectin population, which was present in minute amounts. This population had such a high $M_w$ that it significantly influenced the $M_w$ of the whole pectin extract.

Our results show that the rhamnogalacturonan backbone from ABP contributes little to the $[\eta]_w$. On the other hand treatment with RGase + RGAE causes a loss of the gelling properties with $Ca^{2+}$, as we showed in another study (Oosterveld et al., 2000b).

Modification of the arabinan side-chains by addition of EA + AF to ABP decreased the $M_w$ to 207 kDa. This decrease was somewhat larger than was expected based on the low arabinose content (7 mol%). Both the $Rg_w$ and the

$[\eta]_w$ hardly changed. Upon HPSEC no clear changes were observed.

Modification of the smooth homogalacturonan regions of ABP with PG + PE decreased the average $M_w$ rapidly to a value of 5 kDa. Furthermore, the $[\eta]_w$, and $Rg_w$ also decreased rapidly. This is in agreement with the results found by Guillon and Thibault, who also found a rapid decrease in $M_w$ and $[\eta]_w$ for an ABP during treatment with PG + PE (Guillon & Thibault, 1990). After treatment of ABP with PG + PE only some hairy fragments remained. Based on our results the average $M_w$ of these fractions is 50 kDa (Fig. 5D), which is much lower than those obtained by autoclaving (see above). On the other hand, it is somewhat higher than the results found by Guillon and Thibault, who found a viscosity average molecular weight of 12 kDa after treatment of an ABP with PG + PE (Guillon & Thibault, 1990).

## 4. Concluding remarks

Enzymatic modification of various types of pectic polysaccharides obtained from sugar beet pulp with specific enzymes affected structural characteristics as well as rheological characteristics of these polysaccharides. This allowed studies of the effect of structural changes on the rheological properties.

In most cases we found that the enzymatic modifications employed in this study decreased the $[\eta]_w$ of the polysaccharides investigated. Obviously, this occurred when backbone degrading enzymes were used. However, we found that degradation of the rhamnogalacturonan backbone of an ABP has relatively little influence on the $[\eta]_w$ which made us conclude that the rhamnogalacturonans present in this type of pectin are mainly located at the extremities of the pectin molecules. Some indications were found that the rhamnogalacturonans obtained by autoclave extraction are linked through the arabinose side-chains, probably by diferulic acid cross-links. However, further study is needed to confirm this. Removal of the arabinans present in these rhamnogalacturonans with enzymes had relatively little effect on the $[\eta]_w$, although the $M_w$ of the populations decreased significantly. This shows that the arabinan side-chains contribute little to the $[\eta]_w$ of ABPs.

In our experiments we found that enzymatic modification with glycanases can be used to significantly change the structural characteristics of pectic polysaccharides without a significant loss of viscosity. In this way glycanases can be used for the removal of side-chains of the pectic molecule, which limit the physico-chemical properties, as described for the production of debranched arabinans (Cooper et al., 1994), and for the improvement of the oxidative cross-linking reaction of beet pectins by removal of the arabinan sidechains (Guillon & Thibault, 1990).

An important prerequisite for the introduction of the use of enzymes for the modification of beet pectins on industrial scale, is the availibility of sufficient amounts of enzymes at reasonable prices. The use of cloned polysaccharide degrading enzymes as described by Kofod, Kauppinen, Christgau, Andersen, Heldt-Hansen & Dörreich (1994) is assumed to be an important step towards the introduction of these enzymes on industrial scale.

## Acknowledgements

The research reported in this paper was supported by the Dutch Innovation research Program on Carbohydrates (IOP-k), and by CSM Suiker bv, the Netherlands. The authors wish to thank Dr J. M. De Bruijn of CSM for valuable discussions.

## References

Baron, A., Rombouts, F. M., Drilleau, J. F., & Pilnik, W. (1980). Purification et propriétés de la pectinestérase produite par Aspergillus niger. *Lebensmittel Wissenschaft und Technologie*, (13), 330–333.

Berth, G., Dautzenberg, H., & Rother, G. (1994). Static light scattering technique applied to pectin in dilute solution. Part II: The effects of clarification. *Carbohydrate Polymers*, (25), 187–195.

Colquhoun, I. J., Ralet, M.-C., Thibault, J.-F., Faulds, C. B., & Williamson, G. (1994). Structure identification of feruloylated oligosaccharides from sugar beet pulp by NMR spectroscopy. *Carbohydrate Research*, (263), 243–256.

Cooper, J. M., McCleary, B. V., Morris, E. R., Richardson, R. K., Marrs, W. M., & Hart, R. J. (1992). Preparation, physical properties and potential food applications of enzymically-debranched araban from sugar beet. In G. O. Phillips, *Gums and stabilizers for the food industry* (pp. 451–460). 6. Oxford, UK: Oxford University Press.

Faulds, C. B., & Williamson, G. (1990). Effect of enzymes derived from orange peel on citrus and sugar beet pectins. In G. O. Phillips, P. A. Williams & D. J. Wedlock, *Gums and stabilizers for the food industry* (pp. 277–280). 5. Oxford, UK: IRL Press.

Fishman, M. L., Gillespie, D. T., Sondey, S. M., & El-Atawy, Y. S. (1991). Intrinsic viscosity and molecular weight of pectin components. *Carbohydrate Research*, (215), 91–104.

Guillon, F., & Thibault, J.-F. (1987). Characterization and oxidative cross-linking of sugar beet pectins after mild acid hydrolysis and arabanases and galactanases degradation. *Food Hydrocolloids*, (1), 547–549.

Guillon, F., & Thibault, J.-F. (1988). Further characterization of acid and alkali soluble pectins from sugar beet pulp. *Lebensmittel Wissenschaft und Technologie*, (21), 198–205.

Guillon, F., & Thibault, J.-F. (1989a). Methylation analysis and mild acid hydrolysis of the hairy fragments of sugarbeet pectins. *Carbohydrate Research*, (190), 85–96.

Guillon, F., & Thibault, J.-F. (1989b). Enzymic hydrolysis of the hairy fragments of sugar beet pectins. *Carbohydrate Research*, (190), 97–108.

Guillon, F., & Thibault, J.-F. (1990). Oxidative cross-linking of chemically and enzymatically modified sugar-beet pectin. *Carbohydrate Polymers*, (12), 353–374.

Hwang, J., & Kokini, J. L. (1991). Contribution of the side branches to rheological properties of pectins. *Carbohydrate Polymers*, (19), 41–50.

Kofod, L. V., Kauppinen, S., Christgau, S., Andersen, L. N., Heldt-Hansen, H. P., & Dörreich, K. (1994). Cloning and characterization of two structurally and functionally divergent rhamnogalacturonases from Aspergillus aculeatus. *Journal of Biological Chemistry*, (268), 29,182–29,189.

Matthew, J. A., Howson, S. J., Keenan, M. H. J., & Bolton, P. S. (1990). Gelation of sugar beet and citrus pectins using enzymes extracted from orange peel. *Carbohydrate Polymers*, (13), 295–306.

McCleary, B. V., Cooper, J. M. & Williams, E. L. (1989). Debranched araban and its use as a fat substitute. *Patent Application*, GB 8828380.9.

Michel, F., Thibault,, J.-F., Mercier, C., Heitz, F., & Pouillaude, F. (1985). Extraction and characterization of pectins from sugar beet pulp. *Journal of Food Science*, (50), 1499–1500.

Oosterveld, A., Beldman, G., Schols, H. A., & Voragen, A. G. J. (1996). Arabinose and ferulic acid rich pectic polysaccharides extracted from sugar beet pulp. *Carbohydrate Research*, (288), 143–153.

Oosterveld, A., Grabber, J. H., Beldman, G., Ralph, J., & Voragen, A. G. J. (1997). Formation of ferulic acid dehydrodimers through oxidative cross-linking of sugar beet pectin. *Carbohydrate Research*, (300), 179–181.

Oosterveld, A., Beldman, G., Schols, H. A., & Voragen, A. G. J. (2000a). Characterization of arabinose and ferulic acid rich pectic polysaccharides and hemicelluloses from sugar beet pulp. *Carbohydrate Research*, (328), 185–197.

Oosterveld, A., Beldman, G., Searle-van Leeuwen, M. J. F., & Voragen, A. G. J. (2000b). Effect of deacetylation on gelation of sugar beet pectin in the presence of calcium. *Carbohydrate Polymers*, (43), 249–256.

Pasculli, R., Gereads, C. C. J. M., Voragen, A. G. J., & Pilnik, W. (1991). Characterization of polygalacturonases from yeast and fungi. *Lebensmittel Wissenschaft und Technologie*, (24), 63–70.

Phatak, L., Chang, K. C., & Brown, G. (1988). Isolation and characterization of pectin in sugar beet pulp. *Journal of Food Science*, (53), 830–833.

Pippen, E. L., McCready, R. M., & Owens, H. S. (1950). Gelation properties of partially acetylated pectins. *Journal of the American Chemical Society*, (72), 813–816.

Ralet, M.-C., Thibault, J.-F., Faulds, C. B., & Williamson, G. (1994). Isolation and purification of feruloylated oligosaccharides from cell walls of sugarbeet pulp. *Carbohydrate Research*, (263), 227–241.

Rombouts, F. M., & Thibault, J.-F. (1986). Sugar beet pectins: chemical structure and gelation through oxidative coupling. In M. L. Fishman & J. J. Jen, *Chemistry and function of pectins. ACS Symposium Series 310* (pp. 49–60). Washington, DC: American Chemical Society.

Rombouts, F. M., Voragen, A. G. J., Searle-van Leeuwen, M. J. F., Gereads, C. C. J. M., Schols, H. A., & Pilnik, W. (1988). The arabinases of Apergillus niger purification and characterisation of two (α)-L-arabinofuranosidases and an endo-1,5-(α)-L-arabinanase. *Carbohydrate Polymers*, (8), 25–47.

Schols, H. A., Gereads, C. C. J. M., Searle-van Leeuwen, M. J. F., Kormelink, F. J. M., & Voragen, A. G. J. (1990). Rhamnogalacturonase: a novel enzyme that degrades the hairy regions of pectins. *Carbohydrate Research*, (206), 105–115.

Searle-van Leeuwen, M. J. F., van den Broek, L. A. M., Schols, H. A., Beldman, G., & Voragen, A. G. J. (1992). Rhamnogalacturonan acetylesterase: a novel enzyme from Aspergillus aculeatus, specific for the deacetylation of hairy (ramified) regions of pectins. *Applied Microbiology and Biotechnology*, (38), 347–349.

Thibault, J.-F. (1979). Automatisation du Dosage des substances pectiques par la méthode au méta-hydroxydiphenyl. *Lebensmittel Wissenschaft und Technologie*, (12), 247–251.

Thibault, J.-F. (1986). Some physicochemical properties of sugarbeet pectins modified by oxidative crosslinking. *Carbohydrate Research*, (155), 183–192.

Thibault, J.-F., Guillon, F., & Rombouts, F. M. (1991). Gelation of sugar beet pectins by oxidative coupling. In R. H. Walter, *The chemistry and technology of pectin* (pp. 119–133). San Diego, CA: Academic Press.

Thibault, J.-F., Renard, C. M. G. C., Axelos, M. A. V., Roger, P., & Crepeau, M.-J. (1993). Studies of length of homogalacturonic regions in pectins by acid hydrolysis. *Carbohydrate Research*, (238), 271–286.

Tollier, M., & Robin, J. (1979). Adaptation de la méthode à l'orcinol-sulfurique au dosage automatique des glucides neutres totaux: conditions d'application aux extraits d'origine végétale. *Annals of Technology in Agriculture*, (28), 1–15.

Voragen, A. G. J., Rombouts, F. M., Searle-van Leeuwen, M. J. F., Schols, H. A., & Pilnik, W. (1987). The degradation of arabinans by endo-arabinanase and arabinofuranosidases purified from Aspergillus niger. *Food Hydrocolloids*, (1), 423–437.