Available online at www.sciencedirect.com
SCIENCE DIRECT
Carbohydrate Polymers 58 (2004) 149–161
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

Preparation and properties of enzymatically and chemically modified sugar beet pectins

Hans Christian Buchholt[a,*], Tove Martel Ida Else Christensen[b], Bjarne Fallesen[c], Marie-Christine Ralet[d], Jean-Francois Thibault[d]

[a]*Danisco Innovation, Edwin Rahrsvej 38, DK-8220 Brabrand, Denmark*
[b]*Danisco Innovation, Langebrogade 1, DK-1001 Copenhagen, Denmark*
[c]*Danisco Sugar, Development Centre, DK-4900 Nakskov, Denmark*
[d]*Unité de Recherche sur les Polysaccharides, leurs Organisations et Interactions, Institut National de la Recherche Agronomique, rue de la Géraudière, B.P. 71627, F-44316 Nantes cedex 3, France*

Received 27 May 2003; revised 17 June 2004; accepted 17 June 2004
Available online 28 August 2004

Abstract

Sugar beet pectin was extracted from fresh sugar beet pulp and further deesterified by base, plant or fungal pectin esterase or esterified in acid methanol. The modified sugar beet pectins were characterised by chemical analyses, macromolecular and rheological properties, capillary electrophoresis and enzymatic fingerprinting.

The majority of the modified sugar beet pectins had the same molar mass and neutral sugar content except for loss of arabinose by prolonged acid hydrolysis. The ferulic acid content was almost stable at about 0.8%. It was found that the enzymatically modified sugar beet pectins were more inhomogeneous due to the presence of acetyl groups hindering the attack by pectin esterases.
© 2004 Elsevier Ltd. All rights reserved.

Keywords: Sugar beet pulp; Sugar beet pectin; Chemical modification; Enzymatic modification; Characterisation

1. Introduction

Sugar beet pulp appears in huge amounts as a low valued by-product from beet sugar production, and conventionally sugar beet pectin (SBP) has been extracted from that source. SBP was produced commercially during the 1940s, but because of its poor gelling property, it was unable to compete with apple or citrus pectin (Towle & Christensen, 1973). Today, SBP is produced in small amounts for applications where it has equal or superior properties compared with citrus or apple pectin. These applications include, e.g. stabilisation of flavoured oil emulsions in juice concentrates (Weibel, 1991), lowering of cholesterol absorption from food (Buchholt & Vinther, 1991; Desforges, Cooper, & Williams, 1995; Weibel, 1991), water-soluble pectin fibres (Buchholt & Vinther, 1991; Desforges et al., 1995; Ralet, Thibault, & Della Valle, 1991), and stabilisation of acidified drinking yoghurt (Takahashi, Furuta, Tobe, & Kiwata, 1999).

Various commercial types of apple and citrus pectin are mainly characterised by different degrees of methyl esterification, methyl ester distribution, molar mass and variable content of rhamnogalacturonan ('hairy' regions). Similar types of SBP have not been characterised in detail. Sequential or specific extraction of sugar beet pulp by selective methods, which include extractions with hot water, sequestering agents or alkali, has been widely attempted and gave low yields of rhamnogalacturonan-rich fractions with relative low galacturonan content (Dea & Madden, 1986; Guillon & Thibault, 1988; Marry et al., 2000; Phatak, Chang, & Brown, 1988; Turquois, Rinaudo, Taravel, & Heyraud, 1999). Acid extraction of sugar beet pulp gave the highest galacturonan content and a good yield of sugar beet pectin (Guillon & Thibault, 1988; Phatak et al., 1988; Sun & Hughes, 1998).

* Corresponding author. Tel.: +45-8943-5271; fax: +45-8625-1077.
E-mail address: g8hcb@danisco.com (H.C. Buchholt).

0144-8617/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2004.06.043

The chemical structure of pectic polysaccharides from sugar beet root consists of backbone sequences of α-(1→4)-linked, partly methyl-esterified D-galacturonic acid residues interrupted by branched rhamnogalacturonan sequences with L-arabinose, D-galactose, L-rhamnose and D-galacturonic acid as the major sugar constituents (Marry et al., 2000; Sun & Hughes, 1998). Feruloyl groups are ester-linked to arabinose and galactose, and part of galacturonic acid groups have acetyl substitution (Thibault, Renard, & Guillon, 2000).

The properties of sugar beet pectin are reflected by its chemical structure. The most important differences from apple and citrus pectin are: shorter galacturonan chain length, more prominent rhamnogalacturonan regions, and significant substitution by acetyl groups in combination to lower molar mass (Rombouts & Thibault, 1986b; Thibault, Guillon, & Rombouts, 1991). The gelling property of high ester pectin in acidic, aqueous solutions at high sugar concentrations is related to plenty of stable intermolecular hydrogen bonds between the galacturonan chains, sugar molecules and water. In addition, low ester pectin forms multiple intermolecular Ca^{2+} bonds which stabilise a strong polymer network in low ester pectin gels. The lower galacturonan content, shorter molecule length and high degree of acetyl substitution of sugar beet pectin significantly reduces intermolecular hydrogen bonding possibilities to a level where gelling in acid/sugar or Ca^{2+}/sugar systems is no longer possible (Phatak et al., 1988).

Pippen, McCready and Owens (1950) investigated the gelation properties of acetylated pectins and concluded that acetyl groups hindered the gelation of SBP. Several deesterification attempts have been made to improve the gel formation of sugar beet pectin. Partial deacetylation of sugar beet pulp by mild alkaline treatment gave low-ester SBP which gelled in the presence of Ca^{2+} (Turquois et al., 1999). Treatment of sugar beet pulp with concentrated acid at 45 °C for several days resulted in almost completely deacetylated sugar beet pectin of a medium- or high-ester type which gelled in an acid/sugar system (Grand & Stevens, 1991). Improved gelation of SBP was obtained by incubation of SBP with an enzyme preparation from *Aspergillus niger* containing arabinase, pectin esterase and pectin acetyl esterase (PAE) (Matthew, Howson, Keenan, & Belton, 1990). The SBP gelled with Ca^{2+} ions after treatment with a crude mixture of pectin acetyl esterase and plant pectin esterase (p-PE) from oranges (Williamson et al., 1990). SBP also gelled in the presence of Ca^{2+} ions after removal of 14.8% of the methyl ester groups with fungal pectin esterase (f-PE) or by treatment with a combination of f-PE and PAE from *Aspergillus niger*, which released 13.8% of the acetyl groups and 27.2% of methyl ester groups under the same incubation conditions and gave a much stronger gel (Oosterveld, Beldman, Searle van Leeuwen, & Voragen, 2000).

The purpose of the present work was to use the same SBP raw material to prepare a series of model sugar beet pectins that had been enzymatically or chemically modified to represent systematically coherent series. The intention was that the isolated SBPs should be comparable regarding neutral sugar composition, average molar mass and galacturonan content, and that they were to be characterised by chemical analyses, physico-chemical properties—including interaction with cations, capillary electrophoresis (CE), and enzymatic fingerprinting.

2. Experimental

2.1. Materials

Fresh sugar beet pulp was obtained from a sugar factory (Danisco Sugar and Sweetener in Assens, DK) and kept in sealed containers at 56–67 °C during the 3 h transportation to Danisco Sugar Development Centre in Nakskov. Fresh sugar beet roots were taken from production on the same day. Methanol, isopropyl alcohol, sodium hexametaphosphate and reagents for pH adjustment were of technical grade, while analytical grade reagents were used for analytical purposes. Plant pectin esterase was isolated from oranges (Christensen, Nielsen, Kreiberg, Rasmussen, & Mikkelsen, 1998), and fungal pectin esterase was isolated and purified from *Aspergillus niger* (Limberg et al., 2000a). One unit (U) of pectin esterase releases 1 μmol of protons per minute from a solution of high-ester pectin at specified conditions. The p-PE activity was measured tritrimetrically by monitoring the release of protons from a 1% aqueous solution of high-ester pectin at pH 6.8 at 22 °C in the presence of 150 mM NaCl. The f-PE activity was determined at pH 4.5 at 22 °C without salt addition. The extractions and uses of endo-PG were described in Limberg et al. (2000a).

2.2. Extraction of sugar beet pectin from sugar beet pulp

Fresh sugar beet pulp (754 kg, 11% dry matter) was hydrolysed (70 °C, pH 1.5) in 2000 l acidified water purified by reverse osmosis prior to use. After 5 h the hydrolysis was stopped by addition of a 10% sodium carbonate solution until reaching pH 3.0, and insoluble pulp residue was then separated in a decanter centrifuge (Alfa Laval NX 409). The pectin juice was further clarified in a disc centrifuge (Westfalia SAOH 205) followed by a cloth filter (Schenk KFP) precoated with Clarcel CBL filter aid. Multivalent cations were removed from the clarified juice by passing it through an ion exchange column (resin: Dowex Upcore Mono C600). The pectin juice was further purified by combined diafiltration and concentration using an ultrafiltration module with a membrane cut-off value of 50 kDa (Danish Separation System). The temperature of the juice was kept above 50 °C throughout the processes. In total 314 kg of 3.0% sugar beet pectin concentrate was produced and preserved by addition of 200 ppm of sorbic acid.

The SBP concentrate was frozen and stored below −16 °C until use. Sugar beet pectin was isolated from the concentrate by precipitation of one volume part of concentrate in three volume parts of pure isopropyl alcohol followed by constant agitation for 1 h. Filtering the mixture through cotton canvas separated the precipitated SBP, which was further washed by suspension in 80 vol.% aqueous isopropyl alcohol. After filtration the material was pressed and dried in a well-ventilated oven at 50 °C overnight. SBP6230 with a 62% degree of methylation (DM) and a 30% degree of acetylation (DAc) was achieved after milling of the dried pectin to pass a 0.5 mm sieve (Retsch ZM1 centrifugal mill).

2.3. Extraction of sugar beet pectin from fresh sugar beet on lab scale

Washed sugar beet roots (30.4 kg) were sliced, hand-cut into strips and minced in a meat chopper through an 8 mm perforated plate. The minced material was immediately blanched at 85 °C for 5–10 min to inactivate enzymes and denature plant protein. Sugar was extracted from the material by suspension in 20 l of water at 60 °C for 2 h. The pulp was then separated on a screen and pressed at 15 bar in a 5 l hydraulic piston press (Hubert Schwanke HP5M). The press cake was suspended in 40 l of water at 70 °C, and the pH was lowered to 1.5 by addition of diluted nitric acid. After slow agitation for 2 h at 70 °C the extraction mixture was passed over the screen to separate the viscous liquid. The pulp was then washed twice by suspension in 8 l of water at 70 °C for 2 h. The filtrates were neutralised to pH 3.0 by addition of a 20% sodium carbonate solution, mixed and centrifuged at 2500 g for 15 min. The supernatant was clarified by passing it through a precoated (Clarcel DIT-R) vacuum plate filter. Cations were exchanged by passing the clear filtrate through a column of cationic exchange resin in sodium form. The refined sugar beet pectin juice was concentrated to approx. 2.5% pectin in solution by evaporation in vacuum. Sugar beet pectin was isolated by precipitation of the concentrate in 3 volume parts of pure isopropyl alcohol and constant agitation of the mixture for 1 h. Passing the mixture through cotton canvas separated precipitated sugar beet pectin, which was further washed by suspension in 2 l of 80 vol.% aqueous isopropyl alcohol. After a second separation the material was pressed and dried overnight in a well-ventilated oven at 50 °C. After milling of the dried pectin to pass a 0.5 mm sieve, 335 g sugar beet pectin BRP5926 (DM = 59% and DAc = 26%) was isolated.

2.4. Modification of sugar beet pectin by plant pectin esterase

Sodium chloride was added to 4000 g of sugar beet pectin concentrate (3.0% sugar beet pectin) in a 5 l stirred reactor to obtain a 0.1 M solution. The solution was heated to 40 °C and the pH was adjusted to 7.0 by addition of a 1 M sodium hydroxide solution. Subsequently, 65–300 units of purified plant pectin esterase were added, and the mixture was stirred at constant pH and temperature, while 0.24–0.8 mmol of NaOH per g of SBP was consumed during the incubation period of 3–25 h. The pH was lowered to 3.6 and the solution was heated to 75 °C for 5 min to inactivate the pectin esterase. The solution was then cooled to below 40 °C and added to 8 l isopropyl alcohol during slow agitation to precipitate the modified SBP. The isolation of the modified SBPs (P series) was made according to the description in Section 2.3.

2.5. Modification of sugar beet pectin by fungal pectin esterase

Sugar beet pectin concentrate (4000 g of 3.0% SBP) was heated to 40 °C in a 5 l stirred reactor. The pH was increased to 4.2 by drop-wise addition of a 1 M sodium hydroxide solution. Subsequently 750–4000 units of fungal pectin esterase from *Aspergillus niger* were added, and the mixture was stirred at constant pH and temperature, while 0.2–0.9 mmol of sodium hydroxide solution per g of SBP was consumed during incubation for 1.5–25 h. The pH was lowered to 3.2 by drop-wise addition of 34% nitric acid to stop the incubation, and the solution was heated to 75 °C for 5 min to inactivate the pectin esterase. The concentrate was then cooled to below 40 °C and added to 8 l of isopropyl alcohol under slow agitation to precipitate the SBP. The isolation of the modified SBPs (F series) was similar to the procedure described in Section 2.3.

2.6. Partial saponification of sugar beet pectin (B series)

Sugar beet pectin concentrate (4000 g, 3.0% SBP) was cooled to 0–2 °C in a 5 l stirred reactor using an ice bath. The pH was increased to 11.0 by drop-wise addition of a 1 M sodium hydroxide solution under good agitation. The solution was stirred at constant pH and temperature, while 0.25–1.2 mmol of sodium hydroxide per g of SBP was consumed over 2.5–24 h. For the preparation of B0100 the mixture was heated to room temperature at the end of the process to complete the saponification. Then 10% nitric acid was added drop-wise to lower the pH to approx. 4.5. The partially saponified SBPs were isolated as described in Section 2.3.

2.7. Partial deacetylation of sugar beet pectin (B' series)

SBP was precipitated from 4000 g of 3.0% SBP concentrate by mixing into 8 l of slowly agitated methanol at room temperature. After stirring for approx. 1 h the suspension of precipitated SBP was filtered through cotton canvas, and the filter cake was resuspended twice in 4 l of methanol for another hour. After final wash the precipitated SBP was pressed and suspended in 3 l of methanol and cooled to 2 °C in a 5 l stirred reactor. The pH of the agitated

suspension was raised to 11–13 by addition of 1 M sodium methylate in methanol. The mixture was stirred at constant pH and temperature, while 0.25–1.2 mmol of sodium methylate in methanol per g of SBP was consumed over 24–28 h. The pH was then lowered to 3.5 by slow addition of 10% nitric acid to stop the process, and SBP was separated from the solution by filtration in vacuum through a Büchner funnel covered with a piece of cotton canvas. The filter cake was resuspended twice in 2 l of 80 vol.% aqueous methanol followed by filtration. The modified SBPs were isolated according to the procedure in Section 2.3.

2.8. Esterification of sugar beet pectin (E series)

A sample of SBP6230 (200 g) was suspended in a mixture of 500 g of methanol and 40 g of 96% sulphuric acid. Temperatures between 8 and 26 °C were used and the reaction time varied from 24 to 120 h according to the required degree of esterification. The reaction was stopped by vacuum filtration of the mixture through a Büchner funnel, followed by wash with 3 portions of 200 ml of methanol. The mixture was suspended in 500 ml of 60 vol% aqueous methanol, while the pH of the suspension was adjusted to 3.0 by addition of a 20% aqueous sodium carbonate solution. After filtration and a final wash the esterified SBP was dried overnight at 50 °C in a ventilated oven.

2.9. Chemical analyses of sugar beet pectins

The degree of methyl esterification and content of anhydrogalacturonic acid (GalA) were determined by potentiometric titration using a modified FCC method in which end point pHs were determined by parallel use of phenolphthalein indicator (Anon., 1981). Separately, the released amount of acetate from the saponification process was determined by a Boehringer Mannheim acetic acid test kit (E 0148261), and the degree of methyl esterification was determined from the difference between the total esterification equivalents and the acetic acid equivalents. The total free galacturonic acid plus methyl ester equivalents give the anhydrogalacturonic acid equivalents.

$$\text{The degree of methylation} = 100 \times \frac{\text{equival. total ester} - \text{equival. acetic acid}}{\text{equival. free acid} + \text{equival. total ester} - \text{equival. acetic acid}}$$

$$\text{The degree of acetylation} = 100 \times \frac{\text{equival. acetic acid}}{\text{equival. free acid} + \text{equival. total ester} - \text{equival. acetic acid}}$$

$$\%\text{GalA} = \left(176 \times \frac{\text{equival. free acid}}{\text{weight of pectin}} + 190 \times \frac{\text{equival. methyl ester}}{\text{weight of pectin}}\right) \Big/ 100$$

The feruloyl ester content in sugar beet pectin was determined by dissolution of 1.000 g of sugar beet pectin/l in an aqueous carbonate buffer (consisting of 0.025 M Na_2CO_3 + 0.025 M $NaHCO_3$) at pH 10.0 and subsequent measurement of the UV light absorbance at 375 nm. The content of feruloyl ester was calculated from a molar extinction coefficient of 31,600 (Rombouts & Thibault, 1986a).

The weight-average molar mass (M_w) was determined by viscometry according to the procedure described in Limberg et al. (2000a).

Molar mass and intrinsic viscosity were determined by HPSEC: SBP (3 mg/ml) was gently suspended in ultrapure water. After dissolution overnight under magnetic stirring, 0.05 M of $NaNO_3$ was added. The solutions were centrifuged and filtered through 0.45 μm Minisart RC15 Sartorius membranes and then injected on a high-performance size exclusion chromatography (HPSEC) system consisting of a Shodex OH SB-G precolumn followed by two Shodex OH-pack 804 and 805 columns in series. The elution was performed at room temperature with 0.05 M of $NaNO_3$, containing 0.02% NaN_3 as preservative, at a constant 42 ml/h flow rate. On-line intrinsic viscosity and molar mass determinations were performed using a differential viscometer (Viscotek, T-50A) and a differential refractometer (ERC 7517 A, $dn/dc = 0.146$ ml/g). The intrinsic viscosity was calculated using TriSEC software (version 3.0, Viscotek). The weight-average molar mass (M_w) and number-average molar mass (M_n) were determined using an universal calibration curve (pullulan 5–800 kDa).

Dry matter was determined after drying of the SBP at 105 °C for 2 h and all the data were calculated on a moisture-free basis.

Galacturonic acid was also quantified by the automated *m*-phenylphenol method (Thibault, 1979) after saponification (0.05 M NaOH, 30 min., room temperature) and neutralisation. The individual sugars were analysed after hydrolysing pectin samples in 2 M trifluoroacetic acid (2 h, 121 °C), which were then reduced, acetylated and analysed by gas-liquid chromatography (Englyst & Cummings, 1988; Thibault, Renard, & Guillon, 2000).

2.10. Investigation of the physical properties of modified SBP at varying pHs, ionic environments and water activities

The solutions were prepared from 5.00 ml of 2% SBP in aqueous solution by adding 5.00 ml of an aqueous or aqueous-alcoholic solution, which adjusted the pH and ionic compositions to predetermined values at room temperature. The sample was thoroughly mixed and centrifuged for 15 min. at 2500*g* after resting for 1 h at room temperature. The variables were:

- Decreasing pH from 2.25 to 0.92.
- Increasing Ca^{2+} concentration (0–60 mM of Ca^{2+} in 0.5 M sodium acetate buffer at pH 4.50).

- Increasing Ca^{2+} concentration at 8% isopropyl alcohol (0–60 mM of Ca^{2+} and 0.5 M sodium acetate buffer at pH 4.50).
- Increasing Ca^{2+} concentration at 16% isopropyl alcohol (0–60 mM of Ca^{2+} and 0.5 M sodium acetate buffer at pH 4.50).

A phase separation of the centrifuged mixture was characterised visually. The pH was measured and the relative viscosity of supernatants was determined at 22 °C by measuring the time for emptying the reservoir of a 2.00 ml analytical DIN transfer pipette, using the same pipette for all experiments. Its efflux time for pure water was 1.83 s. The relative viscosity is the ratio between the efflux time for the SBP solution and the efflux time for the solvent used.

2.11. Capillary electrophoresis

Capillary electrophoresis (CE) was performed on a Hewlett Packard 3D instrument equipped with a bubble cell capillary (Agilent), with a length of 64.5 cm and a 75 μm internal diameter. Between operations the capillary was conditioned with 1 M NaOH for 5 min followed by 0.1 M NaOH, water and buffer for 2 min each. The buffer was 30 mM phosphoric acid at pH 7.0. The electrophoretic conditions were 25 kV potential differences at 30 °C. SBP (5 mg/ml) was dissolved in pure water and filtrated through a 0.45 μm nylon filter before hydrodynamic injection at 50 mbar over 5 s. The elution profile was monitored by UV detection at 192 nm.

2.12. Enzymatic fingerprinting with combined endo-/exo-PG

Enzymatic fingerprinting of the sugar beet pectin samples was performed by incubation with endo-polygalacturonase II (endo-PG II) followed by exo-polygalacturonase (exo-PG), both from *Aspergillus*, as described by Limberg et al. (2000b). SBP (5 mg/ml) and glucuronic acid as internal standard (1 mg/ml) were dissolved in 50 mM of sodium acetate at pH 4.5 by shaking over night at room temperature. One ml of pectin solution was incubated with 0.2 U of endo-PG II for 20 h at 40 °C. The incubation was stopped by heating for 5 min in boiling water. After cooling 0.1 U of exo-PG was added, and after incubation at 40 °C for 20 h the reaction was stopped by reheating in boiling water. The sample was analysed for GalA content using HPAEC on a Dionex AI 450 system equipped with a PAD detector (Limberg et al., 2000b).

3. Results and discussion

3.1. Analytical characterisation of SBP

The chemical analytical data for the two extracted SBPs and the five series of modified SBPs are shown in Table 1: By acid-catalysed methyl esterification of SBPs over time some acetyl groups were lost by hydrolysis (E series). The molar mass increased a little, probably favoured by extended acid hydrolysis and removal of small SBP molecules and neutral sugars during wash and neutralisation of the SBP.

The analysis of the B series showed that half of the methyl ester groups were removed by alkaline saponification within a few hours at 0–2 °C. A significant amount of acetyl esters was also saponified, when the saponification process was continued. At room temperature the saponification process was complete at pH 12 within short time.

SBP was almost selectively deacetylated at pH 11–13 in a methanolic suspension according to data from the B′ series in Table 1. The degree of acetylation was easily controlled by pH and base consumption. Loss of acetyl groups and possibly some neutral sugars increased the galacturonic acid content in these products.

The modification of SBP by fungal pectin esterase purified from *Aspergillus niger* was rapid until the DM approached 50%. Further deesterification was slow and only possible by high f-PE addition and a much longer incubation time (F series, Table 1). The deesterification process stopped at nearly the same DM and DAc: approx. 30%. This may be because f-PE does not attack methyl-esterified galacturonic acid groups, which are also acetylated, and the rate of deesterification is low if a neighbouring galacturonic acid group is acetylated. In contrast, the deesterification of lime pectin by f-PE proceeded at decreasing rate until the degree of esterification was approx. 11% (Ralet, Dronnet, Buchholt, & Thibault, 2001).

The corresponding modification of SBP by plant pectin esterase was also rapid until the DM was reduced to 55%. Further deesterification was slow and only possible by high p-PE addition and a longer incubation time (P series, Table 1). It was possible to split off almost half of the methyl ester groups, but the deesterification process stopped at nearly the same DM and DAc. It seems that p-PE does not attack a methyl-esterified galacturonic acid group which is acetylated. Lime pectin could be deesterified to an approx. 16% degree of esterification by p-PE (Ralet et al., 2001).

The content of feruloyl ester was approximately 8 mg/g in all of the isolated SBPs (Table 1). A small decrease of feruloyl ester was found in base-treated samples. The feruloyl ester group was quite resistant in the alkaline condition used for the modification.

According to Table 1 the content of galacturonic acid was quite stable at 550–700 mg/g in samples from modified SBP. The GalA content was increased by deacetylation and decreased by higher cation content during deesterification.

The sugar composition of the SBPs is presented in Table 1. SBP6230 exhibited an overall sugar composition in close agreement with previously published data (Thibault, Renard, & Guillon, 2000) with 548 mg of galacturonic acid/g of dry matter, 123 mg/g of arabinose, 104 mg/g of galactose and 53 mg/g of rhamnose. Traces of glucose

Table 1
Composition of modified sugar beet pectins (mg/g dry matter)

Pectin type	GalA[a] (mg/g of dry matter)	GalA[b] (mg/g of dry matter)	Rha (mg/g of dry matter)	Ara (mg/g of dry matter)	Xyl (mg/g of dry matter)	Gal (mg/g of dry matter)	Glc (mg/g of dry matter)	FerA[c] (mg/g of dry matter)	DM (%)	DAc (%)
Extracted from sugar beet pulp										
SBP6230	548	589	53	123	3	104	5	8.3	61.6	29.8
Extracted from sugar beet root										
BRP5926	–	635	–	–	–	–	–	8.2	59.0	25.9
Esterified (E series)										
E7329	581	617	54	82	2	100	4	8.2	73.2	28.7
E8614	591	695	54	35	3	102	5	7.3	86.3	14.4
E9409	625	717	59	17	3	104	5	7.2	93.6	8.6
Base deesterified (B series)										
B5326	579	583	55	121	5	103	4	8.4	52.6	26.2
B4626	598	560	56	113	3	98	4	8.3	46.4	26.2
B3124	577	546	56	117	3	98	4	8.1	31.4	24.0
B2516	547	561	53	120	3	100	4	8.2	24.8	16.0
B0915	569	533	48	102	2	93	3	7.9	9.0	14.9
B0100	579	538	50	105	2	94	4	6.9	1.1	0.2
Base deacetylated (B'series)										
B′6126	556	647	50	106	3	85	5	7.7	61.0	26.1
B′6023	574	667	49	111	3	89	6	8.4	59.6	23.1
B′6109	592	674	49	108	3	84	5	7.3	61.1	9.2
B′5803	604	698	50	101	3	78	5	7.4	58.2	2.8
Fungal PE deesterified (F series)										
F5129	567	590	51	116	3	94	2	8.7	50.6	29.4
F4429	567	580	52	110	3	92	3	8.2	43.9	29.3
F3331	570	559	54	117	3	93	3	8.2	32.5	30.7
F2830	573	582	53	115	3	96	2	8.4	28.3	29.8
Plant PE deesterified (P series)										
P5328	582	582	55	109	2	99	4	8.3	52.7	28.1
P4628	572	578	54	111	0	96	4	8.5	45.6	27.6
P3429	656	576	51	109	2	96	4	8.4	34.3	29.0

[a] Determined by the *m*-phenylphenol method.
[b] Determined by titrimetry.
[c] Ferulic acid.

and xylose were also detected. Pectins from the F and P series exhibited a sugar composition very similar to that of SBP6230, revealing no specific losses of any sugar and thereby showing that the pectin esterases were not contaminated by any hydrolases. Pectins from the B and B′ series were only slightly impoverished with regard to arabinose (101–121 mg/g of dry matter) and galactose (78–103 mg/g of dry matter) the more the DM and/or the DAc decreased. The SBPs from the E series were drastically impoverished with regard to arabinose (17–82 mg/g dry matter) the more the DM increased. Arabinan side chains are, indeed, known to be particularly acid labile, and from the composition of neutral sugars it can be roughly estimated that rhamnogalacturonan constitutes approx. 33% of the acid-extracted SBP.

3.2. Macromolecular characteristics of modified sugar beet pectins

Macromolecular characteristics of the modified SBPs are shown in Table 2. SBP6230 exhibited a fairly high intrinsic viscosity (344 ml/g). Values of $[\eta]$ ranging from 110 to 493 ml/g were reported for acid-extracted SBPs (Levigne, Ralet, & Thibault, 2002; Oosterveld, Beldman, & Voragen, 2002; Ralet et al., 1991; Rombouts & Thibault, 1986a; Arslan, 1995). A radius of gyration (Rg_w) of 31 nm was observed, in close agreement with previously published data (Oosterveld et al., 2002). The $[\eta]$ and Rg_w values obtained fit with the correlation between log ($[\eta]$) and log (Rg_w) established by Fishman, Gillespie, Sondey and Barford (1989). The model of aggregated rods for citrus pectins, whose aggregation number depends on the degree of methyl esterification and the nature and placement of side chains, hypothesised by these authors could therefore be valid for SBPs; although another explanation—such as an expanded coil conformation—cannot be precluded. The molar mass analysed by HPSEC was particularly high compared with values currently found in the literature and the viscometric analysis. However, Levigne et al. (2002) and Oosterveld et al. (2002) also reported high M_w values for acid-extracted SBPs when using a Right Angle Laser-Scattering detector and a differential viscometer with a universal calibration

Table 2
Macromolecular properties of sugar beet pectins

Pectin type	Intrinsic viscosity [η] (ml/g)	M_w[a] (kDa)	M_w[b] (kDa)	M_n[a] (kDa)	Polydispersity ($=M_w/M_n$)	Rg_w (nm)
Extracted from sugar beet pulp						
SBP6230	344 (20)	351 (33)[c]	50	55 (3.9)[c]	6.5	31
Extracted from sugar beet root						
BRP5926	–	–	54	–	–	–
Esterified (E series)						
E7329	354	316	52	8.2	38.4	30
E8614	307	247	51	8.5	29.2	28
E9409	356	218	54	9.1	24.0	27
Base deesterified (B series)						
B5326	338	318	49	55	5.8	30
B4626	310	312	47	53	5.9	29
B3124	292	316	45	57	5.6	9
B2516	272	330	43	59	5.6	29
B0915	232	321	43	59	5.4	27
B0100	236	308	41	65	4.8	27
Base deacetylated (B′ series)						
B′6126	348	346	58	29	11.8	31
B′6023	328	358	55	30	12.1	31
B′6109	228	470	40	25	18.8	27
B′5803	90	325	11	19	17.2	17
Fungal PE deesterified (F series)						
F5129	333	343	51	53	6.4	31
F4429	318	345	48	53	6.5	30
F3331	302	364	45	55	6.6	30
F2830	378	359	41	53	6.8	29
Plant PE deesterified (P series)						
P5328	292	395	42	53	7.5	30
P4628	276	338	43	56	6.1	29
P3429	258	335	40	57	5.9	28

[a] Determined by HPSEC.
[b] Determined by viscometry.
[c] Value in parenthesis indicates standard deviation ($n=6$).

curve, respectively. The well-known tendency of pectic substances to associate often leads to overestimated M_w values when using light scattering techniques (Berth, Dautzenberg, & Hartmann, 1994). On the other hand, the use of a universal calibration curve using pullulans to estimate M_w may not be perfectly adequate as pectins have been shown to behave as aggregated rods (Fishman et al., 1989). Another explanation could be that cross-linking between pectic molecules through diferulic bridges may occur to some extent (Levigne et al., 2002; Oosterveld et al., 2002). Finally, the contribution of arabinan side chains to molar mass may be quite important. The M_n was low (55,000 g/mol), and the sample was therefore highly polydisperse ($I=6.5$; Fig. 1), in agreement with literature data (Kontaminas & Kokini, 1990; Levigne et al., 2002). The elution pattern of SBP6230 showed two broad populations eluting at 12.8 ml and 14.5 ml (Fig. 1).

Pectins from the F series exhibited macromolecular characteristics and elution patterns similar to that of SBP6230 (Table 2, Fig. 1), showing that no changes in the molar mass or in the overall conformation of sugar beet pectin occurred during this enzymatic deesterification process. Compared with SBP6230, pectins in the P series exhibited lower [η] and Rg_w values, while the M_w and M_n values remained unchanged. The elution patterns of pectins from the P series did not differ significantly from that of SBP6230 (Fig. 1). Pectins from the B series exhibited decreasing [η], Rg_w and M_w values with decreasing degree of esterification. The M_n values remained unchanged, and therefore pectins from the B series were slightly less polydisperse. The elution profile of B0100 is, however, quite similar to that of SBP6230. Slight depolymerisation due to a β-elimination reaction occurred for the B series. Compared with SBP6230, B′6126 and B′6023 exhibited similar [η], Rg_w and M_w, while the M_n values were severely reduced, leading to higher polydispersity. B′6109 and B′5803 appeared to be severely depolymerised (Table 2), and the elution profile of B′5803 (Fig. 1) revealed that the population at 12.8 ml was still present, but that the deacetylation process generated an important low-molar mass population eluting at 17.5 ml. Finally, compared with SBP6230, pectins from the E series exhibited similar [η] values, lower M_w and Rg_w values and drastically decreased M_n values (Table 2). The pectins from the E series were

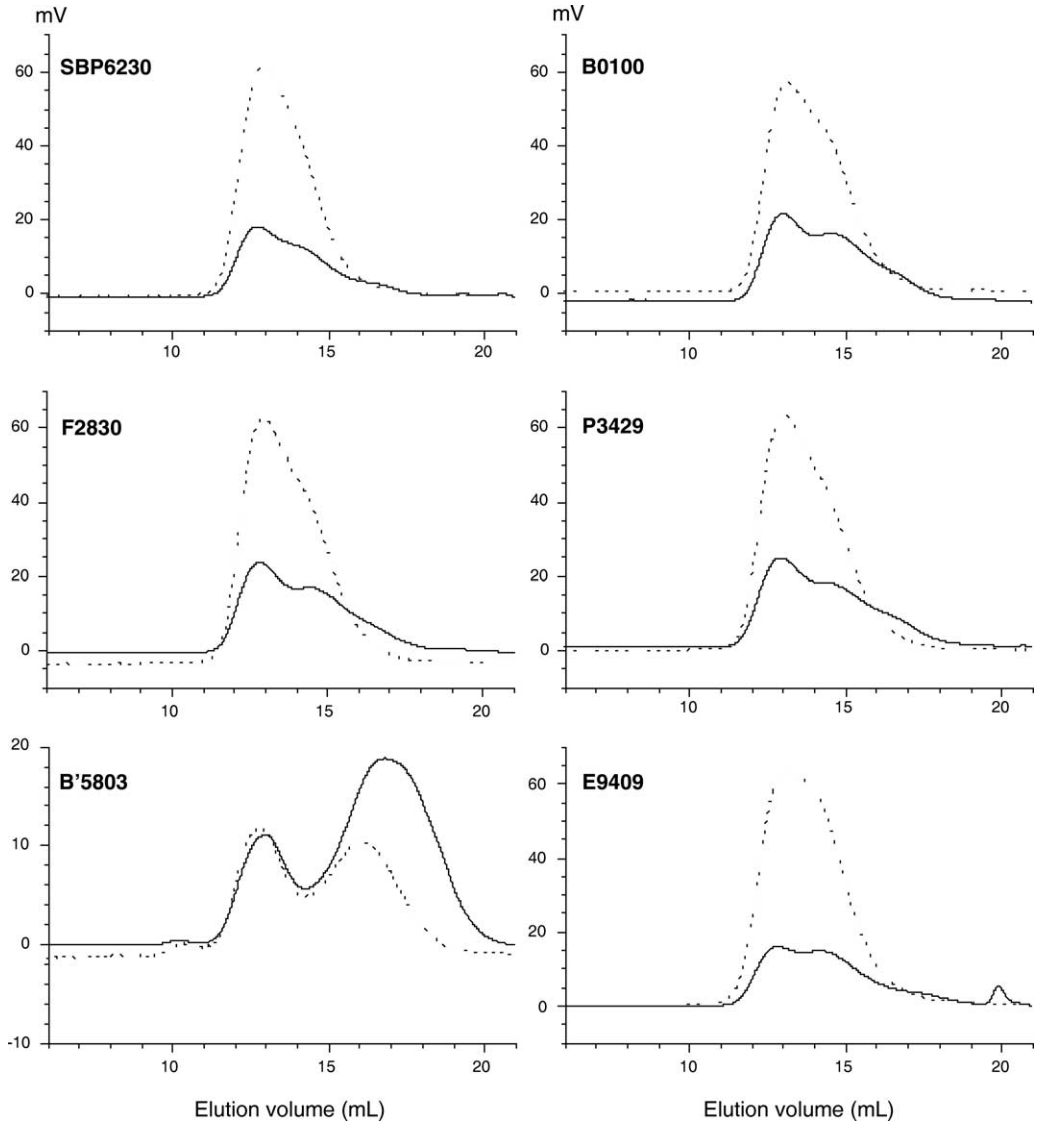

Fig. 1. HPSEC elution pattern of SBP6230 and one of each modified series. Solid line: refractive index, dotted line: viscosity.

therefore characterised by a very high polydispersity index. Pectins from the E series were drastically impoverished in arabinose (Table 1). Oosterveld et al. (2002) also showed that the extensive degradation of arabinan side chains has only little influence on the [η] values. The elution profile of E9409 revealed the presence of the two populations at 12.8 and 14.5 ml observed for the SBP6230, but the high-molar mass population was decreased in favour of the low-molar mass population.

3.3. Rheological characterisation of modified SBPs

Table 3 shows the characterisation of 1% solutions of modified sugar beet pectin at various solvent compositions. Partial precipitation was observed visually and could be estimated qualitatively by the decrease of relative viscosity. By complete precipitation the relative viscosity of the mixture approximated 1.00. Increased relative viscosity indicated the presence of strong intermolecular forces. Hydrogen bonds were responsible for an increase in viscosity at low pH and in the absence of added Ca^{2+}, while in the presence of Ca^{2+} ions at pH above pK_a the calcium bonds contributed to increase the viscosity if the pectin was calcium sensitive or induced precipitation of SBP followed by reduced viscosity of the solution if the pectin was calcium reactive.

From Table 3 it can be seen that lowering the pH did not precipitate the enzymatically modified SBPs: F5129, F4429 and P5328. A fraction of F3331, F2830, P4628 and P3429 was precipitated below pH 1.7, while a relative viscosity of 1.3–1.4 remained and showed that a significant fraction of these modified sugar beet pectins was unaffected by a decrease in pH. This shows that these enzymatically modified sugar beet pectins have an inhomogeneous methyl ester distribution. The briefly esterase-treated SBPs showed calcium-sensitive properties and were partly

Table 3
Relative viscosity of modified sugar beet pectins

	pH	SBP6230	BRP5926	E7329	E8614	E9409	F5129	F4429	F3331	F2830	P5328	P4628
Acidified samples												
0.025 M HCl	2.25	1.55	1.60	1.65	1.68	1.74	1.55	1.58	1.56	1.51	1.47	1.49
0.050 M HCl	1.70	1.54	1.61	1.63	1.65	1.70	1.51	1.52	1.45[a]	1.44	1.47	1.38[a]
0.10 M HCl	1.35	1.56	1.65	1.62	1.61	1.72	1.51	1.52	1.42[a]	1.39[a]	1.46	1.35[a]
0.25 M HCl	0.92	1.54	1.62	1.60	1.60	1.73	1.51	1.50	1.40[a]	1.37[a]	1.46	1.36[a]
Addition of Ca^{2+} ions												
0 mM Ca^{2+}	4.59	1.58	1.67	1.65	1.69	1.80	1.57	1.50	1.47	1.41	1.48	1.40
20 mM Ca^{2+}	4.60	1.59	1.66	1.61	1.68	1.80	1.57	1.50	1.44[a]	1.38[a]	1.54	1.36[a]
40 mM Ca^{2+}	4.61	1.69	1.65	1.62	1.68	1.80	1.57	1.54	1.34[a]	1.33[a]	1.59[b]	1.27[a]
60 mM Ca^{2+}	4.62	1.62	1.67	1.62	1.68	1.74	1.60	1.57	1.33[a]	1.22[a]	1.65[b]	1.25[a]
Addition of Ca^{2+} ions and 8% isopropyl alcohol												
0 mM Ca^{2+}	4.68	1.66	1.75	1.71	1.82	1.86	1.58	1.54	1.52	1.44	1.49	1.47
20 mM Ca^{2+}	4.68	1.67	1.76	1.76	1.75	1.82	1.60	1.67[b]	1.35[a]	1.29[a]	1.68	1.29[a]
40 mM Ca^{2+}	4.69	1.69	1.77	1.75	1.76	1.82	1.69	1.40[a]	1.26[a]	1.20[a]	1.80[b]	1.20[a]
60 mM Ca^{2+}	4.69	1.68	1.77	1.75	1.75	1.88	1.72	1.40[a]	1.18[a]	1.13[a]	1.89[b]	1.15[a]
Addition of Ca^{2+} ions and 16% isopropyl alcohol												
0 mM Ca^{2+}	4.74	1.77	1.81	1.84	1.87	2.01	1.68	1.58	1.48[a]	1.38[a]	1.57	1.50
20 mM Ca^{2+}	4.72	1.75	1.80	1.79	1.81	1.87	1.81[b]	1.47[a]	1.26[a]	1.17[a]	1.86[b]	1.22[a]
40 mM Ca^{2+}	4.72	1.78	1.88	1.83	1.82	1.96	1.51[a]	1.37[a]	1.11[a]	1.06[a]	1.56[a]	1.13[a]
60 mM Ca^{2+}	4.73	1.80	1.87	1.87	1.88	1.95	1.48[a]	1.32[a]	1.11[a]	1.02[c]	1.47[a]	1.05[c]

	pH	P3429	B5326	B4626	B3124	B2516	B0915	B0100	B′6126	B′6023	B′6109	B′5803
Acidified samples												
0.025 M HCl	2.25	1.53	1.51	1.51	1.52	1.67	1.59	1.51	1.67	1.58	1.49	1.18
0.050 M HCl	1.70	1.35[a]	1.48	1.46	1.46	2.20[b]	28.6[b]	13.4[b]	1.63	1.59	1.45	1.09
0.10 M HCl	1.35	1.30[a]	1.48	1.47	1.46	2.71[b]	1.03[c]	1.01[c]	1.62	1.62	1.43	1.09
0.25 M HCl	0.92	1.27[a]	1.47	1.45	1.41	2.52[b]	1.00[c]	0.98[c]	1.61	1.54	1.38	1.09
Addition of Ca^{2+} ions												
0 mM Ca^{2+}	4.59	1.37	1.51	1.48	1.42	1.38	1.21[a]	1.02[c]	1.66	1.63	1.44	1.10
20 mM Ca^{2+}	4.60	1.21[a]	1.51	1.49	1.44	1.25[a]	1.01[c]	1.01[c]	1.72	1.65	1.47	1.10
40 mM Ca^{2+}	4.61	1.16[a]	1.50	1.49	1.62[b]	1.06[a]	1.00[c]	1.01[c]	1.68	1.59	1.48	1.10
60 mM Ca^{2+}	4.62	1.13[a]	1.52	1.50	1.54[a]	1.00[c]	1.00[c]	1.01[c]	1.70	1.65	1.50	1.09
Addition of Ca^{2+} ions and 8% isopropyl alcohol												
0 mM Ca^{2+}	4.68	1.30[a]	1.55	1.54	1.45	1.43	1.06[a]	1.02[c]	1.71	1.69	1.52	1.09
20 mM Ca^{2+}	4.68	1.15[a]	1.57	1.57	1.82[b]	1.04[c]	1.01[c]	1.02[c]	1.76	1.70	1.53	1.09
40 mM Ca^{2+}	4.69	1.12[a]	1.61	1.57	1.29[a]	1.03[c]	0.98[c]	1.01[c]	1.81	1.75	1.56	1.10
60 mM Ca^{2+}	4.69	1.05[c]	1.63	1.57	1.15[a]	1.00[c]	0.99[c]	0.99[c]	1.77	1.74	1.53	1.10
Addition of Ca^{2+} ions and 16% isopropyl alcohol												
0 mM Ca^{2+}	4.74	1.30[a]	1.68	1.57	1.47	1.25[a]	1.02[c]	0.99[c]	1.84	1.79	1.56	1.09
20 mM Ca^{2+}	4.72	1.12[a]	1.65	1.59	1.27[a]	1.01[c]	0.99[c]	1.00[c]	1.85	1.85	1.60	1.08
40 mM Ca^{2+}	4.72	1.03[c]	1.69	1.62	1.09[a]	1.01[c]	0.99[c]	1.00[c]	1.86	1.82	1.59	1.09
60 mM Ca^{2+}	4.73	1.03[c]	1.70	1.68	1.03[c]	1.01[c]	0.99[c]	0.99[c]	1.85	1.85	1.60	1.10

[a] Partially precipitated.
[b] Increase of viscosity from calcium sensitivity or weak acid gelation.
[c] Completely precipitated.

precipitated in excess of Ca^{2+} ions, while the longer esterase-treated SBPs were partially precipitated at 20 mM Ca^{2+} and were almost completely precipitated at reduced water activity.

SBP6230, BRP5926, the series of base-deacetylated sugar beet pectin (B′ series), the weakly base-deesterified SBPs B5326 and B4626, and the methyl-esterified sugar beet pectin gave no viscosity increase and did not show phase separation upon pH decrease, addition of Ca^{2+} ions, and reduction of the water activity. B0915 and B0100 gave high-viscous solutions already when the pH decreased to 2.4–2.6 and precipitated completely at lower pH. B2516 gave a small increase in viscosity by lowering the pH, while the viscosity of B3124 was unaffected at low pH. At pH 4.7 B0100 was almost insoluble, while B0915 was partially soluble in the absence of Ca^{2+} but precipitated completely in the presence of Ca^{2+} ions. B2516 was soluble in the absence of Ca^{2+} ions but precipitated gradually with increasing Ca^{2+} ion concentration. B3124 was weakly calcium sensitive. B0915 precipitated completely at reduced water activity, while B2516 and B3124 required the presence of Ca^{2+} ions to precipitate.

SBP6230, BRP5926, the B′ series as well as B5326 and B4626 from the B series were not calcium sensitive.

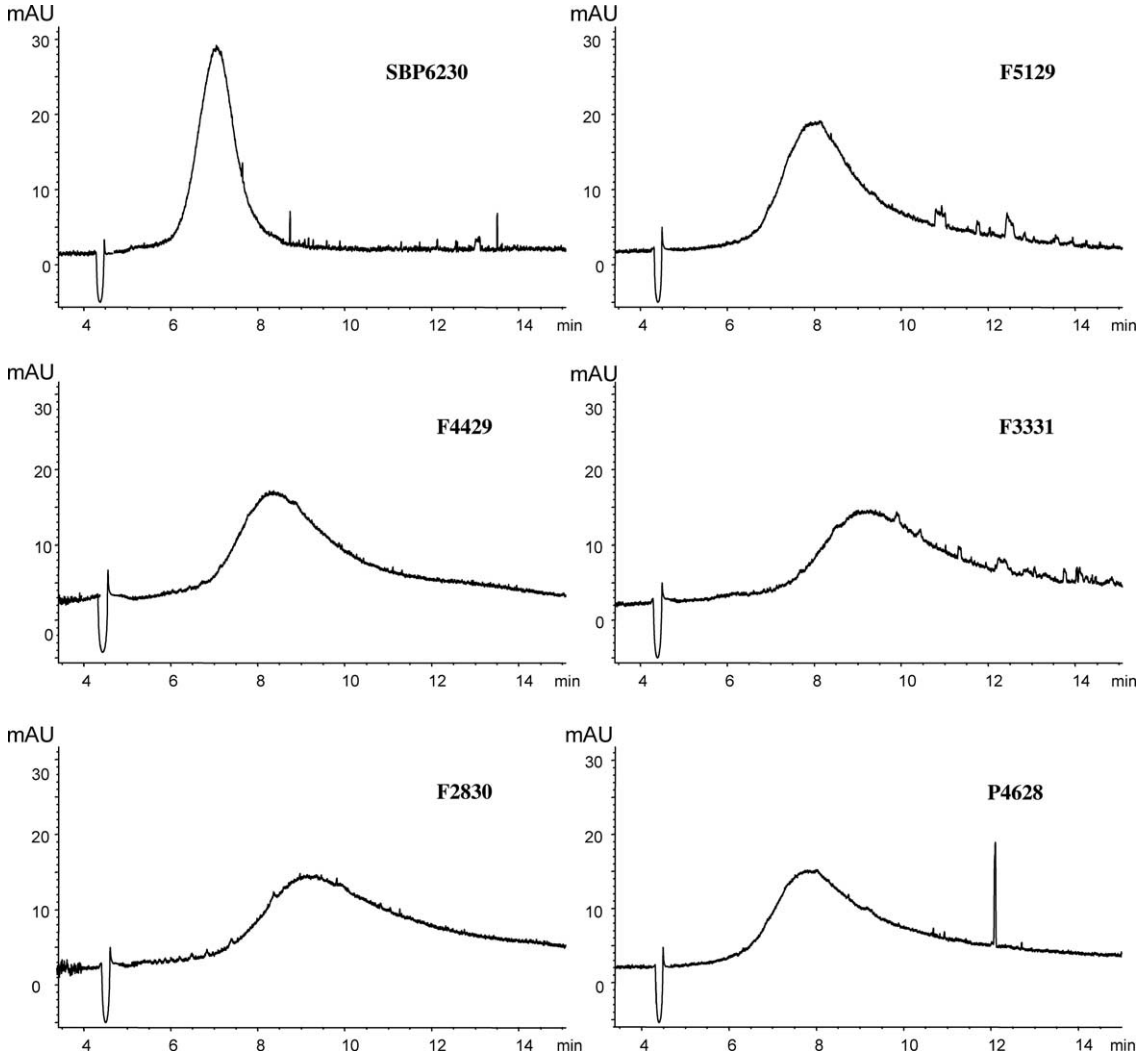

Fig. 2. CE electropherograms of the F series pectin and the starting material SBP6230.

3.4. Electrophoretic characterisation of modified SBPs

CE elution profiles of modified SBPs are shown in Figs. 2 and 3. The mobility of the SBP sample depends on the number of charges per unit of hydrodynamic volume or the charge density of the SBP molecules, essentially reflecting the intermolecular distribution of non-esterified galacturonic acid groups. Deesterification of SBP with fungal pectin esterase gave pectins with broader peak widths compared with SBP6230, indicating a broader variation of intermolecular distribution of non-esterified galacturonic acid groups (Fig. 2). The deesterified SBPs eluted later than the starting material (SBP6230) due to a lower degree of methyl esterification. Similar electrophoretic elution patterns were seen in SBPs deesterified with plant pectin esterase and chemically deacetylated SBPs (the results are not included). The samples B3124, B2516, B0915 and B0100, which were all significantly demethylated and deacetylated, showed two or three separated peaks in the electropherograms (Fig. 3). This clearly demonstrated the presence of several fractions of SBP with different charge densities. The increased degree of methyl esterification by methylation of SBP resulted in a DM distribution similar to that of the starting material, SBP6230, but the peaks were eluting earlier. The migration time relative to the electro-osmotic flow showed a linear correlation to DM with $R^2 = 0.94$ for SBPs randomly demethylated by fungal pectin esterase or by partial saponification. Migration times were not significantly different between the F series and the B series of modified SBPs (Fig. 4). The P series of modified SBPs showed slightly lower migration times than for the F and B series. Jiang, Wu, Chang, and Chang (2001) found that the migration time and DM in block-wise deesterified citrus pectins did not correlate linearly but gave a concave curve. The few results from the SBP P series did not show a similar pattern. A commonly accepted hypothesis (Micheli, 2001) is that p-PE deesterifies pectin via a processive mechanism producing blocks of deesterified galacturonic acid regions. However, the CE analysis suggested that p-PE may demethylate sugar beet pectin in a random, block-wise manner due to the steric hindrance of the acetyl ester group present.

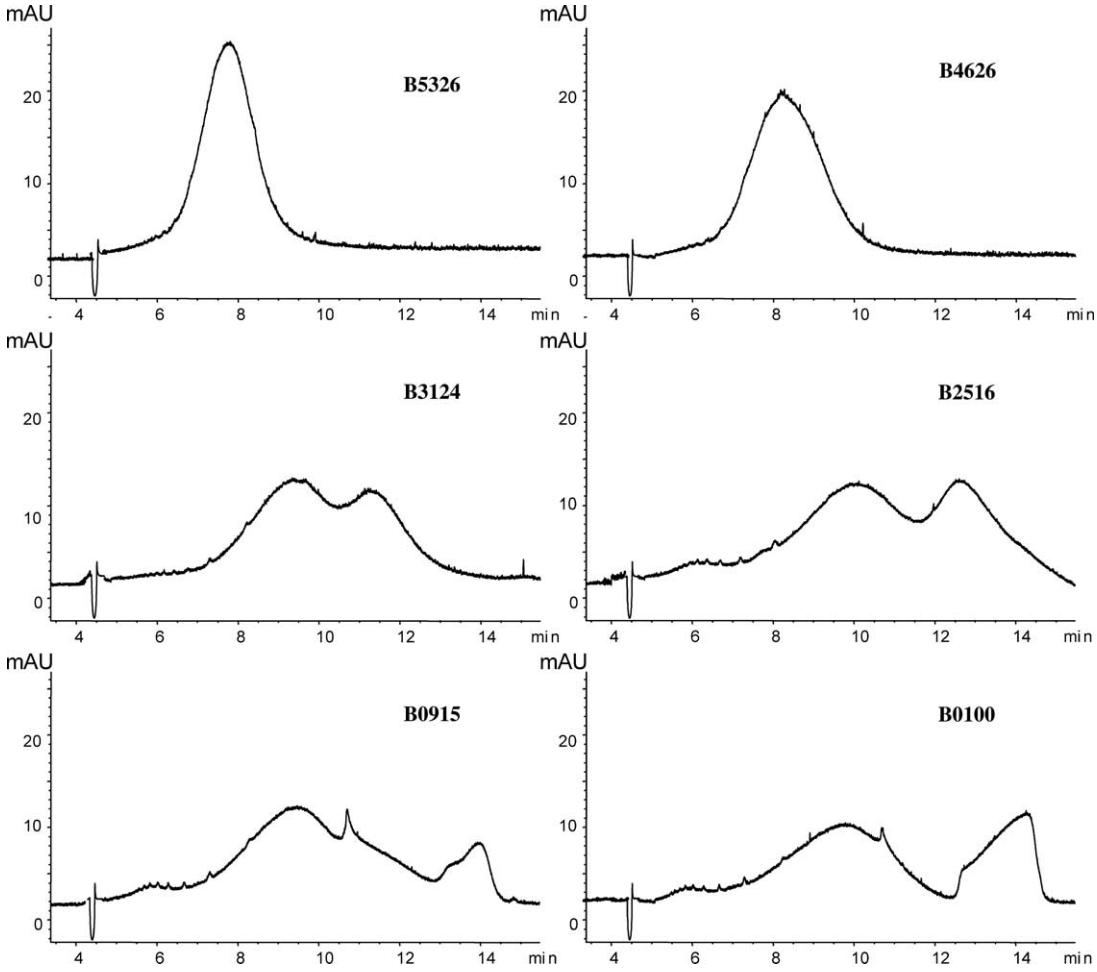

Fig. 3. CE electropherograms of the B series of SBP pectin.

3.5. Characterisation of modified SBPs by enzymatic fingerprinting

Results from the use of combined endo- and exo-PGs in Fig. 5 showed that the level of digestible regions, determined as the amount of released galacturonic acid, increased linearly as the degree of methyl esterification decreased. The amount of released GalA was a little higher for the P series at the same degree of methyl esterification compared with the SBP B and F series.

It also appeared that a slightly higher amount of GalA was released from the F series compared with similar SBPs from the B series. This could be explained by a slightly higher DM of the galacturonan in the B series, while chemical deesterification would be expected to proceed at similar rates in hairy regions and galacturonan chains. Demethylation by p-PE as well as by f-PE proceeded only in the galacturonan chain, which was consequently expected to be more demethylated compared with the same type of

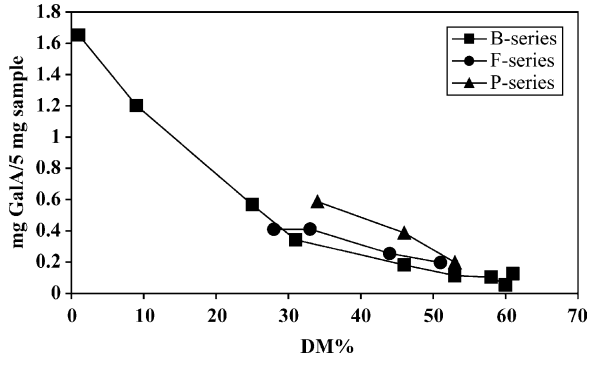

Fig. 4. Relative migration time in CE versus DM% of modified SBPs.

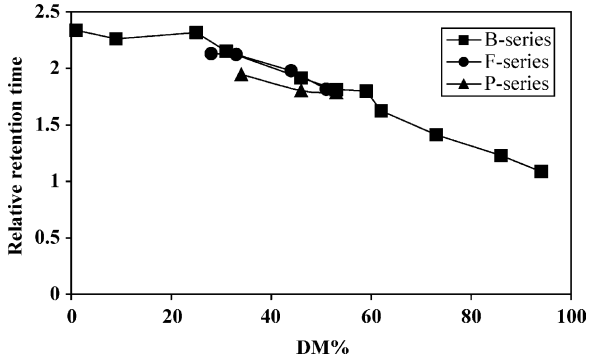

Fig. 5. Release of galacturonic acid by digestion of modified SBPs with endo-/exo-PG.

base-deesterified SBP. This could be the cause for the higher release of GalA by the enzymatic fingerprinting analysis. Similar results were found by enzymatic fingerprinting analysis of lime pectins deesterified by the same methods (Limberg et al., 2000b). The higher release of GalA from SBPs when using p-PE showed that this enzyme was demethylating SBP (as well as lime pectin) in a block-wise manner.

The enzymatic fingerprinting quantifies the amount of GalA residues present in block sequences of galacturonan. When comparing results from enzymatic fingerprinting of modified SBPs with similar series of modified lime pectins, no significant difference was found in the increase in the amount of GalA between B, F and P series of SBPs and corresponding series of lime pectins. This indicates that the deesterifying mechanism is the same, irrespective of the source of pectin, and that the p-PE causes block-wise demethylation, whereas f-PE demethylates randomly.

By further demethylation of the SBPs in the B series the amount of released GalA increased almost linearly from approx. 0.06 g of GalA/g of sample for B3124 to 0.33 g of GalA/g of sample for B0100. The amount of released GalA was increased with decreasing DM of the SBP. Deacetylation of the SBP significantly increased the PG activity, which was reflected by combined endo-/exo PG digestion.

4. Conclusion

Acid-extracted SBPs and chemically or enzymatically modified SBPs were composed of 55–70% anhydrogalacturonic acid, approx. 0.8% ferulic acid, 5–5.5% rhamnose, 10–12% arabinose, 10% galactose and traces of xylose and glucose. The last 6–10% consisted of moisture and salts.

Solutions (1%) of esterified, weakly base-deesterified and base-deacetylated sugar beet pectin as well as non-modified sugar beet pectin showed no phase transitions or viscoelastic changes in properties by lowering of pH, addition of Ca ions and lowering of water activity by addition of alcohol. Base-deesterified products were completely precipitated by an excess of Ca^{2+} ions below a degree of methylation of approx. 25, while enzymatically deesterified products with a DM of 34–28 were only partially precipitable, showing that enzymatically deesterified products have a more heterogeneous methyl ester distribution.

The modified SBPs showed fairly high intrinsic viscosity values. The E series showed a higher polydispersity index, and HPSEC analyses showed the presence of two populations.

CE further supported the heterogeneous composition of the modified SBPs, where the electropherograms in general showed broad peaks, reflecting broad intermolecular distributions of free acid groups.

Enzymatic fingerprinting clearly showed that the amount of galacturonic acid released from the P series was higher than from the B and F series for SBPs with the same degrees of methylation and acetylation. This showed a higher content of block sequences of free acid groups in SBPs modified by p-PE.

Acknowledgements

Financial support from the European Community within the Europectin QLK3-1999-00089 Program is gratefully acknowledged. We also wish to thank Hanne Hoberg, Lise Andersen, Lone Rasmussen and Dorthe Stenbaek for skillful technical assistance and Jannie Rohde Nielsen for preparing the manuscript.

References

Anon. (1981). Pectin. In *Food chemicals codex* (3rd ed., pp. 283–286). Washington DC: National Academy Press.

Arslan, N. (1995). Extraction of pectin from sugar beet pulp and intrinsic viscosity–molecular weight relationship of pectin solutions. *Journal of Food Science and Technology*, *32*, 381–385.

Berth, G., Dautzenberg, H., & Hartmann, J. (1994). Static light scattering technique applied to pectin in dilute solution. Part III: The tendency for association. *Carbohydrate Polymers*, *25*, 197–202.

Buchholt, H. C., & Vinther, H. (1991). Pectin containing product and method for producting same from agricultural waste. PCT international application. WO 91 15,517.

Christensen, T. M. I. E., Nielsen, J. E., Kreiberg, J. D., Rasmussen, P., & Mikkelsen, J. D. (1998). Pectin methyl esterase from orange fruit: Characterization and localization by in situ hybridization and immunohistochemistry. *Planta*, *206*, 493–503.

Dea, I. C. M., & Madden, J. K. (1986). Acetylated pectic polysaccharides of sugar beet. *Food Hydrocolloids*, *1*, 71–88.

Desforges, M., Cooper, J. M., & Williams, E. L. (1995). Process for making soluble beet fibre. *European Patent Application*, 0,649,602.

Englyst, H. N., & Cummings, J. H. (1988). Improved method of measurement of dietary fiber as non-starch polysaccharides in plant foods. *Journal of Association of Official Analytical Chemistry*, *71*, 808–814.

Fishman, M. L., Gillespie, D. T., Sondey, S. M., & Barford, R. A. (1989). Characterization of pectins by size exclusion chromatography in conjunction with viscosity detection. *Journal of Agricultural and Food Chemistry*, *37*, 584–591.

Grand, F. le, & Stevens, N. C. (1991). Process for producing pectin with a high-to-medium methoxyl content from beet pulp. *United States Patent*, 5,071,970.

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.

Jiang, C.-M., Wu, M.-C., Chang, W.-H., & Chang, H. M. (2001). Determination of random- and blockwise-type deesterified pectins by capillary zone electrophoresis. *Journal of Agricultural and Food Chemistry*, *49*, 5584–5588.

Kontaminas, M. G., & Kokini, J. L. (1990). Measurement of molecular parameters of water soluble apple pectin using low angle laser light scattering. *Lebensmittel Wissenschaft und Technologie*, *23*, 174–177.

Levigne, S., Ralet, M.-C., & Thibault, J.-F. (2002). Characterisation of pectins extracted from fresh sugar beet under different conditions using an experimental design. *Carbohydrate Polymers*, *49*, 145–153.

Limberg, G., Körner, R., Buchholt, H. C., Christensen, T. M. I. E., Roepstorff, P., & Mikkelsen, J. D. (2000a). Analysis of different deesterification mechanisms for pectin by enzymatic fingerprinting using endopectin lyase and endopolygalacturonase II from *A. niger*. *Carbohydrate Research*, *327*, 293–307.

Limberg, G., Körner, R., Buchholt, H. C., Christensen, T. M. I. E., Roepstorff, P., & Mikkelsen, J. D. (2000b). Quantification of the amount of galacturonic acid residues in block sequences in pectin homogalacturonan by enzymatic fingerprinting with exo- and endo-polygalacturonase II from *Aspergillus niger*. *Carbohydrate Research*, *327*, 321–332.

Marry, M., McCann, M. C., Kolpak, F., White, A. R., Stacey, N. J., & Roberts, K. (2000). Extraction of pectic polysaccharides from sugar-beet cell walls. *Journal of Science of Food and Agriculture*, *80*, 17–28.

Matthew, J. A., Howson, S. A., Keenan, M. H. J., & Belton, P. S. (1990). Improvement of the gelation properties of sugar beet pectin following treatment with an enzyme preparation derived from *Aspergillus niger*—Comparison with a chemical modification. *Carbohydrate Polymers*, *12*, 295–306.

Micheli, F. (2001). Pectin methyl esterases: Cell wall enzymes with important roles in plant physiology. *Trends in Plant Science*, *6*, 414–419.

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

Oosterveld, A., Beldman, G., & Voragen, A. G. J. (2002). Enzymatic modification of pectic polysaccharides obtained from sugar beet pulp. *Carbohydrate Polymers*, *48*, 73–81.

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 American Chemical Society*, *72*, 813–816.

Ralet, M.-C., Dronnet, V., Buchholt, H. C., & Thibault, J.-F. (2001). Enzymatically and chemically deesterified lime pectins: Characterisation, polyelectrolyte behaviour and calcium-binding properties. *Carbohydrate Research*, *336*, 117–125.

Ralet, M.-C., Thibault, J.-F., & Della Valle, G. (1991). Solubilization of sugar-beet pulp cell wall polysaccharides by extrusion-cooking. *Lebensmittel Wissenschaft und Technologie*, *24*, 107–112.

Rombouts, F. M., & Thibault, J.-F. (1986a). 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* (Vol. 310) (pp. 49–60). Washington, DC: American Chemical Society.

Rombouts, F. M., & Thibault, J.-F. (1986b). Feruloylated pectic substances from sugar-beet pulp. *Carbohydrate Research*, *154*, 177–188.

Sun, R., & Hughes, S. (1998). Extraction and physico-chemical characterization of pectins from sugar beet pulp. *Polymer Journal*, *30*, 671–677.

Takahashi, T., Furuta, H., Tobe J., & Kiwata, R. (1999). Acidic protein foods and process for their production. *European Patent Application*, 0,958,746.

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

Thibault, J.-F., Guillon, F., & Rombouts, F. M. (1991). Gelation of sugar beet pectins by oxidative coupling. In R. Walter (Ed.), *The chemistry and technology of pectins* (pp. 109–118). New York: Academic Press.

Thibault, J.-F., Renard, C. M. G. C., & Guillon, F. (2000). Sugar beet fiber. Production, composition, physicochemical properties, physiological effects, safety, and food applications. In S. S. Cho, & M. L. Dreher (Eds.), *Handbook of dietary fiber* (pp. 553–582). New York: Marcel Dekker.

Towle, G. A., & Christensen, O. (1973). Pectin. In R. Whistler (Ed.), *Industrial gums*2nd ed. (p. 430). New York: Academic Press.

Turquois, T., Rinaudo, M., Taravel, F. R., & Heyraud, A. (1999). Extraction of highly gelling pectic substances from sugar beet pulp and potato pulp: Influence of extrinsic parameters on their gelling properties. *Food Hydrocolloids*, *13*, 255–262.

Weibel, M. K. (1991). Sugar beet pectins and their use in comestibles. *United States Patent* 5,008,254.

Williamson, G., Faulds, C. B., Matthew, J. A., Archer, D. B., Morris, V. J., Brownsey, G. J., & Ridout, M. J. (1990). Gelation of sugar beet and citrus pectins using enzymes extracted from orange peel. *Carbohydrate Polymers*, *13*, 295–306.