

Polyelectrolyte behaviour and calcium binding properties of sugar beet pectins differing in their degrees of methylation and acetylation

Marie-Christine Ralet^{a,*}, Marie-Jeanne Crépeau^a, Hans-Christian Buchholt^b,
Jean-François Thibault^a

^a *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*

^b *Danisco, Innovation, Edwin Rahrs Vej 38, DK-8220 Brabrand, Denmark*

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Abstract

Five series of pectins with different levels and patterns of methyl esterification and/or different levels of acetyl esterification were produced by chemical or enzymatic treatment of an acid-extracted sugar beet pectin. The relationship between pK_a and the degree of dissociation and the free fractions of monovalent and calcium counterions were determined on dilute pectin salt-free solutions. Calcium sensitivity was determined in more concentrated solutions. The presence of acetyl groups hindered the formation of calcium-pectinate precipitates in different manners according to the demethoxylation mode and the pectin concentration. The base-deesterified pectins appeared to have the most homogeneous methyl ester distribution. Pectins demethoxylated by fungus-PME exhibited a peculiar distribution of free galacturonic acid residues. Blocks of contiguous free galacturonic acid were generated by treatment of sugar beet pectin with a plant-PME. Those blocks were not long enough to induce abnormal polyelectrolyte behaviour or to promote dimerisation of pectic molecules in dilute solution but allow the formation of calcium-pectinate precipitates in concentrated medium for high DM.

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1. Introduction

Pectins are ionic plant polysaccharides that are widely used in food industry for their gelling properties. Their main structural features include a backbone of (1 → 4)-linked α -D-galacturonic acid units. These “smooth” homogalacturonic regions are interrupted by “hairy” rhamnogalacturonic regions in which galacturonic acid residues are interspersed with (1 → 2)-linked α -L-rhamnopyranosyl residues. Some rhamnosyl residues are substituted by arabinose- and galactose-containing side chains while galacturonic acid residues can be partially esterified by methanol on the carboxyl group and by acetyl on the secondary hydroxyls [1]. Highly methoxylated pectins (HM) gel in an acidic medium on addition of sucrose. Lowly methoxylated pectins (LM) cross-link *via* calcium bridges. The impact of both the degree of methyl-esterification (DM) and the repartition of the methyl groups along the pectic molecules on the binding of calcium ions has been extensively studied [2–5]. The gel

forming ability in the presence of calcium ions increases with decreasing DM and it is generally agreed that a transition in calcium affinity towards randomly charged pectins (base or fungus-PME deesterified) occurs around a DM of 40 [4–6]. Pectins (DM < 60%) with a blockwise distribution of free carboxyl groups, such as those deesterified by plant-PME, were characterised by low calcium activity coefficients, close to that of calcium-pectate [3–5]. Such pectins were shown to be highly calcium sensitive [7].

Native pectins are usually highly methoxylated and lowly acetylated. However, there are some highly acetylated pectins, especially in sugar beet, but also in carrot, potato and Cythere plum [1]. Sugar beet pectins exhibit several peculiar structural characteristics: (i) a high content in neutral sugars side-chains, especially arabinans; (ii) the presence of ferulic acid residues ester-linked to arabinose and galactose units within the pectic side-chains [8,9]; (iii) a high acetyl content [10]. *O*-acetyl groups can be attached on both of the available ring positions (2 and 3) of galacturonic acid units [11]. Furthermore, Rombouts and Thibault [12] have shown that 80–90% of the acetyl groups are located in the “smooth” regions through O-2 and/or O-3 of the galacturonic

* Corresponding author. Tel.: +33-240675064; fax: +33-240675066.
E-mail address: ralet@nantes.inra.fr (M.-C. Ralet).

acid units and that they are fairly regularly distributed along the chains. Sugar beet pectins poor gelling properties have been attributed mainly to their high acetyl content [13].

Pippen et al. [10], by chemical acetylation of citrus pectins, showed that gelation with calcium was rapidly hindered (degree of acetylation, DAc 15–20 as calculated by Renard and Jarvis [14]). A selective removal of acetyl groups led to the re-establishment of gelling properties. Acetylation decreased the stability of binding of calcium by pectin and pectic acid [15,16]. This was ascribed to a steric effect of acetyl groups that prevent, to a certain extent, the access of calcium ions to a close proximity of two neighbouring carboxyl groups. More recently, Renard and Jarvis [17], by using partially methylated and acetylated homogalacturonans, showed that acetylation had drastic effects on the binding of calcium. Effects of acetylation may be attributed to modifications of conformation and complexation. Presence of acetyl groups could lower the strength of binding of the cation to individual galacturonic acid residues or hinder adoption by the polymer of binding-favourable conformations.

Several attempts have been made to improve gel formation of beet pectins with calcium, mainly by decreasing their content in acetyl groups. Matthew et al. [18] treated sugar beet pectins with an enzymatic preparation derived from *Aspergillus niger*. They induced some deacetylation and demethoxylation, and also a large reduction in the arabinose content. The resulting pectins exhibited largely enhanced gelling properties. Williamson et al. [19] induced gelation of the same sugar beet pectin after treatment with a partially purified pectin acetyl esterase containing some pectin methyl esterase activity. The sugar beet pectin used in these studies exhibited a particularly low degree of acetylation (DAc \sim 7) and the removal of only a small percentage of these acetyl groups led to greatly improved gelling properties. Recently, Oosterveld et al. [20] used highly purified enzymes to study the effect of methyl esters, acetyl groups and neutral sugar side-chains on the gelation properties of sugar beet pectin with calcium. The removal of only 13.8% of the acetyl groups was shown to increase the action of pectin methyl esterase and to improve the gelling capacity of sugar beet pectin with calcium. Arabinose side chains had a relatively small influence on gel formation with calcium. The arabinose content of sugar beet pectins used in this study was however low and the arabinofuranosidase action might have been very limited.

In the present study, an acid-extracted sugar beet pectin (SBP 62-30; DM: 62%; Dac: 30%) has been treated with plant and fungal pectin methyl esterases. Two series of enzymatically de-methoxylated pectins were thereby generated. SBP 62-30 was also treated with base in aqueous and in methanol medium to generate a series of de-methoxylated and de-acetylated pectins, and a series of de-acetylated pectins, respectively. Finally, SBP 62-30 was esterified in methanolic medium. The dissociation of these pectin samples in dilute salt-free solutions was followed by po-

tentiometric titration and the degree of calcium binding was determined by conductimetry and spectrophotometry. Pectins calcium sensitivity was followed by viscosimetry on more concentrated pectin solutions. The effect of methyl esters (content and repartition), acetyl groups and, to a certain extent, neutral sugar side-chains on the polyelectrolyte behaviour and calcium binding properties of sugar beet pectins are discussed on the basis of these results.

2. Experimental

2.1. Material

Polygalacturonic acid (PGA) and tetramethylmurexide (TMMX) are from Sigma, LiOH from Prolabo and NaOH, KOH and Ca(OH)₂ from Merck.

2.2. Preparation of model pectins

The acid-extraction of the mother pectin SBP 62-30 and the preparation of modified model pectins has been performed by Danisco (Denmark) and detailed reaction conditions are described elsewhere [21].

SBP 62-30 has been modified by enzymatic and chemical methods to generate five series of model pectins (Fig. 1). SBP 62-30 was treated with plant or fungal pectin methyl esterases. Two series of enzymatically de-methoxylated pectins (P- and F-series) were thereby generated. SBP 62-30 was also treated with base in aqueous, and in methanolic medium to generate a series of de-methoxylated and de-acetylated pectins (B-series), and a series of de-acetylated pectins (B'-series), respectively. Finally, SBP 62-30 was esterified at low temperature in acidified methanol to generate an E-series.

2.3. Analytical

All values were calculated on a moisture-free basis. Galacturonic acid (GalA) and neutral sugars (expressed as arabinose) were quantified colorimetrically by the automated *m*-phenylphenol [22] and orcinol [23] methods, respectively, the latter being corrected for interfering galacturonic acid.

2.4. Ion-exchange chromatography

Chromatography on DEAE-Sepharose CL-6B was performed on a column (31 cm \times 2.6 cm) equilibrated with 0.05 M sodium succinate pH 4.5 at a flow-rate of 100 ml/h. Samples (50 ml of a solution at 4 mg/ml in distilled water) were loaded onto the column and the gel was washed with 500 ml of buffer. The bound material was eluted with a linear NaCl gradient (0–0.6 M; 2 l); 15 ml fractions were collected and analysed.

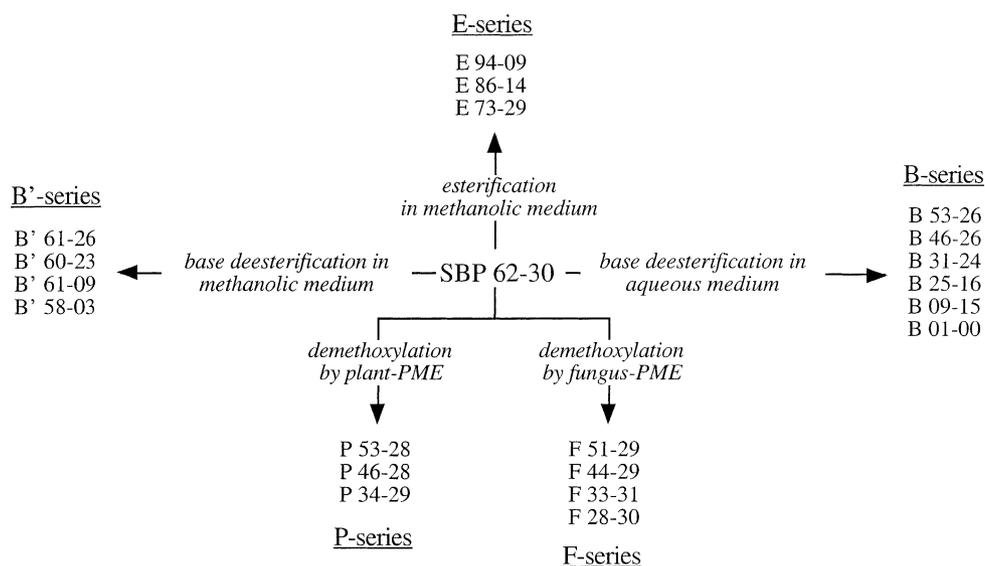


Fig. 1. Schematic presentation of the different series of model sugar beet pectins.

2.5. Potentiometric measurements

Pectin samples were extensively washed with 65% aqueous ethanol in order to eliminate salt traces. Pectins were dissolved in ultra-pure water at ~ 7 meq/l under magnetic stirring overnight at room temperature. Percolating the sample through a strong H^+ -exchanger (Rohm & Hass Amberlite IR 120) allowed the recovery of pectin samples in the acidic form at a concentration (C_p) of ~ 1 meq/l.

pH measurements were performed at 25.0 ± 0.2 °C with a pH-meter LPH 430 T (Radiometer Analytical SA) fitted out with a combined pH-electrode Ingold (type U 402-S7/120) and a temperature probe (XT 130, Radiometer). The pH-meter response was calibrated before each set of measurements between pH 4 and 7. The titrations were performed on pectin samples in the acidic form ($C_p \sim 1$ meq/l) with freshly prepared 10 meq/l NaOH solution. From the degree of neutralisation α' and the pH value for each neutralisation step, the degree of dissociation α and the apparent pK (pK_a) of the polyelectrolyte were calculated from Eqs. (1) and (2):

$$\alpha = \alpha' + \frac{[H^+]}{C_p} \quad (1)$$

$$pK_a = pH + \log \left(\frac{1 - \alpha}{\alpha} \right) \quad (2)$$

The treatment of experimental data was deduced from the model of Lifson and Katchalsky [24]. The structural charge density ($\bar{\xi}$) was calculated from Eq. (3):

$$\bar{\xi} = \frac{\varepsilon_2}{bDkT} \times \frac{100 - DM}{100} = 1.61 \times \frac{100 - DM}{100} \quad (3)$$

where ε is the electron charge, kT the Boltzmann term, b the length of the monomeric unit (0.435 nm) [25], D the

dielectric constant of the solvent and DM is the degree of methylation. The effective charge density (ξ) can be calculated from Eq. (4) and compared to the critical value (ξ_0) given by Eq. (5):

$$\xi = \alpha \bar{\xi}_0 \quad (4)$$

$$\xi_0 = \frac{\ln(R/a)}{1 + \ln(R/a)} \quad (5)$$

where R , the radius of the cylindrical subvolume, is calculated from C_p , and a , the minimum distance of approach, taken as 0.6 nm [26].

The intrinsic dissociation constant (pK_0) can be obtained by superimposing the experimental curves $pK_a = f(\alpha)$ on the theoretical ones $\Delta pK = f(\alpha)$, as described by Rinaudo and Milas [26].

2.6. Conductimetric measurements

Transport parameters were determined using conductimetric measurements as already described [4,27]. They are related to the free fraction of the considered counterion. All conductimetric measurements were carried out in triplicate (S.E. < 5%) at 25.0 ± 0.2 °C with a CDM 83 conductimeter (Radiometer Analytical SA) equipped with a double platinum electrode CDC 241U (Radiometer Analytical SA). The cell constant was determined with 0.05% (w/w) NaCl before each set of measurements. The titrations were performed with freshly prepared 10 meq/l solutions of KOH, LiOH and $Ca(OH)_2$. The limiting law for the equivalent conductivity of polyelectrolyte without external salts is given by

$$\Lambda = f(\lambda_c + \lambda_p) \quad (6)$$

where Λ is the equivalent conductivity ($S\text{cm}^2/\text{eq}$) of the salts in solution, λ_p , the equivalent conductivity of the active

monomer carried by the polyelectrolyte, λ_c , the equivalent conductivity of the isolated counterion in pure solvent at infinite dilution at 25 °C and f is the transport parameter.

By measuring the conductivity of three ionic forms of the polyelectrolyte (Li-, K- and Ca-forms) and by considering transport parameter independent of the nature of the monovalent counterion, the transport parameters for monovalent ($f_{\text{Li}^+, \text{K}^+}$) and calcium ($f_{\text{Ca}^{2+}}$) cations, and the equivalent conductivity of the polyelectrolyte (λ_p), can be calculated.

The calcium activity coefficients at the neutralisation point ($\gamma_{\text{Ca}^{2+}}$) were determined in triplicate (S.E. < 5%) by means of a dual-wavelength spectrophotometric method using TMMX as an activity probe for calcium ions [28]. A calibration curve was obtained using CaCl_2 solutions. Values reported correspond to the ratio of the activity of calcium ions in the presence of pectins to the activity of calcium ions in ideal CaCl_2 solutions at the same ionic concentrations.

The transport parameter values and activity coefficients were compared with theoretical predictions from Manning's model [29–31]. For $z\bar{\xi} < 1$:

$$f = 1 - \frac{0.55(|z|\bar{\xi})^2}{|z|\bar{\xi} + \pi} \quad (7)$$

$$\gamma = e^{-((|z|\bar{\xi})/2)} \quad (8)$$

and for $z\bar{\xi} \geq 1$:

$$f = \frac{0.87}{|z|\bar{\xi}} \quad (9)$$

$$\gamma = \frac{e^{-1/2}}{|z|\bar{\xi}} \quad (10)$$

where z is the charge of the counterion.

2.7. Determination of calcium sensitivity

Calcium sensitivity (CS) is defined as the relative increase of viscosity of a pectin solution in the presence of calcium ions [32]. 2 ml of a 1 M sodium acetate buffer pH 4.5 (reference –Ca) or 2 ml of a 40 mM CaCl_2 in 1 M sodium acetate buffer pH 4.5 (test +Ca) were added to 2 ml of a stirred 2% aqueous solution of sugar beet pectin. The solutions were stirred for 30 min at room temperature and centrifuged at $2500 \times g$ for 10 min to separate any precipitated material. The viscosity of the clear supernatants was measured at 22 °C in a thermostatically controlled bath by measuring the time ($t_{+\text{Ca}}$, $t_{-\text{Ca}}$) for emptying the reservoir of a 2 ml analytical DIN transfer pipette, using the same pipette for all experiments. The calcium sensitivity is defined as follows:

$$\text{CS} = \frac{t_{+\text{Ca}} - t_{-\text{Ca}}}{t_{-\text{Ca}}} \quad (11)$$

3. Results

Four series of demethoxylated and/or deacetylated pectins were prepared by chemical or enzymatic treatment of acid-extracted sugar beet pectin (SBP 62-30; DM: 62%; DAc: 30%). The sugar composition and macromolecular characteristics of sugar beet pectins are published elsewhere [21]. SBP 62-30 exhibited an overall sugar composition in close agreement with previously published data [33] with 548 mg/g dry matter of galacturonic acid, 123 mg/g of arabinose, 104 mg/g of galactose and 53 mg/g of rhamnose. Traces of glucose and xylose were also detected. Pectins from the F- and P-series (fungus and plant-PME, respectively) exhibited a sugar composition similar to that of SBP 62-30. The methyl groups (18–55 and 15–45%) were released for F- and P-series, respectively, while no acetyl groups were released. Pectins from the B- and B'-series (deesterified by base in aqueous or methanolic medium, respectively) were only slightly impoverished in arabinose (101–121 mg/g dry matter) and galactose (78–103 mg/g dry matter), all the more the DM and/or DAc decreased. The methyl groups (15–98 and 0%) were released for B- and B'-series, respectively. The acetyl groups (13–100 and 13–90%) were released for B- and B'-series, respectively. A fifth series of pectins of high DM was obtained by esterification of SBP 62-30 (E-series). Pectins from the E-series were drastically impoverished in arabinose (17–82 mg/g dry matter), all the more the DM increased. Methyl content was increased by 118–152 and 3–70% of the acetyl groups were released for this series.

3.1. Ion-exchange chromatography

Ion exchange chromatography on DEAE-Sepharose CL-6B was used to fractionate the initial beet pectin SBP 62-30, and three pectins of similar DM but obtained by different deesterification means (B 31-24, F 33-31 and P 34-29) (Fig. 2). Recoveries in galacturonic acid and neutral sugars were >95% for all samples. Neutral polysaccharides that were not bound to the column represented 4% of the total neutral and acidic sugars injected (14% of the neutral sugars injected) for SBP 62-30 and around 2% of the total sugars (around 9% of the neutral sugars) for B 31-24, F 33-31 and P 34-29. SBP 62-30 was eluted as a broad peak at an ionic strength of 0.19, in agreement with its DM [34]. B 31-24, F 33-31 and P 34-29 were eluted at similar ionic strengths (0.29, 0.30 and 0.30, respectively) in compliance with their close DMs. However, their elution patterns differed significantly. B 31-24 was eluted as a very thin peak in agreement with the random distribution of demethoxylated units on all pectic chains (single attack mechanism) reported for alkaline deesterification [35]. F 33-31 was eluted as a slightly broader peak. Limberg et al. [7] reported that fungus-PME and base treatment led to two different forms of homogeneous methyl esterification patterns on citrus pectins. This seems to be also the case for beet pectins. P 34-29 was

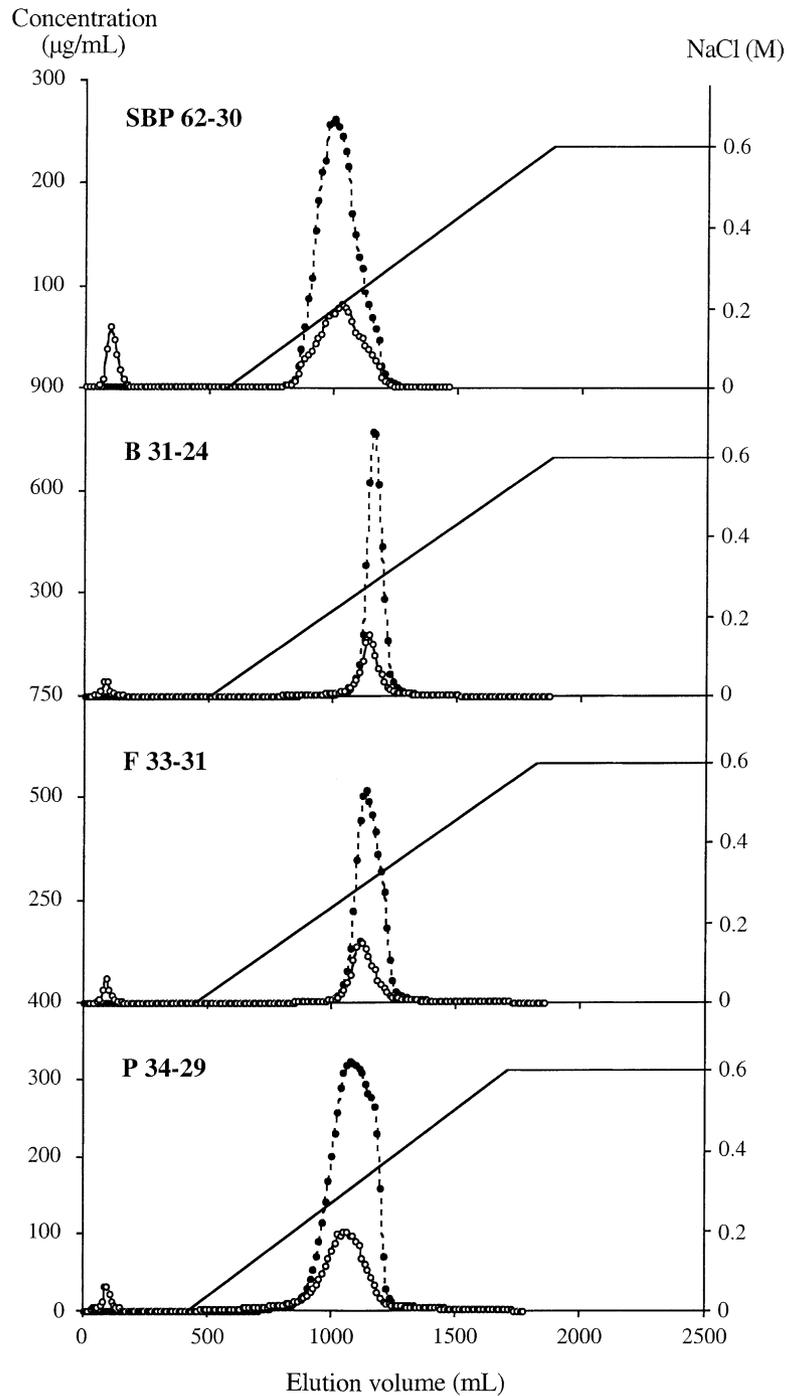


Fig. 2. Elution profiles of the mother pectin SBP 62-30 and of model pectins of similar DM but differing in their obtention mode (B 31-24, F 33-31, P 34-29) on ion-exchange chromatography (DEAE-Sepharose CL-6B): (●) galacturonic acid; (○) neutral sugars; (—) NaCl molarity.

eluted as a broader unsymmetrical peak. As previously reported for citrus pectins [34], a multiple attack mechanism leading to at least short blocks of demethoxylated units can be hypothesised for plant-PME treated beet pectins.

3.2. Polyelectrolyte behaviour

The relationships between pK_a and the degree of dissociation α of some pectins from the B-, E-, F- and P-series are

presented in Fig. 3 together with the Lifson and Katchalsky's theoretical curves $\Delta pK = f(\alpha)$. Pectins from the B'-series are not shown since they all behave as the initial pectin SBP 62-30. For pectins from the B-, B'-, and F-series, the polyelectrolyte behaviour observed was very similar to that of citrus pectins demethoxylated by base or fungus-PME [5]. For a given degree of dissociation, the pK_a decreased with an increase of DM. Experimental points were fitted satisfactorily by the theoretical curves for intermediate DMs (45% <

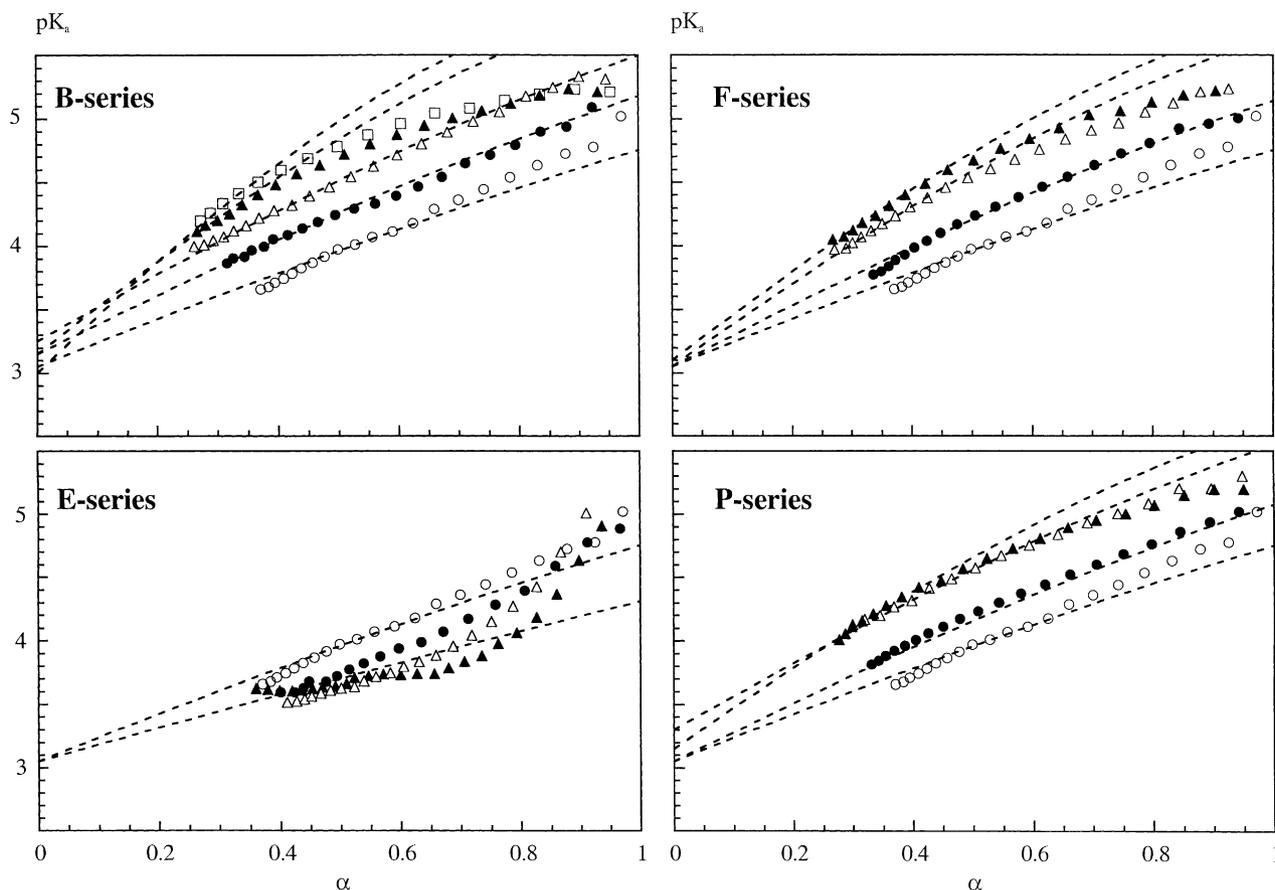


Fig. 3. Variation in pK_a with the degree of dissociation (α). Dotted lines are the corresponding theoretical $\Delta pK_a = f(\alpha)$ functions. B-series: (○) SBP 62-30; (●) B 53-26; (△) B 46-26; (▲) B 25-16; (□) B 09-15. E-series: (○) SBP 62-30; (●) E 73-29; (△) E 86-14; (▲) E 94-09. F-series: (○) SBP 62-30; (●) F 51-29; (△) F 33-31; (▲) F 28-30. P-series: (○) SBP 62-30; (●) P 53-28; (△) P 46-28; (▲) P 34-29.

DM < 62%). For lower DMs, the experimental values were in good agreement with the theoretical ones for low degrees of dissociation and progressively became lower than the theoretical ones for high degrees of dissociation. This can be attributed to ionic condensation that progressively takes place around a DM of 40–45% [36]. An intrinsic dissociation constant pK_0 of 3.1 ± 0.1 could be extrapolated for beet pectins from the B-, B'- and F-series. No differences in pK_0 could be detected neither between pectins of similar DM and different DAc (B'-series) nor between pectins of different DM and similar DAc (F-series), in agreement with previously published data [16,37]. For pectins from the E-series, pK_a curves revealed a concave curvature in agreement with previously published data on very highly methoxylated pectins [5,27]. The agreement between experimental and theoretical curves was very poor especially for high degrees of dissociation for which pectins from the E-series exhibited much higher values than expected from the Lifson and Katchalsky's theory. Rinaudo and Milas [26] suggested that for low-charge density, the uniformly charged-rod model is incorrect as long as the distance between two sites is larger than about 0.7 nm.

Citrus pectins demethoxylated by plant-PME were shown to have peculiar polyelectrolyte behaviour [5]. The block-wise arrangement of free carboxyl groups in these pectins

led to an excess of condensation that was evidenced by abnormally high initial slopes of the pK_a curves [5]. Conversely, beet pectins from the P-series exhibited the same behaviour than the other beet pectins studied. The adequacy between theoretical and experimental curves was good for intermediate DMs and, similarly to what was observed for pectins from the F- and B-series of close DM (F 33-31 and B 31-24), some condensation was evidenced for P 34-29. But contrary to what was observed for citrus pectins demethoxylated by plant-PME, no excess of condensation was revealed for beet pectins from the P-series. If blocks of deesterified galacturonic residues are generated by the plant-PME treatment, they might be not long enough to induce abnormal polyelectrolyte behaviour. Another hypothesis could be that long blocks of demethoxylated galacturonic acid residues are generated but that some acetyl groups are present within the blocks. This last hypothesis is quite improbable since acetyl groups are known to hinder the action of PME [20].

3.3. Interactions of pectins with cations

Interactions of chemically and enzymatically treated beet pectins with monovalent cations and calcium in dilute solution were quantified by conductimetry (Table 1). Calcium

Table 1
Monovalent and calcium transport parameter, calcium activity coefficient and calcium sensitivity of sugar beet model pectins

	f^+ exp	f^+ theo	f^{2+} exp	f^{2+} theo	γ^{2+} exp	γ^{2+} theo	t_{-Ca} (s)	t_{+Ca} (s)	CS
Acetate buffer	–	–	–	–	–	–	1.92	1.93	0.005
SBP 62-30	0.760 (0.014)	0.945	0.415 (0.007)	0.711	0.505 (0.035)	0.496	3.04	3.06	0.007
E 73-29	0.805 (0.007)	0.971	0.540 (0.028)	0.896	0.635 (0.007)	0.647	3.17	3.11	–0.019
E 86-14	0.800 (0)	0.992	0.655 (0.007)	0.969	0.800 (0.014)	0.798	3.25	3.24	–0.003
E 94-09	0.805 (0.021)	0.998	0.670 (0)	0.994	0.900 (0.014)	0.908	3.45	3.48	0.009
B 53-26	0.783 (0.025)	0.919	0.370 (0.010)	0.575	0.447 (0.021)	0.401	2.89	2.91	0.007
B 46-26	0.840 (0.020)	0.896	0.363 (0.012)	0.500	0.430 (0)	0.349	2.84	2.87	0.011
B 31-24	0.695 (0.021)	0.783	0.260 (0.014)	0.392	0.340 (0.028)	0.273	2.72	2.77	0.018
B 25-16	0.615 (0.007)	0.720	0.210 (0)	0.360	0.280 (0.028)	0.251	2.64	2.41	Partial precipitation
B 09-15	0.680 (0.085)	0.594	0.180 (0.014)	0.297	0.250 (0)	0.207	2.32	1.94	Complete precipitation
B 01-00	0.705 (0.021)	0.546	0.120 (0)	0.273	0.065 (0.035)	0.190	1.95	1.94	Complete precipitation
B' 61-26	0.775 (0.021)	0.942	0.425 (0.021)	0.693	0.530 (0.028)	0.483	3.18	3.31	0.041
B' 60-23	0.780 (0)	0.940	0.425 (0.007)	0.695	0.520 (0.014)	0.471	3.12	3.19	0.022
B' 61-09	0.765 (0.035)	0.942	0.420 (0.003)	0.693	0.505 (0.035)	0.483	2.77	2.83	0.022
B' 58-03	0.810 (0)	0.934	0.435 (0.007)	0.643	0.545 (0.021)	0.448	2.12	2.13	0.005
F 51-29	0.750 (0.014)	0.913	0.335 (0.007)	0.551	0.443 (0.030)	0.384	3.01	3.02	0.003
F 44-29	0.705 (0.021)	0.889	0.290 (0)	0.482	0.390 (0.028)	0.336	2.87	2.89	0.007
F 33-31	0.605 (0.007)	0.807	0.215 (0.007)	0.403	0.375 (0.021)	0.281	2.82	2.77	Partial precipitation
F 28-30	0.630 (0.017)	0.751	0.230 (0.010)	0.375	0.327 (0.021)	0.262	2.71	2.67	Partial precipitation
P 53-28	0.735 (0.106)	0.919	0.335 (0.032)	0.575	0.480 (0.017)	0.401	2.84	2.97	0.046
P 46-28	0.695 (0.035)	0.896	0.290 (0.014)	0.500	0.390 (0.014)	0.349	2.68	2.63	Partial precipitation
P 34-29	0.710 (0.057)	0.819	0.245 (0.021)	0.409	0.325 (0.007)	0.285	2.63	2.33	Partial precipitation

Values in parenthesis are standard deviations ($n = 3$).

binding properties were also determined as calcium activity coefficients by a metallochromic indicator method (Table 1). The transport parameter of monovalent counterions (f^+) decreased fairly regularly with decreasing DM in agreement with previously reported data on non-acetylated pectins [4,5,27]. As previously shown on citrus pectins [5], no difference could be observed between the different series.

In agreement with previously reported data [4,5,27], calcium activity coefficient values (γ^{2+}) were slightly higher than calcium transport parameter ones (f^{2+}) except for the virtually fully demethoxylated and deacetylated sample B 01-00 (Fig. 4). The values of γ^{2+} and f^{2+} decreased regularly with decreasing DM down to DM $\sim 10\%$ and then plunge between DM $\sim 10\%$ and DM $\sim 0\%$. No clear difference was observed between the different series (Fig. 4).

The f^{2+} values of deesterified beet pectins were compared to those observed for citrus pectins belonging to homologous series [5] (Fig. 5). Some differences were observed between chemically treated pectins from beet and citrus, beet pectins exhibiting f^{2+} values slightly lower than citrus pectins for $40\% < DM < 80\%$ and higher for $3\% < DM < 30\%$. Similar f^{2+} values were observed for virtually fully deesterified pectins from beet and polygalacturonic acid. Beet and citrus pectins demethoxylated by fungus-PME had comparable behaviours while beet pectins demethoxylated by plant-PME exhibited slightly higher f^{2+} values than citrus pectins demethoxylated by the same enzyme. Citrus and beet pectins were also compared on the basis of their γ^{2+} values (Fig. 5). For chemically deester-

ified and plant-PME deesterified pectins, the observations made by comparing f^{2+} values were confirmed, differences between beet and citrus pectins being amplified. Furthermore, pectins demethoxylated by fungus-PME differed moderately, beet pectins exhibiting slightly higher γ^{2+} values than citrus pectins.

The ratio of experimental to theoretical values of the calcium transport parameter (f^{2+} exp/theo) and of the calcium

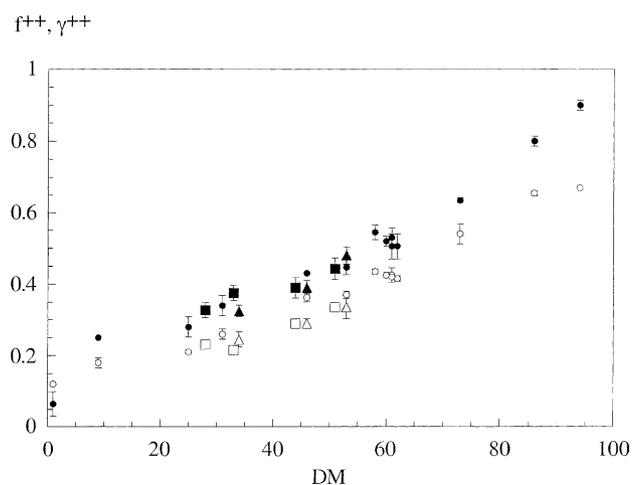


Fig. 4. Variations of values of calcium transport parameter (f^{2+}) and calcium activity coefficient (γ^{2+}) with the degree of methylation (DM). Calcium transport parameter (f^{2+}): (○) SBP 62-30, B-, B'- and E-series; (□) F-series; (△) P-series. Calcium activity coefficient (γ^{2+}): (●) SBP 62-30, B-, B'- and E-series; (■) F-series; (▲) P-series.

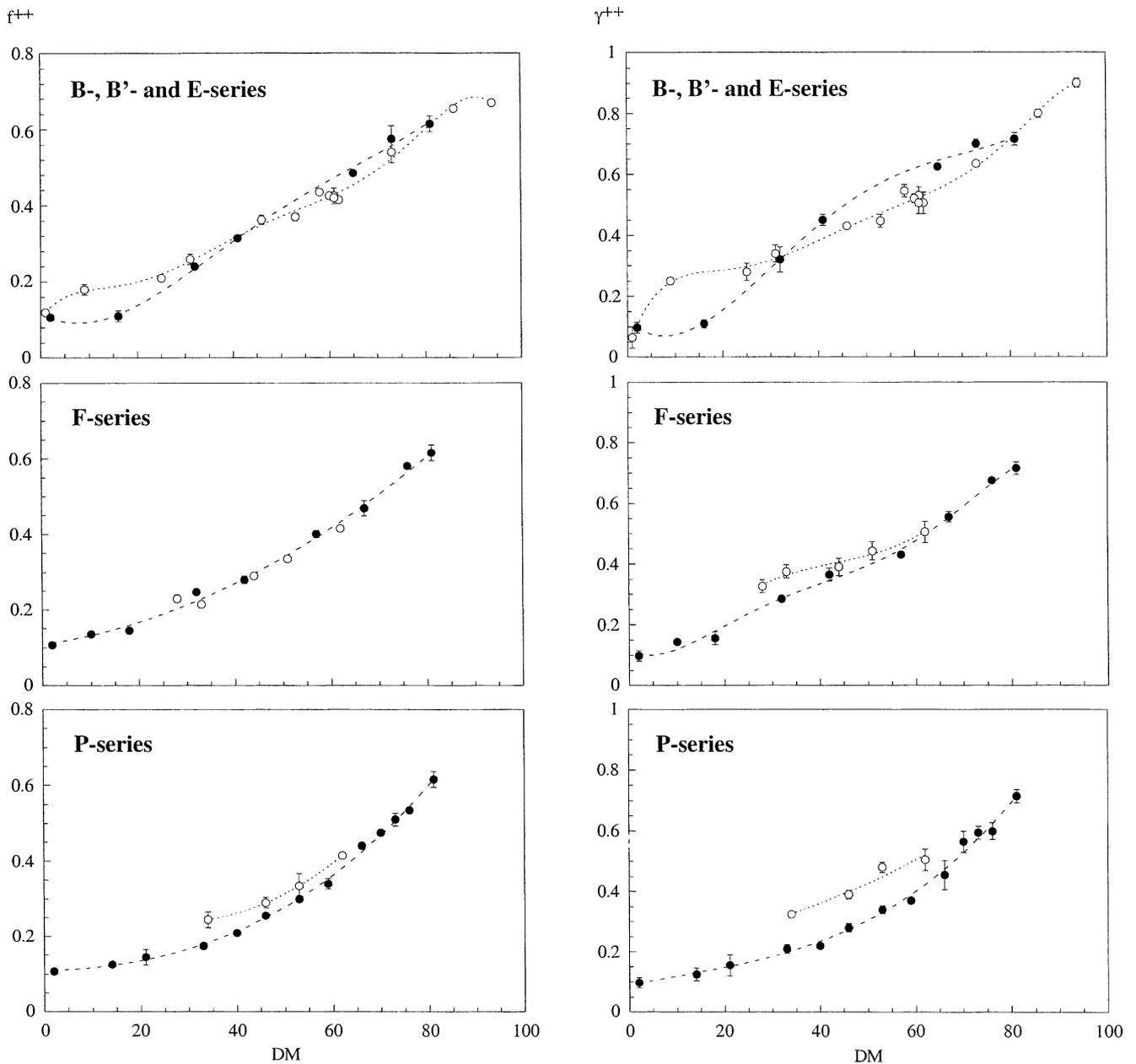


Fig. 5. Variations of values of calcium transport parameter (f^{2+}) and calcium activity coefficient (γ^{2+}) with the degree of methylation (DM): (●) citrus pectins; (○) sugar beet pectins.

activity coefficient (γ^{2+} exp/theo) was plotted versus the degree of methylation (Fig. 6). Alike patterns were obtained by these two approaches of the calcium binding properties. A clear transition was evidenced at a DM of 35–40% for citrus pectins from the F- and B-series. The sudden drop in f^{2+} exp/theo and γ^{2+} exp/theo values observed at this DM was attributed to an intermolecular binding of the calcium ions to carboxyl groups of two molecules leading to the formation of dimers [4,5]. It has to be noticed that for DM > 30–35%, pectins demethoxylated by fungus-PME exhibited lower f^{2+} exp/theo and γ^{2+} exp/theo values than alkali-deesterified pectins. As said above, fungus-PME and

base treatment led to two different forms of homogeneous methyl esterification patterns on citrus pectins [7]. Citrus pectins from the P-series exhibited a radically different behaviour. A roughly continuous decrease in f^{2+} exp/theo and γ^{2+} exp/theo values was observed. It was suggested that short sequences of demethoxylated galacturonic acid residues were generated together with longer ones able to form “egg-boxes”, long demethoxylated sequences being all the more numerous when the DM decreases [34].

When examining f^{2+} exp/theo versus DM for beet pectins, it can be observed that beet pectins treated by base, fungus-PME or plant-PME, behave similarly to fungus-PME

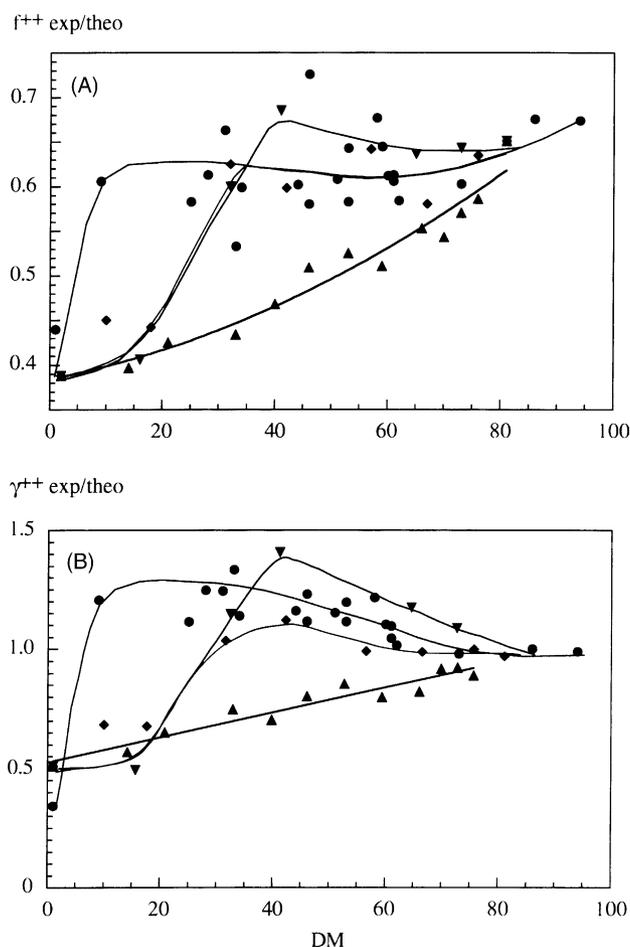


Fig. 6. Variations of the ratio of experimental to theoretical values of (A) calcium transport parameter (f^{2+}) and (B) calcium activity coefficient (γ^{2+}) with the degree of methylation (DM): (●) sugar beet pectins; (▼) citrus pectins from the B-series; (◆) citrus pectins from the F-series; (▲) citrus pectins from the P-series.

deesterified citrus pectins down to DM \sim 35%. However, while fungus-PME deesterified citrus pectins exhibited a fairly constant $f^{2+} \text{ exp/theo}$ values of 0.616 ± 0.026 down to DM \sim 35%, beet pectins exhibited an analogous constant ratio of 0.613 ± 0.044 down to DM \sim 10%. Below this DM, the ratio value dropped suddenly to reach a value of 0.440 for the virtually fully deesterified sample B 01-00. This value is in close agreement with values observed for polygalacturonic acid or virtually fully deesterified pectins from apple [4,5,27] although B 01-00 is very rich in neutral sugar side-chains (ara + gal = 199 mg/g pectin) [21]. This suggests that inter-chain association is not hindered by arabinose and galactose side-chains. It has to be noticed that beet pectins deesterified by plant-PME do not differ from the other beet pectin samples. As suggested by potentiometric measurements, if sequences of deesterified galacturonic acid residues are generated by plant-PME, they are not long enough to promote dimerisation of beet pectins in dilute solutions. The action of plant-PME is probably hindered by the presence of acetyl groups as suggested for fungus-PME

by Oosterveld et al. [20]. The fact that long sequences of demethoxylated and deacetylated galacturonic acid units cannot be generated, even when the DM is lowered to 34%, is in favour of a random repartition of acetyl groups along the beet pectic molecule, in agreement with Rombouts and Thibault [12] suggestion.

When studying $\gamma^{2+} \text{ exp/theo}$ versus DM, it appeared that above DM \sim 35% beet pectins have an intermediate behaviour between fungus-PME and alkali deesterified citrus pectins. The “mother sugar beet pectin” having a DM of 62%, some small blocks of non methoxylated and non acetylated galacturonic acid residues are probably present leading to enhanced calcium binding properties compared to randomly deesterified citrus pectins arising from a “mother pectin” having a DM of 81%. Below DM \sim 35%, the observations made by studying $f^{2+} \text{ exp/theo}$ versus DM were confirmed with a sudden drop in $\gamma^{2+} \text{ exp/theo}$ values below DM \sim 10%. Below DM \sim 20%, citrus pectins were able to fully dimerize, whatever their deesterification mode. Conversely, B 09-15 is unable to dimerize in dilute solution, probably because of its residual acetyl content.

Interaction of pectins with calcium was also studied in a more concentrated medium with an excess of calcium ions by measuring calcium sensitivity. The initial sugar beet pectins SBP 62-30, pectins from the E-series and pectins from the B'-series were not calcium sensitive. Some pectins from the B-series were calcium reactive. B 25-16 partly precipitated and a voluminous precipitated calcium-pectinate was observed, while the supernatant viscosity decreased compared with the viscosity level in the solution without added calcium. B 09-15 and B 01-00 precipitated completely in the presence of excess calcium ions, and the supernatant viscosity fell to the level of the buffer. Some pectins from the F-series were also calcium reactive: F 33-31 and F 28-30 partly precipitated in the presence of excess calcium ions. These results confirm that pectins from the B-series have the most homogenous methyl ester distribution. Partial precipitation was observed only from DM 25%, while pectins which had been de-methylated by fungus-PME already started to precipitate at 33% DM during the test. Pectins from the P-series started to precipitate at 46% DM, in agreement with the blockwise distribution of free galacturonic acid units. No complete precipitation was however observed neither for pectins from the F- nor for pectins from the P-series.

4. Discussion

Five series of pectins with different levels and patterns of methyl esterification and/or different levels of acetyl esterification were produced by chemical or enzymatic treatment of an acid-extracted sugar beet pectin. Although some differences in the pattern of methylesterification between pectins deesterified by base, fungus-PME and plant-PME were revealed by ion-exchange chromatography profiles, hardly any differences could be evidenced between the series, neither

in the polyelectrolyte behaviour nor in the calcium binding properties in dilute solution. By comparison with unacetylated citrus pectins belonging to homologous series [5], it was obvious that acetyl groups in sugar beet pectins strongly hindered dimerization of pectic chains through calcium ions in dilute solution. Citrus pectins with a random (B-series) or random-like (F-series) distribution of free galacturonic acids were able to dimerize below DM \sim 35% while sugar beet pectins were able to dimerize only below DM \sim 10%, the residual DAc being furthermore below 15%. These results are in good agreement with the findings of Renard and Jarvis [14] who calculated from Phippen et al. [10] results that gelation was seriously hindered at a DAc of 15%, and completely inhibited at 20%. Plant-PME are known to result in a blockwise arrangement of free carboxyl groups in the pectin molecule [3–5,7] leading to an important calcium reactivity for high DMs. Sugar beet pectins demethoxylated by plant-PME were however not able to dimerize through calcium ions in dilute solution. Acetyl groups are known to hinder the action of PME [10,20] and only small blocks of demethoxylated galacturonic acid residues seem to be produced by plant-PME on sugar beet pectins. Calcium sensitivity tests performed on more concentrated pectin solutions and in an excess of calcium ions allowed to discriminate the different series of sugar beet pectins. Calcium sensitivity (CS) is a property of high ester pectin which causes the viscosity of an appropriate test system containing dissolved pectin to be positively correlated to the amount of calcium ions [33]. The calcium sensitivity of high ester pectin occurs in the presence of inhomogeneous methyl ester distribution. Regions of free galacturonic acid groups form multiple calcium bridges, which create a domain of strong, intermolecular association between the galacturonan chains, resulting in increased viscosity. Consequently, extensive pectin de-methylation in the presence of excess calcium ions causes pectin gelling or precipitation, and the same is the case when multiple regions of predominant free galacturonic acid groups are present in the same pectin molecule. Low ester pectin often has sufficient free galacturonic acid chains to show increased calcium sensitivity or to gel/precipitate with the addition of calcium. From a physical point of view it is evident that increased molecular interaction caused by increased pectin concentration as well as long molecular chains promote a viscosity increase in calcium-sensitive pectin when calcium ions are present. The measurement of calcium sensitivity was carried out at a pH above the pK_a value, where chain associations in the form of hydrogen bonding are suppressed, and significant repulsions exist between negative galacturonate ions. An efficient buffer system was used to stabilise the pH and to suppress long-range intermolecular forces. Complete precipitations were observed only for base-deesterified pectins for which DAc decreased with decreasing DM. As observed in dilute solution, DM and DAc have to be deeply lowered to allow calcium reactivity and partial precipitation was observed only from DM 25%. Complete precipitation was observed

only for low DM and DAc (9 and 15%, respectively). Partial precipitations were observed for higher DM for fungus-PME demethoxylated pectins (from DM 33%) and for much higher DM for plant-PME demethoxylated pectins (from DM 46%). For fungus-PME treated citrus pectins, the endo-PG II digestibility is greatly enhanced and simultaneously the pectin-lyase digestibility is decreased compared with pectins with similar DM obtained from base treatment [7]. Correspondingly, fungus-PME deesterified citrus pectins seem to bind calcium more tightly than pectins deesterified by alkali [5]. This seems to be also the case for sugar beet pectins. Similarly, as observed for plant-PME demethoxylated citrus pectins [5,34], plant-PME demethoxylated sugar beet pectins seem to rapidly contain blocks of free galacturonic acid residues long enough to promote calcium reactivity in concentrated solution, even for high DM. Complete precipitations could however not be observed for fungus-PME or plant-PME demethoxylated sugar beet pectins, probably because the DAc of these pectins is too high. In concentrated solution, Oosterveld et al. [20] obtained calcium-gels from a sugar beet pectin (DM 58%, DAc 15%) treated by fungus-PME, although DM was only lowered to 49%. Treating the same pectin with PME and an acetyl esterase active on smooth regions led to a modified pectin (DM 42%, DAc 13%) with enhanced gelling properties. Specific removal of acetyl groups in the “smooth” homogalacturonic regions of sugar beet pectins together with the use of plant-PME could allow the synthesis of sugar beet pectins with largely improved gelling properties with calcium ions.

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