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Chromatographic study of highly methoxylated lime pectins deesterified by different pectin methyl-esterases

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Abstract

The inter-molecular distribution of free carboxyl groups of two highly methoxylated pectins enzymatically deesterified by plant and fungus pectin methyl-esterases were investigated by size-exclusion (SEC) and ion-exchange chromatography (IEC). ''Homogeneous'' populations with respect to molar mass or charge density were thereby obtained and their chemical composition and physico-chemical properties (transport parameter for monovalent cations and calcium, calcium activity coefficient) were studied. Chemical analysis showed that the composition varies from one SEC fraction to another, the highest molar mass fraction being richer in rhamnose and galactose and exhibiting a slightly higher degree of methylation. Separation of pectins by IEC revealed a quite homogeneous charge density distribution for F58 contrary to P60 which exhibited a large distribution of methoxyl groups. The free carboxyl groups distributions and calcium binding behaviours of SEC and IEC fractions were shown to differ widely for highly methoxylated pectins deesterified by plant and fungus pectin methyl-esterases.

Keywords: Pectins; Pectin methyl-esterases

1. Introduction

Pectins are a family of complex polysaccharides mainly present in the primary cell walls and intercellular regions of dicotyledons [1,2]. Pectin structure is composed of several subunits, the main one consisting of homogalacturonan (''smooth'' region) made up of ~100 (1→4)-linked α-D-galacturonic acid units [3] that may be partially methyl esterified at position 6. Their methoxyl content, expressed as their degree of methylation (DM), implies specific industrial applications [4]. Homogalacturonans are interrupted by rhamnogalacturonans (''hairy'' regions) in which galacturonic acid residues are interspersed with (1→2) linked α-L-rhamnopyranosyl residues carrying neutral sugar side-chains [5]. In addition to the complex nature of a single pectin molecule, pectins are characterised by a high degree of inter-molecular heterogeneity in terms of composition, molar mass and DM [6,7].

In the plant cell walls, pectins are usually highly methoxylated (HM pectins). After extraction, HM pectins form gels in an acidic medium on addition of sucrose. Pectins of lower ester content (LM pectins) can be prepared by controlled deesterification. For such ionic polymers, the ability to form gels in the presence of calcium leads to many food applications [1]. The interaction of calcium ions with carboxyl groups of pectins has been extensively studied, mainly to elucidate the effect of DM on calcium

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binding properties [8–13]. It was shown that the interaction of calcium with HM pectins (DM>40–50%) has a character of a ''pure electrostatic'' intramolecular binding [8,12,14] while pectins of lower DM bind these cations strongly through inter-chain association. The nature of the calcium cross-slinks is here believed to follow the ''egg-box'' type interaction proposed by Powell and co-workers [11,15] for alginates. Besides the sole DM parameter, the distribution pattern of free and esterified carboxyl groups was shown to have a marked effect on the strength of calcium binding for LM pectins [12]. LM citrus pectins (DM<40%) with blockwise distribution of free carboxyl groups, prepared by deesterification of an HM pectin by a plant pectin methyl-esterase, were characterised by low calcium activity coefficients, close to that of calcium pectate [12]. From studies using oligogalacturonates or block deesterified pectins, it seems that 7 to 15 consecutive non-esterified galacturonic acid units are necessary to achieve a chain dimerization through calcium ions [16]. Most of the above cited works have been carried out on LM pectins and only few data are available on calcium binding properties of HM pectins [17]. Furthermore, preceding studies have been achieved on unfractionated pectins while pectins are known to be highly heterogeneous with respect to sugar composition, molar mass and charge density [6,7].

The purpose of this work was to obtain ''homogeneous'' populations, with respect to molar mass or charge density, by fractionating HM pectins (DM~60%) with different patterns of esterification by preparative size-exclusion (SEC) or ion-exchange chromatography (IEC). The inter-molecular distribution of sugars and methoxyl groups was studied in the different fractions and related to calcium binding properties.

2. Experimental

2.1. Pectins

A commercial pectin (L72), from Mexican lime peel (*Citrus aurantifolia*), with a DM of 72% was esterified in acid–methanol medium to give a pectin (E81) of a DM of 81%. P60 and F58 were produced by treatment with plant pectin methyl-esterase or fungal pectin methyl-esterase, respectively. A detailed description of reaction conditions has been published elsewhere [18].

2.2. Analytical

All values were calculated on a moisture-free basis. Galacturonic acid and neutral sugars (expressed as galactose) were quantified colorimetrically by the automated *m*-phenylphenol [19] and orcinol [20] methods, respectively, the latter being corrected for interfering galacturonic acid. Except for chromatographic fractions, galacturonic acid was quantified after saponification of the pectin samples (0.05 *M* NaOH, 30 min, room temperature) and neutralisation (0.05 *M* HCl). Pectins were hydrolysed in 2 *M* trifluoroacetic acid (2 h, 121°C). The individual sugars were reduced, acetylated, and analysed by gas–liquid chromatography (GLC) [21]. Pectins were first recovered in their acidic form (cf. Section 2.4). Free carboxylic functions were quantified at the neutralisation point by conductimetric titrations with a base of known molarity and total carboxylic functions by colorimetry on the same solutions after saponification. DM was calculated by:

$$\mathrm{DM} = 100 \times (\text{total carboxylic functions} - \text{free carboxylic functions}) / \text{total carboxylic functions}$$

2.3. Chromatography

2.3.1. Size-exclusion chromatography

SEC was performed on a column (92×5 cm) of Sephacryl S-500 equilibrated with 0.1 *M* sodium succinate, pH 4.5. Thimerosal (0.02%) was added to the buffer as a preservative. Solutions (50 ml at 5 mg/ml) were loaded onto the column and eluted by upward elution at 250 ml/h. Fractions (15 ml) were collected and analysed.

2.3.2. Ion-exchange chromatography

Chromatography on DEAE-Sepharose CL-6B was performed on a column (31×2.6 cm) equilibrated with 0.05 *M* sodium succinate, pH 4.5 at a flow-rate

of 100 ml/h. Thimerosal (0.02%) was added to the buffer as a preservative. Samples (50 ml of a solution at 4 mg/ml) 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 (0–0.4 *M*; 2 l); 15-ml fractions were collected and analysed.

2.4. Physico-chemical characterisation

2.4.1. Molar mass determination

Pectin samples were gently dispersed into ultra-pure water (~3 mg/ml) and left for dissolution overnight under vigorous magnetic stirring; $NaNO_3$ was then added (0.05 *M* final). The solution was centrifuged in a bench top centrifuge and filtered on 0.45-μm Minisart RC15 Sartorius membranes. Sample was then injected on a high-performance size-exclusion chromatography (HPSEC) system constituted of a Shodex OH SB-G pre-column followed by two Shodex OH-pack 804 and 805 columns used in series. The elution was performed at room temperature with 0.05 *M* $NaNO_3$, containing 0.02% NaN_3 as preservative, at a constant 36 ml/h flow-rate. On-line molar mass and intrinsic viscosity determinations were performed using a multiangle laser light scattering (MALLS) detector (mini-Dawn, Wyatt, Santa Barbara, CA, USA) operating at three angles (41, 90 and 138°), a differential viscosimeter (T-50A, Viscotek) and a differential refractometer (ERC 7517 A) ($dn/dc = 0.146$ ml/g). Molar mass were determined using a universal calibration curve (pullulans $5 \cdot 10^3$ to $1600 \cdot 10^3$ g/mol).

2.4.2. Transport parameters and calcium activity coefficients

Transport parameters were determined using conductimetric measurements as already described [12,22]. They are related to the free fraction of the considered counterion. Pectin samples were extensively washed with 65% aqueous ethanol before being dried by solvent exchange, in order to eliminate salt traces. Pectins were dissolved in ultra-pure water at ~7 mequiv./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 of ~1 mequiv./l. All conductimetric measurements were carried out at 25.0±0.2°C with a CDM 83 conductimeter (Radiometer Analytical) equipped with a double platinum electrode CDC 241U (Radiometer Analytical). The cell constant was determined with 0.05% (w/w) NaCl before each set of measurements. The titrations were performed with freshly prepared 10 mequiv./l solutions of KOH, LiOH and $Ca(OH)_2$. The limiting law for the equivalent conductivity of polyelectrolyte without external salts is given by:

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

with: Λ, the equivalent conductivity (S cm^2/equiv.) 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*, 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_{Li^+,K^+}) and calcium ($f_{Ca^{2+}}$) cations, and the equivalent conductivity of the polyelectrolyte (λ_p), can be calculated.

The calcium activity coefficients at the neutralisation point ($\gamma_{Ca^{2+}}$) were determined by means of a dual-wavelength spectrophotometric method using tetramethylmurexide as an activity probe for calcium ions [23]. 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.

3. Results and discussion

3.1. Characterisation of initial pectins

The main characteristics of F58 and P60 pectins are shown in Table 1. Both pectins were characterised by a high galacturonic acid content. Their content in neutral sugars were low and only galactose, rhamnose and arabinose amounts were higher than 1 mg/g. No significant difference was evi-

Table 1
Composition and physico-chemical properties of F58 and P60 initial pectins

	F58	P60
GalA (mg/g)	883	843
Rha (mg/g)	15	16
Ara (mg/g)	3	3
Gal (mg/g)	38	38
DM (%)	56	59
M_w (1000 g/mol)	117	94
Rg (nm)	26	22
$[\eta]$ (ml/g)	533	400
I (M_n/M_w)	2.1	2.1

denced between F58 and P60 in their chemical composition. Pectin samples were poor in neutral sugars when compared to usual pectins [1] and such results suggest that the number and size of side-chains are limited and that rhamnogalacturonan sequences are few and/or short.

When analysed by HPSEC–MALLS, both samples showed a high and narrow light-scattering signal at the excluded volume for a quasi-absent refractometric signal; this was attributed to the presence of traces of aggregated materials as already pointed out by numerous authors [7,24–27]. The presence of these aggregates led to an overestimation of average molar mass. Considering the uncertainty of light scattering measurements, molar mass were therefore estimated by viscosimetry through universal calibration. The whole elution profile for studied samples was within the calibration curve which permits a good evaluation of molar mass by this method. F58 and P60 exhibited average molar masses of $117 \cdot 10^3$ and $94 \cdot 10^3$ g/mol, respectively, suggesting that the preparative conditions were more degrading for plant-pectin methyl-esterase (PME) deesterified pectins. The average molar mass and intrinsic viscosity values observed were in good agreement with those reported for citrus pectins [28–30]. However, higher molar mass and gyration radii values were obtained by light-scattering [31,32]. Samples exhibited a fairly large distribution of molar mass ($I \sim 2$) and displayed a population distribution that can be decomposed into two wide peaks, the low-molar-mass material constituting the main part of the whole population as previously found for apple pectins [33].

The inter-molecular distribution of sugar units and methoxyl groups as well as the physico-chemical characteristics of F58 and P60 were studied by means of preparative SEC and IEC.

3.2. Fractionation by preparative SEC

The elution patterns obtained by fractionating F58 and P60 pectins on Sephacryl S-500 are shown in Fig. 1, chromatography yields were close to 100% for both samples. Both pectins have large hydrodynamic volumes and wide size distribution, P60 elution pattern being slightly shifted to higher K_{av} compared to F58. This was consistent with the results obtained by HPSEC. Five populations were recovered for both pectins and their chemical and physico-chemical characteristics are summarised in Tables 2 and 3. Fractions 2, 3 and 4 were rich in galacturonic acid, the higher and lower molar mass fractions (1 and 5) being poorer in that sugar. As already pointed out by Kravtchenko et al. [6], the fractions of higher-molar-mass exhibit a higher amount of both rhamnose and galactose and the ratio galacturonic acid/rhamnose was low, indicating the

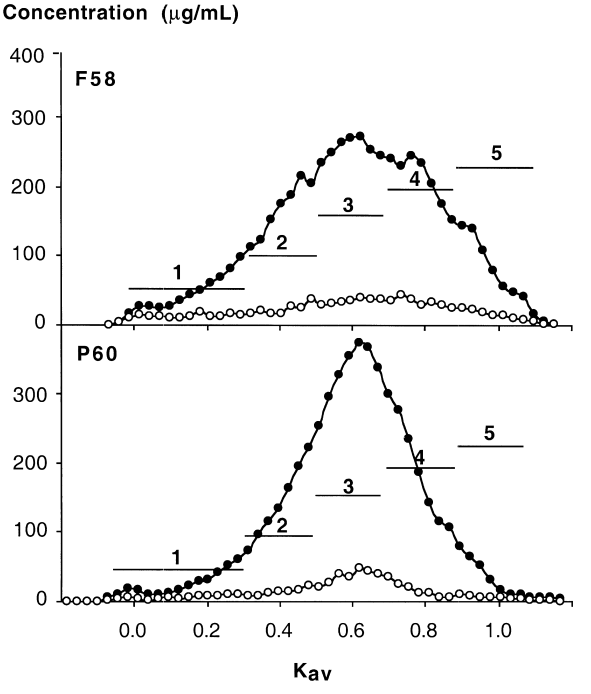

Fig. 1. Size-exclusion chromatography on Sephacryl S-500 of F58 and P60 initial pectins. ●, Galacturonic acid; ○ neutral sugars.

Table 2
Composition and physico-chemical properties of Sephacryl S-500 F58 fractions

	F58 S-500 (1)	F58 S-500 (2)	F58 S-500 (3)	F58 S-500 (4)	F58 S-500 (5)
Yield (%)	13.5	23.2	31.2	22.3	9.9
GalA (mg/g)	720	870	854	853	842
Rha (mg/g)	27	15	15	11	11
Ara (mg/g)	3	3	3	3	3
Gal (mg/g)	40	21	27	30	43
DM (%)	61	59	57	58	57
M_w (1000 g/mol)	300	152	104	69	54
Rg (nm)	44	28	22	18	15
$[\eta]$ (ml/g)	939	481	367	291	225
I (M_n/M_w)	1.6	1.6	1.7	1.3	1.4

presence of longer or more numerous rhamnogalacturonic sequences. The smallest molecules exhibit a higher amount in galactose but not in rhamnose, which is probably due to the presence of free neutral polysaccharides. A similar enrichment of neutral sugars at the beginning and at the end of elution pattern was already observed [6,25,34]. For both pectins, we observed a trend of moderately decreasing DM along the fractionation. Fraction 1, and that was particularly obvious for P60, exhibited a higher DM than the other fractions. This higher DM might be attributed to the presence in these fractions of longer or more numerous rhamnogalacturonic sequences which are known to be very highly methoxylated [35]. Such a variation has been previously evidenced [6,36].

As for unfractionated pectins, molar mass were estimated by viscosimetry. As expected, decreasing average molar mass, intrinsic viscosities and radii of gyration were observed with increasing elution volume. F58 and P60 fractions 1 had similar average molar mass and were representative of the high-molar-mass population observed in the unfractionated pectins (Fig. 2). Fractions 2 also contained part of this high-molar-mass population, while fractions 3, 4 and 5 were devoid of it. Except for fraction 1, P60 fractions were of lower average molar mass than corresponding F58 fractions. This difference may be correlated to distinctive preparative conditions as already mentioned above. The mass heterogeneity of the fractions was significantly reduced ($I = 1.3$ to 1.7) as compared to unfractionated pectins.

3.3. Fractionation by preparative IEC

Ion-exchange chromatography on DEAE-Sepharose CL-6B was used to fractionate F58 and P60 pectins according to their charge density (Fig. 3). Galacturonic acid recovery was $>95\%$ for both samples. The free neutral polysaccharides which are

Table 3
Composition and physico-chemical properties of Sephacryl S-500 P60 fractions

	P60 S-500 (1)	P60 S-500 (2)	P60 S-500 (3)	P60 S-500 (4)	P60 S-500 (5)
Yield (%)	6.9	21.4	41.5	24.3	5.9
GalA (mg/g)	763	896	896	908	746
Rha (mg/g)	31	17	13	15	14
Ara (mg/g)	4	2	3	3	4
Gal (mg/g)	45	21	25	53	71
DM (%)	68	60	58	61	60
M_w (1000 g/mol)	280	117	67	50	36
Rg (nm)	38	24	17	14	12
$[\eta]$ (ml/g)	640	406	255	191	165
I (M_n/M_w)	1.6	1.5	1.4	1.3	1.3

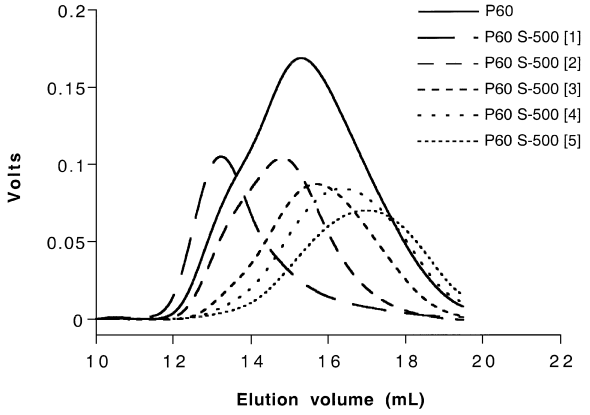

Fig. 2. HPSEC elution patterns (refractive index) for P60 initial pectin and SEC fractions.

not bound to the column represented a very small fraction of the samples (<2%) and was neglected. F58 pectin was eluted as a thin homogeneous peak while P60 pectin exhibited a broad elution pattern. Pectins saponified by acid or alkali were shown to be homogeneous on IEC whereas pectins saponified by plant-PME elute in large fractions [37]. Four and seven populations were recovered for F58 and P60, respectively; their chemical and physico-chemical characteristics are summarised in Tables 4 and 5. The two extreme populations (1 and 4) of F58 exhibited lower amounts in galacturonic acid. Rhamnose, arabinose and galactose contents decreased from population 1 to population 3 and then remained stable or very slightly increased in population 4. The galacturonic acid over rhamnose ratio was low in population 1, indicating, as pointed out above, the presence of longer or more numerous rhamnogalacturonic sequences. Similar results were observed for P60 with an overall decrease in rhamnose, galactose and arabinose and an increase in the galacturonic acid over rhamnose ratio along the fractionation. An examination of the relative proportion of neutral sugars reveals only moderate differences with a trend of slightly increasing rhamnose proportion and slightly decreasing galactose proportion along the fractionations. As predicted by the narrow elution pattern, F58 was fairly homogeneous in terms of DM (49 to 61%). P60 on the opposite was very heterogeneous in terms of DM (34 to 72%). DM of the fractions obtained by IEC decreased regularly and there seemed to be a similar agreement between DM and elution ionic strength for both pectins, contrary to previously reported data [7,37].

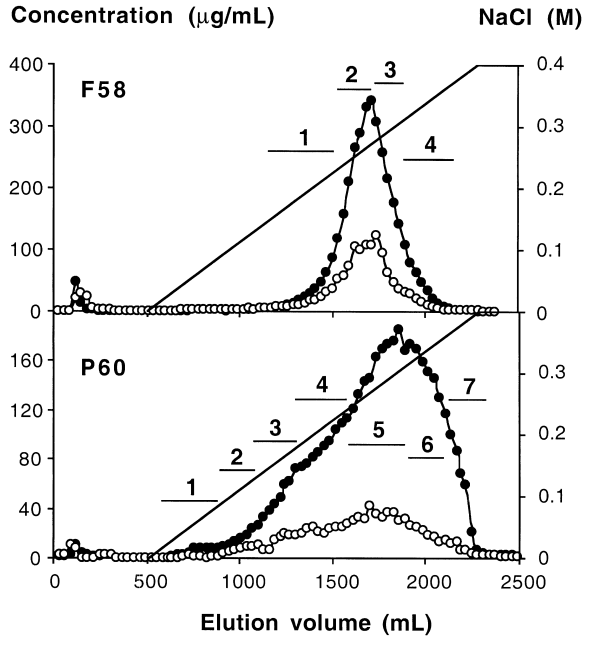

Fig. 3. Ion-exchange chromatography on DEAE-Sepharose CL-6B of F58 and P60 initial pectins. ●, Galacturonic acid; ○ neutral sugars.

For F58, the first population contained a mixture of low- and high-molar-mass molecules. Populations 2 and 3 which were shown to be chemically quite similar, were also of similar molar mass, close to the average value obtained for the main SEC population. The last population was again constituted of a mixture of low- and high-molar-mass molecules. For P60, the two first populations, recovered in very small amounts, were of low average molar mass and might be constituted of ''hairy'' region-rich molecules, considering their sugar composition. Populations 3 and 4 and 7 were constituted of a mixture of low- and high-molar-mass molecules, as F58 populations 1 and 4. P60 DEAE (3) and (4) and F58 DEAE (1) on one hand and P60 DEAE (7) and F58 DEAE (4) on the other hand, were indeed eluted for similar ionic strength (Fig. 3). P60 populations 5 and 6, which represented together around 50% of the whole initial pectin were of similar average molar mass than the main SEC population. Although no values

Table 4
Composition and physico-chemical properties of DEAE Sepharose CL-6B F58 fractions

	F58 DEAE (1)	F58 DEAE (2)	F58 DEAE (3)	F58 DEAE (4)
Yield (%)	14.2	43.0	32.9	9.8
GalA (mg/g)	770	905	912	809
Rha (mg/g)	30	9	7	7
Ara (mg/g)	5	3	3	3
Gal (mg/g)	53	14	10	10
DM (%)	61	60	53	49
M_w (1000 g/mol)	496+50 (25+75%)	95	102	338+64 (18+82%)
Rg (nm)	49+14	23	24	45+17
[η] (ml/g)	753+173	429	446	783+244

of average molar mass were reported, Kratchenko et al. [7] also noticed that IEC fractions did not appear homogeneous on SEC.

3.4. Interactions of pectins with cations

Monovalent ($f_{Li^+,\ K^+}$) and divalent ($f_{Ca^{2+}}$) transport parameters, the equivalent conductivity of the active monomer inside the pectic chain (λ_p) and calcium activity coefficient ($\gamma_{Ca^{2+}}$) are indicated in Table 6. The charge parameter ξ is also as this fundamental parameter governs the behaviour of polyelectrolyte polymers [38].

Experimental data were compared with theoretical ones calculated from Manning's theory [39]. The treatment of Manning is proposed for infinitely dilute solutions of rodlike polyelectrolytes. The transport parameters and activity coefficients are directly deduced from the effective charge density and the counterion valence.

The transport parameter value of monovalent counterion for F58 unfractionated pectins is in agreement with values found by Racape et al. [22] on pectins of similar charge density. The experimental values are slightly lower than the theoretical ones (experimental/theoretical values: 0.83 and 0.76 for F58 and P60, respectively). As already reported [12,22], the interactions between monovalent ions and pectins of DM above ~30% are a classical ''electrostatic'' binding and the sole charge density parameter is able to describe these interactions. Discrepancies between experimental and theoretical values were much larger for calcium transport parameters. F58 calcium transport parameter and calcium activity coefficient were similar to those reported by Thibault and Rinaudo [12] for alkali-

Table 5
Composition and physico-chemical properties of DEAE Sepharose CL-6B P60 fractions

	P60 DEAE (1)	P60 DEAE (2)	P60 DEAE (3)	P60 DEAE (4)	P60 DEAE (5)	P60 DEAE (6)	P60 DEAE (7)
Yield (%)	1.5	3.6	9.8	19.9	27.5	25.7	11.9
GalA (mg/g)	532	776	823	832	861	841	839
Rha (mg/g)	26	14	16	13	9	9	8
Ara (mg/g)	6	4	5	3	3	2	3
Gal (mg/g)	63	27	24	21	13	12	10
DM (%)	n.d.	n.d.	72	68	61	48	34
M_w (1000 g/mol)	28	27	504+36 (7+93%)	434+42 (8+92%)	62	61	261+50 (14+86%)
Rg (nm)	9	10	40+12	45+15	19	17	38+15
[η] (ml/g)	79	130	401+157	631+278	359	304	621+202

Table 6
Charge densities, ion binding data and theoretical values

Sample	DM (%)	ξ	f_{Li^+, K^+} experimental	f_{Li^+, K^+} theoretical	$f_{Ca^{2+}}$ experimental	$f_{Ca^{2+}}$ theoretical	$\gamma_{Ca^{2+}}$ experimental	$\gamma_{Ca^{2+}}$ theoretical	λ_p
F58	56	0.70	0.79	0.93	0.40	0.62	0.43	0.43	35.3
F58 S-500 (1)	61	0.63	0.78	0.94	0.39	0.69	0.48	0.48	40.3
F58 S-500 (2)	59	0.66	0.77	0.94	0.41	0.66	0.47	0.46	31.4
F58 S-500 (3)	57	0.69	0.77	0.93	0.38	0.63	0.38	0.44	39.6
F58 S-500 (4)	58	0.68	0.77	0.93	0.40	0.64	0.41	0.44	35.7
F58 S-500 (5)	57	0.70	0.79	0.93	0.41	0.62	0.49	0.43	33.4
F58 DEAE (1)	61	0.63	0.72	0.94	0.43	0.69	0.46	0.48	45.9
F58 DEAE (2)	60	0.65	0.74	0.94	0.42	0.67	0.43	0.47	41.7
F58 DEAE (3)	53	0.75	0.78	0.92	0.37	0.58	0.43	0.40	44.3
F58 DEAE (4)	49	0.82	0.70	0.91	0.30	0.53	0.32	0.37	46.7
P60	59	0.66	0.72	0.94	0.34	0.66	0.37	0.46	39.8
P60 S-500 (1)	68	0.52	0.76	0.96	0.38	0.84	0.43	0.58	31.4
P60 S-500 (2)	61	0.63	0.74	0.94	0.34	0.69	0.39	0.48	39.5
P60 S-500 (3)	58	0.68	0.72	0.93	0.34	0.64	0.37	0.44	39.8
P60 S-500 (4)	61	0.64	0.72	0.94	0.34	0.68	0.41	0.48	43.1
P60 S-500 (5)	60	0.65	0.76	0.94	0.34	0.67	0.44	0.47	35.2
P60 DEAE (1)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
P60 DEAE (2)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
P60 DEAE (3)	72	0.45	n.d.	0.97	n.d.	0.89	n.d.	0.64	n.d.
P60 DEAE (4)	68	0.52	0.83	0.96	0.44	0.84	0.46	0.58	30.5
P60 DEAE (5)	61	0.63	0.81	0.94	0.38	0.69	0.48	0.48	39.8
P60 DEAE (6)	48	0.84	0.74	0.90	0.27	0.52	0.31	0.36	46.2
P60 DEAE (7)	34	1.07	0.75	0.82	0.21	0.41	0.20	0.28	31.6

deesterified pectins with randomly distributed carboxyl groups. Although F58 and P60 were of similar DM and exhibited the same λ_p, monovalent and divalent transport parameters and calcium activity coefficients were significantly lower for P60. Indeed, PMEs from higher plants are known to create blockwise sequences during the demethylation process while fungal PMEs and chemical deesterification (by using alkali or acid solutions) give rise to a random mode of distribution of the resulting carboxyl functions [17]. Plant-PME deesterified pectins of DM$<$ 40% were characterised by low calcium transport parameters and calcium activity coefficients [12]. According to our results, blocks of un-methylesterified galacturonic acid units, long enough to create strong interactions with calcium ions, seem to be very rapidly generated by plant-PME and to be already present in fairly high DM pectic molecules. SEC and IEC permit the separation of such molecules and fractions recovered by these chromatographic means were further studied.

For F58 and P60 SEC and IEC fractions, transport parameter values of monovalent counterion decreased only slowly with a decrease of DM. Similar results were observed for structural charge densities ranging from 0.45 to 0.9, i.e., DM ranging from 72 to 45% [22]. No significant differences were observed between F58 and P60 fractions in agreement with the very slight differences observed between alkali-deesterified and plant-PME deesterified pectins of DM$<$40% [12]. Equivalent conductivity values of the active monomer inside the pectic chain (λ_p) were also within the same range along the fractionations and values observed were similar to those reported in the literature [12]. Calcium transport parameter values were significantly lower for all P60 SEC and IEC fractions than for F58 ones, showing that the specific repartition of free carboxyl groups was

different for all populations of plant- or fungus-PME deesterified pectins. The ratio of experimental to theoretical values is very constant for both F58 and P60 SEC and IEC fractions (0.611 ± 0.03 and 0.513 ± 0.02, respectively), the ratio observed for F58 fractions being identical to that observed by Thibault and Rinaudo [12] for pectins of DM ranging from 50 to 80%. Calcium activity coefficients followed the same tendency than calcium transport parameters with decreased values when decreasing DM and with overall lower values for P60 fractions than for F58 ones. The discrepancy between plant-PME deesterified samples and fungus-PME deesterified samples was however lower than observed by Kohn et al. [17].

4. Conclusion

Preparative SEC and IEC allowed us to recover quite homogeneous fractions with respect to molar mass or charge density, respectively. Fractions obtained by SEC and IEC were shown to vary in neutral sugar composition. SEC fractions were fairly similar along fractionation with respect to DM. However, IEC revealed that the plant-PME deesterified pectin (P60) contained molecules of a wide range of charge density contrary to the fungus-PME deesterified pectin (F58) which was fairly homogeneous with respect to DM. Furthermore, ion interaction studies showed that plant-PME deesterified SEC and IEC fractions were all characterised by higher calcium binding properties than fungus-PME ones, even for high DM. The presence of high amounts of lowly methoxylated populations able to bind calcium strongly in plant-PME deesterified pectins of high DM can lead to application problems such as pre-gelation due to the presence of divalent cations in water during the HM pectins gelation process. On the other hand the block-wise distribution of free carboxyl groups in these pectins influences significantly their interactions with positively charged molecules (cations, proteins, etc.). The use of both SEC and IEC in combination will permit the recovery of more homogeneous pectins and help to establish clear relations between structure and physico-chemical properties.

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