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Structure and properties of apple and sugar-beet pectins extracted by chelating agents

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ABSTRACT

Pectins were extracted from apple or sugar-beet cell walls (alcohol-insoluble solids) at pH 4.5 and 20°C and at pH 6.5 and 80°C in acetate and phosphate buffer solutions, respectively, and in the same buffers containing cyclohexanediaminetetraacetic acid or ethylenediaminetetraacetic acid. The yields were very different between the two pH and temperature conditions, but variations with the nature of the extractant were small at pH 4.5 and 20°C, and no differences were observed at pH 6.5 and 80°C. The pectins extracted at pH 6.5 and 80°C were richer in neutral sugars and acetic acid. Degradation of the pectins extracted at high pH and temperature was shown by the decrease of the intrinsic viscosity and by the shift of the uronic acid peak toward the total volume in gel-filtration chromatography. These pectins contained a high molecular weight population rich in neutral sugars and a low molecular weight acid population, in contrast to the pectins extracted at low temperature and pH, for which neutral and acidic sugars had similar elution profiles. At pH 6.5 and 80°C, rather extensive degradation of the pectins occurred. Calcium-binding experiments (potentiometry with specific electrode and conductimetry) indicated a statistical distribution of free carboxyl groups. These results do not support the hypothesis that chelating-agent extractable pectins are held in the cell wall by calcium cross-links involving the formation of "egg-boxes".

INTRODUCTION

Pectins are one of the main components of the primary cell walls of dicotyledons. They are composed of a backbone of (1 → 4)-linked α-D-galacturonic acids interpersed with a few (1 → 2)-linked L-rhamnose residues, to which are linked neutral sugar side-chains, composed mostly of arabinose and galactose. The degrees of methylation (dm) and acetylation (da), i.e., the proportion of galacturonic acids esterified by methyl groups (on the carboxylic acid) and acetyl groups

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(on the secondary alcohols), are of paramount importance for the use of pectins in food industries. A high da, as in beet pectins, prevents gelation. High methoxy pectins (dm > 50–60) gel at low pH and water activity (e.g., in jams), and low methoxy pectins in the presence of calcium (e.g., in milk desserts). The gelation of pectates with multivalent cations has been explained by the formation of "egg boxes"[1] involving two negatively charged blocks (with a minimum length of ~ 14 residues[2]) on two pectin chains.

It is commonly assumed that native pectins are cross-linked through calcium ions, and some authors have supposed that "egg-boxes" were present in the cell walls[3–5]. Chelating agents have long been known[6,7] to enhance extractability of pectins and to solubilise additional pectins after water or buffer[5,8,9] treatment. CDTA (cyclohexanediaminetetraacetic acid)[8] has been advocated[9] for use at room temperature. Polysaccharides can be released from potato cell walls[10] isolated in the presence of, or later treated by, a chelating agent. The release was totally inhibited by 1 mM $CaCl_2$ and by treatment with organic solvents. Calcium, added to pickled fruits[11] or cucumbers[12], inhibits their softening; this effect has been attributed to an increase of the cohesion of a pectin network. However, endogenous pectinmethylesterases stay active, albeit at very low level, during the pickling process[13], so that the pectins could be demethylated and able to form "egg-boxes".

However, Knee[14] showed that the rates of extraction of pectins in the presence of chelating agents were closely comparable to the rates of depolymerisation of glycuronan, and that effective extraction was obtained only at temperatures known to cause degradation. McFeeters and Fleming[15] noticed that inhibition of the softening of cucumber tissues by various multivalent cations could not be related to their affinity for pectins. Pectins extracted by chelating agents often have high dm (70–80), though calcium cannot precipitate pectins with dm above 50 or 60 (ref. 16). Joslyn[17] concluded, from his examination of pectin extraction and inhibition of softening of fruits, that protopectin could not be an insoluble calcium pectinate. Doesburg[11] found that "removal of calcium is important in relation to extraction of pectic substances", but that its effect "could not be attributed to the presence of calcium bridges between carboxyl groups of neighbouring filamentous molecules".

Our aim was to compare the yields and characteristics of pectins extracted from apple and sugar-beet cell walls in the presence or absence of chelating agents (EDTA and CDTA) and in different conditions. These two substrates were chosen because sugar-beet pectins are highly acetylated[18], whereas apple cell walls contain more conventional pectins.

EXPERIMENTAL

Plant material.—Fresh apples (Golden Delicious) were purchased in December 1989 from a local store. The fresh sugar beets were from the sugar factory in Artenay (France). Poly(galacturonic acid) was from Sigma.

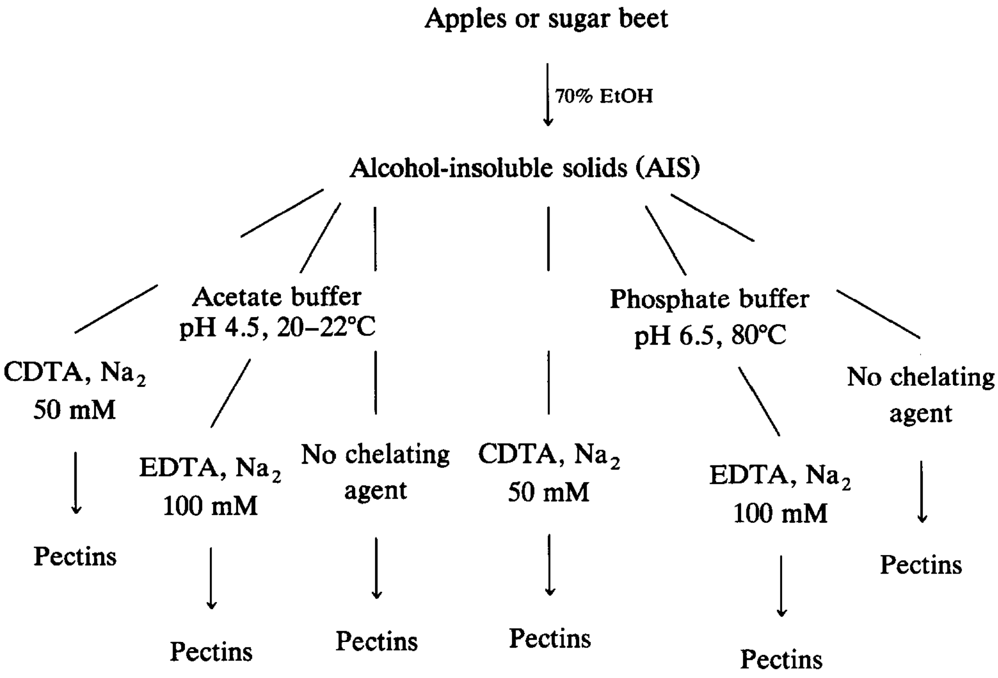


Fig. 1. Extraction of the apple and beet pectins. The details of the procedures are given in the Experimental section.

Preparation of cell wall material.—The cell walls were isolated as alcohol-insoluble solids (AIS) as described earlier[19]. The 70% EtOH extract was sugar-free (phenol–H_2SO_4 test) after 11 washes for the sugar beet (4 L for 2.6 kg of beet) and 9 washes for the apples (3 L for 2 kg of apples). The alcohol-insoluble solids were dried by solvent exchange (96% EtOH and acetone).

Extraction of pectins.—The extraction scheme is shown in Fig. 1. Pectins were extracted with 50 mM CDTA, 100 mM EDTA, or without chelating agent, either at room temperature in sodium acetate buffer (pH 4.5) or at 80°C in phosphate buffer (pH 6.5). The final ionic strength of the extracting solutions was adjusted to 0.2 M by adding different amounts of the buffer salts.

The concentrations of CDTA and EDTA were calculated to give the same effective complexation constant at pH 4.5 (ref 20). In theory, the formation of a complex between a chelating agent Y and a metal ion M is an equilibrium that can be described by:

$$\mathrm{M} + \mathrm{Y} \rightleftharpoons \mathrm{MY} \text{ with the complexation constant } K = [\mathrm{MY}]/[\mathrm{M}][\mathrm{Y}],$$

where [M] is the concentration of free metal ions and [Y] is the concentration of free chelating agent. However, when the chelating agent (or the metal ion) is involved in other equilibria, it is the effective complexation constant K' which matters:

$$K' = [\mathrm{MY}]/[\mathrm{M}'][\mathrm{Y}'],$$

where $[\mathrm{M}']$ is the concentration of metal ions free or engaged in other reactions and $[\mathrm{Y}']$ is the concentration of chelating agent free, protonated, or complexing other ions.

For EDTA and CDTA, the complexing species is the tetravalent ion Y^{4-}, and pH is therefore an extremely important factor. Though the calcium complexation constants of CDTA and EDTA differ by two orders of magnitude, at pH 4.5 the effective complexation constants do not differ so much, and a concentration of EDTA about twice that of CDTA is all that is needed to complex the same amount of calcium ions.

Five grams of alcohol-insoluble solids were suspended in 300 mL of extracting solution. They were left under agitation either for 1 h (at room temperature) or for 0.5 h (at 80°C). The extracts were collected by filtration on G4 sintered-glass filters. The residues were reextracted under the same conditions until the sugar concentration in the extract was lower than 10 μg/mL. Five extractions were necessary to reach that level at room temperature with apples and seven for sugar beets, and only three at 80°C. The final residues were washed extensively with distilled water and dried by solvent exchange. The extracts were pooled, dialysed, and freeze-dried. Pectins were further purified by stirring in 70% EtOH.

General.—Salt-free pectins were obtained by repeated stirring in 70% EtOH. They were converted into the acidic form by percolating the salt-free solutions through a column (15 × 2 cm) of Amberlite IR-120 resin in the H^+ form. Salts of these samples were obtained by exact neutralisation with calcium hydroxide. The neutralisation point was determined on an aliquot by conductimetry.

Physico-chemical methods.—(*a*) *Ion-exchange chromatography.* Pectins (50–100 mg in 50 mL) were injected on a column (20 × 2.6 cm) of DEAE Sepharose CL-6B (Pharmacia, Uppsala, Sweden) equilibrated with 0.05 M succinic acid–sodium succinate buffer at pH 4.8 (containing 0.01% of merthiolate). After injection, the column was washed with the equilibration buffer (100 mL) and eluted with a linear gradient from 0.05 to 1 M, followed by 100 mL of 1 M buffer. The flow rate was 32 mL/h. Fractions of 8 mL were collected and assayed for uronic acid and neutral sugar contents. Appropriate fractions were pooled, dialysed, and freeze-dried.

(*b*) *Gel-filtration chromatography.* Pectins (~20 mg) in 10 mL of buffer were injected on a column (90 × 2.6 cm) of Sephacryl S500 (Pharmacia, Uppsala, Sweden) eluted upward with succinic acid–sodium succinate buffer (pH 4.5, containing 0.01% of merthiolate) at a flow rate of 25.6 mL/h. Fractions of 6.4 mL were collected and assayed for uronic acid and neutral sugar contents. Appropriate fractions were pooled, dialysed, and freeze-dried.

(*c*) *Viscosimetry.* Intrinsic viscosity values ($[\eta]$, mL/g) were calculated by measuring the flow times of solutions of pectins in 0.155 M NaCl (specific viscosity range, 0.2–1.5) at 25.0 ± 0.1°C in an automatic Ubbelhode viscosimeter (Amtec, Nice, France) and by using the double extrapolation to zero concentration based on the Huggins and Kramer equations[21].

(*d*) *Conductimetry.* The conductimetric measurements were carried out with a CDM 83 conductimeter (Radiometer, Copenhagen, Denmark) equipped with a double platinum electrode (Radiometer PP 1042) at 25.0 ± 0.1°C. The cell constant was determined daily with 0.1 M KCl; the potassium, lithium, and calcium hydroxide solutions were prepared daily and were titrated with 1 M HCl (Titrisol, Merck). The pectin solutions had concentrations of ~ 1 mequiv/L at the neutralisation point.

(*e*) *Potentiometry.* The potentiometric measurements were carried out on a PHM 84 pH meter (Radiometer). The calcium activity coefficient was measured at 25.0 ± 0.1°C with gentle stirring and, after stabilisation, with a selective electrode (Radiometer F2112 Ca) calibrated with $CaCl_2$ solutions of calculated activity. A saturated calomel electrode was used as the reference. The pectin solutions had concentrations of ~ 1 mequiv/L. The $CaCl_2$ concentration was obtained by conductimetric titration (platinum electrode) of the chloride with silver nitrate. The activity coefficients given here are the ratio of the activity of calcium ions in the presence of pectins to that in $CaCl_2$ solutions at the same concentrations.

Analytical.—The galacturonic acid and total neutral sugar concentrations were measured by automated *m*-phenylphenol[22] and orcinol assays[23], respectively. An arabinose standard was used for the neutral sugars. Corrections were made for the interference of uronic acids in the orcinol assay. Individual neutral sugars were analysed as their alditol acetate derivatives by gas chromatography[24] after hydrolysis by 1 M H_2SO_4 at 100°C for 3 h, with prehydrolysis in 72% H_2SO_4 for 1 h at room temperature in the case of residues. Proteins were measured in the residues by semi-automatic micro-Kjeldahl and in the extracts by the Lowry method[25] using a BSA standard. Methanol and acetic acid contents were estimated by HPLC according to Voragen et al.[26]. The dm and da were calculated as the molar ratios of MeOH and acetic acid to galacturonic acid. Phenolic acids were determined by HPLC after saponification and extraction[27]. Ferulic acid was measured spectrophotometrically at 375 nm at pH 10 (ref 28). Moisture was determined as the weight loss after drying at 120°C for 2 h. Ash was determined by incineration overnight at 550°C then for 1 h at 900°C.

RESULTS AND DISCUSSION

Composition of the cell wall materials.—Both apple (Table I) and beet (Table II) AIS were composed mainly of polysaccharides. The main sugars in apple AIS were D-glucose and D-galacturonic acid. Its composition was similar to previous results[19,29,30]; it was relatively poor in L-arabinose and rich in D-xylose, and contained no phenolic acids[19]. The main sugars in beet AIS were D-glucose, D-galacturonic acid, and L-arabinose. It was rich in acetic acid and contained

TABLE I

Composition (% dry weight) of the apple alcohol-insoluble solids (AIS) and extracts

	AIS	pH 4.5, 20°C			pH 6.5, 80°C		
		CDTA	EDTA	Buffer	CDTA	EDTA	Buffer
Yields	1.7 [a]	10.6 [b]	7.4 [b]	7.9 [b]	31.9 [b]	nd	31.2 [b]
Galacturonic acid	23.8	55.1	60.2	52.7	49.2	49.3	45.1
Rhamnose	1.7	1.1	1.2	1.1	2.2	1.9	2.5
Fucose	1.2	0.4	0.4	0.4	0.2	0.1	0.2
Arabinose	6.7	3.5	3.6	3.5	9.3	7.9	10.1
Xylose	5.4	1.2	1.2	1.3	2.5	2.1	3.2
Mannose	1.4						
Galactose	5.9	4.6	4.6	4.7	6.4	5.3	7.1
Glucose (cellulose)	32.2 (30.7)	2.1	2.0	2.3	1.4	1.2	1.9
Methanol (dm) [c]	3.4 (80)	8.1 (80)	8.3 (76)	7.5 (78)	6.7 (74)	6.7 (75)	5.2 (63)
Acetic acid (da) [d]	2.1 (26)	1.1 (6)	1.1 (5)	1.0 (6)	2.2 (13)	2.0 (12)	2.1 (14)
Proteins	7.1	11.2	10.7	17	4.5	4.5	5.7
Ash	1.0	nd [e]	nd	nd	nd	nd	nd

[a] From fresh plant material. [b] From AIS. [c] dm, Degree of methylation. [d] da, Degree of acetylation. [e] nd, Not determined.

phenolic acids, mainly ferulic acid (1.3%); the dm of the beet pectins was lower than that of apple pectins. Its composition was similar to that of cell wall isolated from sugar-beet pulps[31–36], which are the residue after extraction of sucrose, but

TABLE II

Composition (% dry weight) of the beet alcohol-insoluble solids (AIS) and extracts

	AIS	pH 4.5, 20°C			pH 6.5, 80°C		
		CDTA	EDTA	Buffer	CDTA	EDTA	Buffer
Yields	3.8 [a]	7.1 [b]	5.6 [b]	5.6 [b]	27.5 [b]	24.5 [b]	28.9 [b]
Galacturonic acid	19.4	48.4	49.8	51.3	48.4	38.6	45.6
Rhamnose	1.8	1.1	1.3	1.3	1.6	1.3	1.9
Fucose	0.2	0.2	0.2	tr [f]			
Arabinose	18.9	8.2	9.6	10.1	14.3	10.9	16.4
Xylose	1.5						
Mannose	1.1						
Galactose	5.8	4.6	5.2	5.1	4.8	4.0	5.5
Glucose (cellulose)	26.3 (25.2)	0.2		tr			
Methanol (dm) [c]	2.5 (72)	4.6 (52)	5.1 (56)	5.8 (63)	4.8 (55)	4.1 (58)	4.3 (52)
Acetic acid (da) [d]	4.4 (67)	4.4 (27)	4.9 (29)	5.6 (32)	5.7 (35)	4.4 (33)	5.3 (34)
Phenolic acids	1.5	nd [e]	nd	nd	nd	nd	nd
Proteins	8.6	10.2	11.3	7.9	5.8	5.4	5.6
Ash	4.1	nd	nd	nd	nd	nd	nd

[a] From fresh plant material. [b] From AIS. [c] dm, Degree of methylation. [d] da, Degree of acetylation. [e] nd, Not determined. [f] tr, Trace.

with a lower ash content (4.1%). The ash content in the beet pulps is usually ~ 10%[32,33,36], due to persistence of soil particles after the industrial process.

Yields of the extracts.—Two different sets of conditions were used for the extractions: "mild" conditions, i.e., pH 4.5 at room temperature; and "harsh" conditions, i.e., pH 6.5 and at 80°C. For each of these extraction conditions, three extractants were used: buffer (acetate at pH 4.5 and phosphate at pH 6.5) alone or with chelating agents, EDTA and CDTA, with a total ionic strength of 0.2 M (Fig. 1).

The yields were much lower under mild conditions (Tables I and II): the maximum yields were 10.6% for apple and 7.1% for sugar beet under mild conditions, but reached ~ 30 and ~ 25% for the apple and beet, respectively, under harsh conditions. The influence of the extracting agent was very limited compared to that of the extraction conditions. Under mild conditions, CDTA did extract more material than the buffer alone, but not EDTA. The yield from CDTA treatment of apple AIS was lower than our previous results[19], for which the apple cell-wall material was not dried, but in good agreement with the results of Selvendran et al.[9]. The yields obtained with EDTA and buffer were similar[37] to, or slightly lower[29] than, those reported earlier. The yields obtained for beet were higher than for pulps treated with cold water or buffer[18,38], probably because, in such by-products, the readily soluble pectins had been removed with the sucrose.

Under harsh conditions, no influence of the chelating agents was visible. The yields obtained for apple were high, comparable to those of Voragen et al.[30] with cold alkali, or Aspinall and Fanous[39] with hot water (17%), followed by oxalate (10%). Yields from beet were similar to those of Phatak et al.[40] with EDTA and oxalate at high temperatures. They were a little higher than with hot dilute acid[18,31,36,40], and correspond approximately to the sum of amounts extracted during sequential extraction schemes up to the dilute-alkali step[18,31].

Composition of the extracts.—The compositions of the extracts (Tables I and II) show differences for each type of cell wall between mild and harsh treatments rather than between the extracting agents. Both for apple and beet, the pectins extracted at high pH and temperatures were richer in neutral sugars, notably arabinose, and had higher da. The protein content of the pectins was lower, though the solubilised amount did increase.

The apple pectins contained galactose, arabinose, rhamnose, xylose, and a little fucose, as reported earlier[19,37]. Under mild conditions, the main neutral sugar was galactose, and, under harsh conditions, arabinose. Rhamnose and xylose contents also increased, whereas galacturonic acid, glucose, and fucose diminished. The pectins extracted under harsher conditions had a slightly lower methoxyl content. The da of the extracted pectins stayed lower than the value calculated for the AIS. Part of the acetyl substituents might be carried by other cell-wall constituents.

The pectins from sugar beet had lower contents of galacturonic acid than those of apples. They were rich in arabinose, contained no xylose and mannose, and only traces of glucose and fucose. The dm were lower, and the da higher, than those of apple pectins. Differences between mild and harsh extracts were less marked than for apple. The compositions of these pectins are close to those reported for cold-water-soluble pectins[18,38].

Ion-exchange chromatography.—The extracts were chromatographed on DEAE Sepharose CL-6B (Figs. 2 and 3), giving indication on the intermolecular distribution of the methoxyl groups and on the attachment of neutral sugars to the pectin backbone. In all cases, there was one main population, composed mostly of galacturonic acid and eluting at an ionic strength compatible with its dm. There were minor non-retained fractions, varying in amount between 6 and 0.7% of the total material.

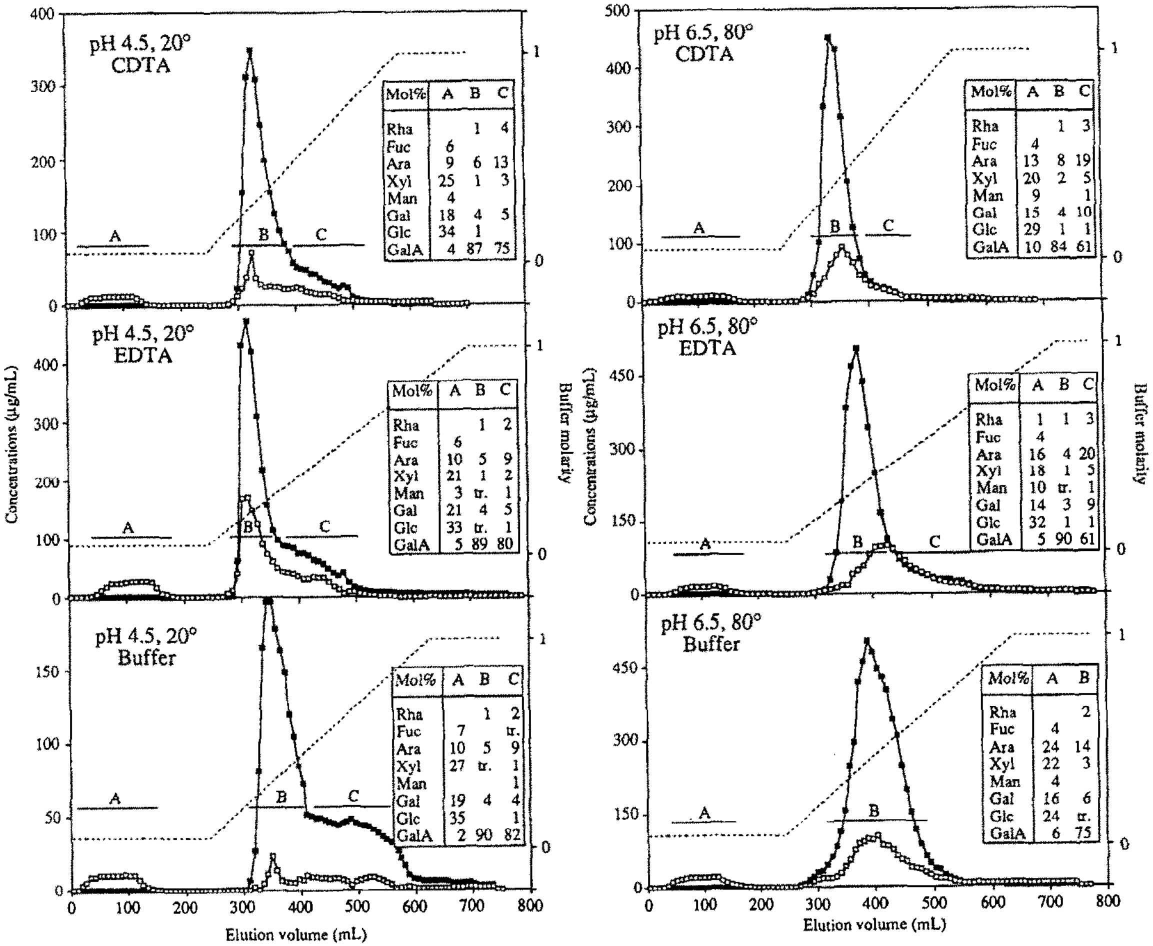
The non-retained fractions represented 4 to 6% of the apple extracts under mild conditions and 3 to 4% under harsh conditions (Fig. 2). They contained high proportions of glucose and xylose, some arabinose, galactose, and fucose, and various amounts of mannose. They still contained some uronic acids, albeit in very low proportions. The molar ratios of glucose, xylose, galactose, and fucose were close to those previously found for apple fucogalactoxyloglucan[41]. The retained fractions contained arabinose, galactose, xylose, rhamnose, and a little glucose. Extracts from mild conditions showed some tailing at higher ionic strengths (Fig. 2). The more retained fractions had increased neutral sugar contents, as noted for pectins extracted from apple cell walls by buffer or chelating agent[19,42], but not pectin from apple juice[43], which had a constant neutral–acid sugars ratio in the retained peak.

In sugar-beet extracts (Fig. 3), the non-retained fractions represented less than 2% of the extracts and were composed mostly of arabinose and galactose. There was less tailing of the main peak than for apple extracts. The extracts under harsh conditions (Fig. 3) showed a very minor fraction, eluting at the very beginning of the gradient, and rich in neutral sugars. The chromatograms obtained here were comparable to those of water-soluble pectins[18] for extracts under mild conditions and cold-alkali-soluble pectins[18] for extracts under harsh conditions.

The pectins in these extracts had very little contaminating material, and were used without purification for further experiments.

Intrinsic viscosities and molecular weight distribution.—High temperature and pH resulted in a marked degradation of the pectins, which can be seen both in the intrinsic viscosities and in the gel-filtration patterns (Figs. 4 and 5).

The pectins extracted under harsh conditions had much lower intrinsic viscosities (Table III) than for mild conditions. The intrinsic viscosities of the apple pectins extracted under mild conditions were very high (between 630 and 750 mL/g), comparable to the values obtained by Knee[44] (in 0.1 M NaPi, pH 6.5, at 20°C) and by Rouau and Thibault[43] (in 0.155 M NaCl, at 25°C) for pectins from apple juice. Apple pectins extracted under harsh conditions had intrinsic viscosities around 100–140 mL/g. Though the sugar-beet pectins extracted under mild conditions did not have such high intrinsic viscosities (around 200 mL/g), extracts in harsh conditions also showed a marked diminution, to around 55–100 mL/g.



pH 4.5, 20° CDTA

Mol%	A	B	C
Rha		1	4
Fuc	6		
Ara	9	6	13
Xyl	25	1	3
Man	4		
Gal	18	4	5
Glc	34	1	
GalA	4	87	75

pH 4.5, 20° EDTA

Mol%	A	B	C
Rha		1	2
Fuc	6		
Ara	10	5	9
Xyl	21	1	2
Man	3	tr.	1
Gal	21	4	5
Glc	33	tr.	1
GalA	5	89	80

pH 4.5, 20° Buffer

Mol%	A	B	C
Rha		1	2
Fuc	7		tr.
Ara	10	5	9
Xyl	27	tr.	1
Man			1
Gal	19	4	4
Glc	35		1
GalA	2	90	82

pH 6.5, 80° CDTA

Mol%	A	B	C
Rha		1	3
Fuc	4		
Ara	13	8	19
Xyl	20	2	5
Man	9		1
Gal	15	4	10
Glc	29	1	1
GalA	10	84	61

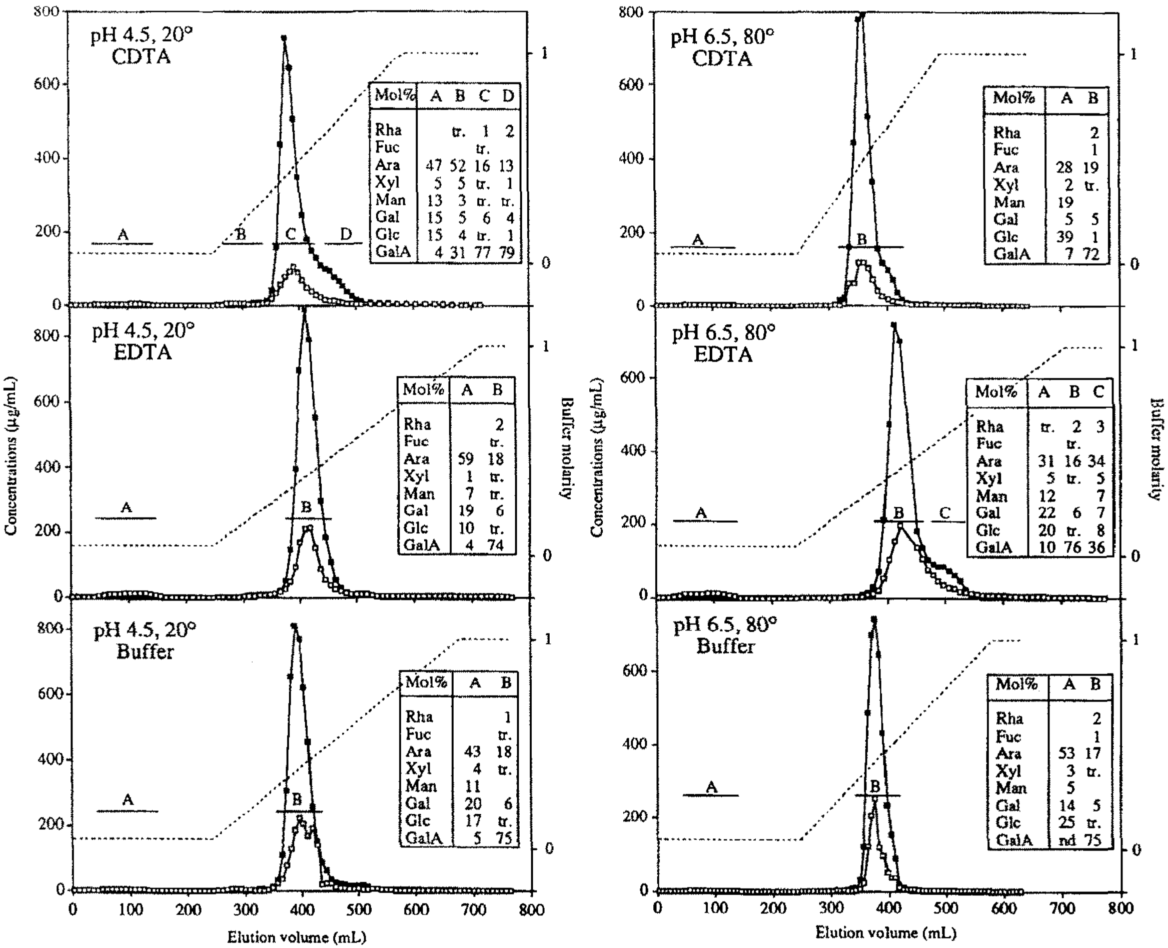
pH 6.5, 80° EDTA

Mol%	A	B	C
Rha	1	1	3
Fuc	4		
Ara	16	4	20
Xyl	18	1	5
Man	10	tr.	1
Gal	14	3	9
Glc	32	1	1
GalA	5	90	61

pH 6.5, 80° Buffer

Mol%	A	B
Rha		2
Fuc	4	
Ara	24	14
Xyl	22	3
Man	4	
Gal	16	6
Glc	24	tr.
GalA	6	75

Fig. 2. Ion-exchange chromatography on DEAE Sepharose CL-6B (eluted with succinate buffer at pH 4.8) of the material extracted from apple AIS under mild (pH 4.5, 20°C) and harsh conditions (pH 6.5, 80°C): ■, galacturonic acid; □, neutral sugars; — — —, buffer molarity.



pH 4.5, 20° CDTA

Mol%	A	B	C	D
Rha		tr.	1	2
Fuc			tr.	
Ara	47	52	16	13
Xyl	5	5	tr.	1
Man	13	3	tr.	tr.
Gal	15	5	6	4
Glc	15	4	tr.	1
GalA	4	31	77	79

pH 4.5, 20° EDTA

Mol%	A	B
Rha		2
Fuc		tr.
Ara	59	18
Xyl	1	tr.
Man	7	tr.
Gal	19	6
Glc	10	tr.
GalA	4	74

pH 4.5, 20° Buffer

Mol%	A	B
Rha		1
Fuc		tr.
Ara	43	18
Xyl	4	tr.
Man	11	
Gal	20	6
Glc	17	tr.
GalA	5	75

pH 6.5, 80° CDTA

Mol%	A	B
Rha		2
Fuc		1
Ara	28	19
Xyl	2	tr.
Man	19	
Gal	5	5
Glc	39	1
GalA	7	72

pH 6.5, 80° EDTA

Mol%	A	B	C
Rha	tr.	2	3
Fuc	tr.		
Ara	31	16	34
Xyl	5	tr.	5
Man	12		7
Gal	22	6	7
Glc	20	tr.	8
GalA	10	76	36

pH 6.5, 80° Buffer

Mol%	A	B
Rha		2
Fuc		1
Ara	53	17
Xyl	3	tr.
Man	5	
Gal	14	5
Glc	25	tr.
GalA	nd	75

Fig. 3. Ion-exchange chromatography on DEAE Sepharose CL-6B (eluted with succinate buffer at pH 4.8) of the material extracted from beet AIS under mild (pH 4.5, 20°C) and harsh conditions (pH 6.5, 80°C): ■, galacturonic acid; □, neutral sugars, — — —, buffer molarity.

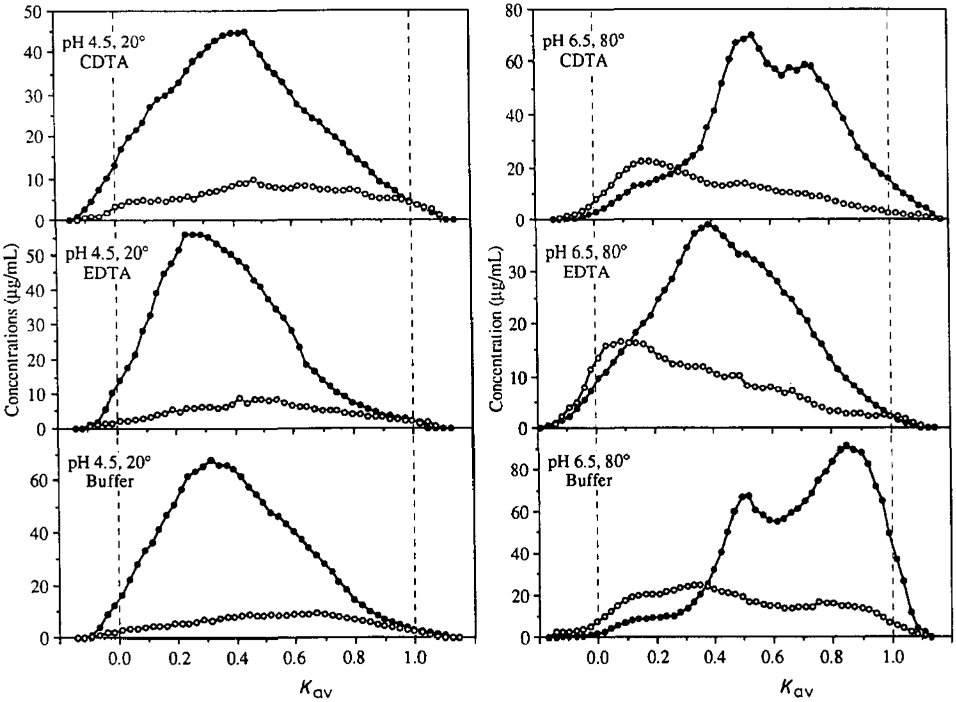


Fig. 4. Chromatography on Sephacryl S500 (eluted with 0.1 M sodium succinate buffer, pH 4.5) of the material extracted from apple AIS under mild (pH 4.5, 20°C) and harsh conditions (pH 6.5, 80°C): ●, galacturonic acid, ○, neutral sugars.

Dea and Madden[38], and Rombouts and Thibault[18] found intrinsic viscosities of 200 mL/g (in 0.5 M NaCl, at 25°C) and 260 mL/g (in 0.155 M NaCl, at 25°C) for cold-water-soluble beet pectins.

There were also differences in the molecular weight distributions (Figs. 4 and 5). For mild conditions, the pectins were highly polydisperse, with one wide uronic acid peak (between K_{av} 0.2 and 0.4 for apple, and 0.4–0.6 for beet), and the neutral sugars followed more or less the same distribution as the galacturonic acid. For harsh conditions, the main galacturonic acid peaks were shifted to higher K_{av} values, while some high molecular weight, neutral-sugar-rich fractions appeared. The gel-filtration patterns of the apple extracts from mild conditions (Fig. 4) were very similar to that reported earlier for apple CDTA-soluble pectin[19]. The patterns observed after harsh extractions (Fig. 4) resembled more those of dilute-alkali-soluble pectins[19], with high molecular weight, neutral-sugar-rich fractions, such as obtained from the "hairy regions" of apple pectins after degradation by alkali[43] or pectolytic enzymes[45,46]. The sugar-beet extracts (Fig. 5) could be compared to the water-soluble pectins of Rombouts and Thibault[18] for the mild conditions extracts and to the cold-NaOH-extract for the harsh conditions extracts. The high molecular weight, neutral-sugar-rich fraction was less important than for the apple extracts.

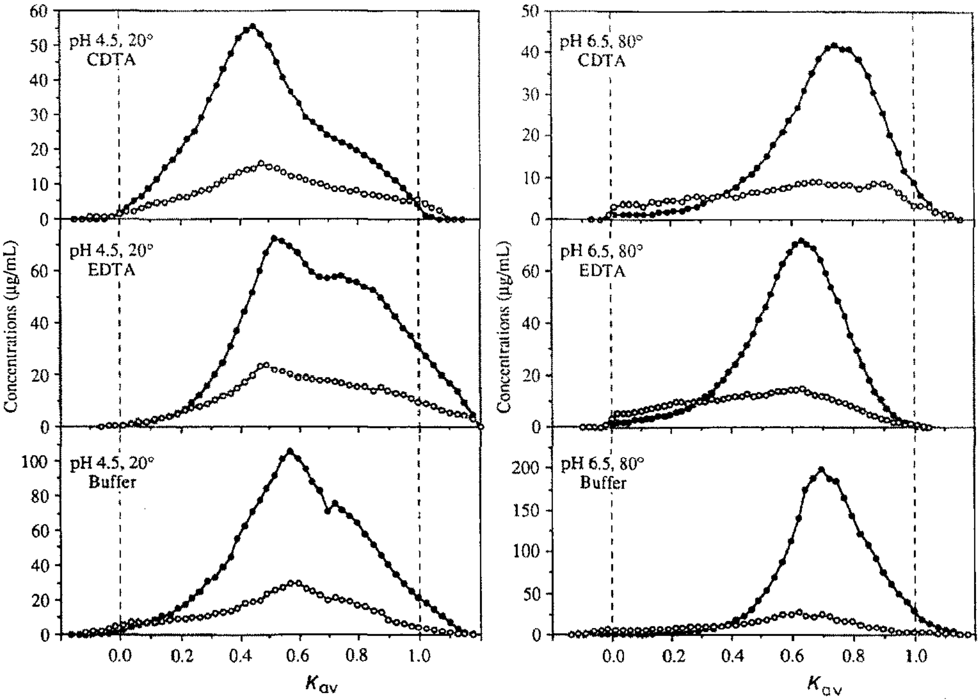


Fig. 5. Chromatography on Sephacryl S500 (eluted with 0.1 M sodium succinate buffer, pH 4.5) of the material extracted from beet AIS under mild (pH 4.5, 20°C) and harsh conditions (pH 6.5, 80°C): ●, galacturonic acid; ○, neutral sugars.

Interaction with monovalent cations and calcium.—The measure of the free fractions of counterions, and notably of calcium, gives indications of the distribution of free carboxyl groups in pectins[16]. The calcium activity coefficient and calcium, potassium, and lithium transport parameters were determined in dilute salt-free solutions of pectins. Results are compared to theoretical values (Table IV).

The behavior of polyelectrolyte polymers is governed by the structural charge parameter ξ, introduced by Lifson and Katchalsky[47]. Its value at 25°C is given by: $\xi = e^2/bDkT(1 - \mathrm{dm}/100) = 1.61(1 - \mathrm{dm}/100)$,

TABLE III

Intrinsic viscosities ([η], mL/g) and Huggins coefficient (κ_H) of the extracts

		pH 4.5, 20°C			pH 6.5, 80°C		
		CDTA	EDTA	Buffer	CDTA	EDTA	Buffer
Apple	[η]	760	680	750	115	143	56
	κ_H	0.74	0.64	0.69	0.58	0.62	1.844
Beet	[η]	257	165	187	100	105	70
	κ_H	0.73	0.58	0.82	0.73	0.74	1.03

TABLE IV

Potentiometric and conductimetric data and theoretical values [a]

Sample	Experimental values				Theoretical values			
	λ_p	f_+	f_{++}	$\gamma_{Ca^{2+}}$	ξ	f_+	f_{++}	$\gamma_{Ca^{2+}}$
Apple, pH 4.5, 20°C								
CDTA	25.6	0.87	0.61	0.58	0.32	0.98	0.94	0.72
EDTA	47.5	0.84	0.49	0.52	0.39	0.98	0.92	0.68
Buffer	21.1	0.84	0.51	0.60	0.35	0.98	0.93	0.70
Apple, pH 6.5, 80°C								
CDTA	25.5	0.86	0.44	0.60	0.42	0.97	0.90	0.66
EDTA	25.2	0.86	0.55	0.55	0.40	0.97	0.91	0.67
Buffer	30.8	0.85	0.44	0.49	0.60	0.95	0.73	0.51
Beet, pH 4.5, 20°C								
CDTA	27.0	0.90	0.45	0.54	0.77	0.92	0.56	0.39
EDTA	48.0	0.76	0.49	0.58	0.71	0.93	0.61	0.43
Buffer	41.0	0.85	0.54	0.38	0.60	0.95	0.73	0.51
Beet, pH 6.5, 80°C								
CDTA	24.9	0.83	0.42	0.48	0.72	0.93	0.60	0.42
EDTA	24.5	0.86	0.45	0.53	0.68	0.93	0.64	0.45
Buffer	38.2	0.82	0.41	0.53	0.77	0.92	0.56	0.39
Galacturonic acid	20.6	0.98	1.02	0.97		1.00	1.00	1.00
Poly(galacturonic acid)	46	0.58	0.10	0.10	1.61	0.54	0.27	0.19

[a] λ_p, Equivalent conductivity of the pectin; f_+, transport parameter of the monovalent ions; f_{++}, transport parameter of the divalent ion; $\gamma_{Ca^{2+}}$, calcium activity coefficient; ξ, structural charge parameter.

where e is the electron charge, kT is the Boltzmann term, and b is the length of the monomeric unit (taken as 0.435 nm[48]).

In infinitely dilute solutions of rod-like polyelectrolytes, the activity coefficients γ are directly imposed by the charge parameter, and Manning's theory[49] gives:

$$\gamma = e^{-1/2|Z|\xi} \text{ if } |Z|\xi \leq 1, \text{ and}$$

$$\gamma = e^{-1/2} \times (|Z|\xi)^{-1} \text{ if } |Z|\xi > 1,$$

with Z the charge of the counterion.

In conductimetry, the limiting law for the equivalent conductivity of a pure polyelectrolyte solution can be written as:

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

where Λ is the equivalent conductivity (in S · cm^2/equiv) of the salt in solution, λ_c is the equivalent conductivity of the counterion in pure solvent, λ_p is the equivalent conductivity of the monomeric unit of the polyelectrolyte, and f is the transport parameter, a quantity related to the free fraction of counterions. The theoretical transport parameter values from Manning's theory are:

$$f_Z = 1 - 0.55(|Z|\xi)^2/(|Z|\xi + 3.14) \text{ if } |Z|\xi \leq 1, \text{ and}$$

$$f_Z = 0.87 \times (|Z|\xi)^{-1} \text{ if } |Z|\xi > 1.$$

By measuring the conductivity for three ionic forms of the polymer (calcium, lithium, and potassium) and assuming that the f_+ value is unaffected by the nature of the monovalent counterion (lithium and potassium), λ_p and the transport parameters (f_+, f_{++}) for monovalent and calcium ions can be calculated. The transport parameter f_+ obtained with lithium and potassium was close to the values calculated by Manning's theory; the same was true of the calcium activity coefficient $\gamma_{Ca^{2+}}$, whereas f_{++} was lower than expected from the theory, as is usually found for pectins[16,50]. The experimental values were in good agreement with results[16,50] for pectins with similar ξ and a random distribution of free carboxyl groups. Kohn[2] and Thibault and Rinaudo[16] showed that the experimental values obtained with monovalent ions are in reasonable agreement with the theoretical values, whereas calcium ions bind strongly to highly charged pectins (low dm) and to pectins with a blockwise distribution of the free carboxyl groups, thus giving lower $\gamma_{Ca^{2+}}$ and f_{++} values. For the same dm, the values obtained with a blockwise distribution of the free carboxyl groups are thus markedly lower than with a random distribution. The very low values (0.10) of $\gamma_{Ca^{2+}}$ and f_{++} of calcium counterions measured for poly(galacturonic acid) are in agreement with previously reported work[16,50].

CONCLUSIONS

CDTA did increase (slightly) extraction of pectins at pH 4.5 and room temperature, but EDTA did not give the same effect, though the concentrations of EDTA and CDTA had been adjusted so that they had the same complexing power for calcium ions. Chelation of calcium does not appear to be a determinant factor for pectin extraction in those conditions. A specific effect of CDTA, other than the chelating effects, is also possible, and could be due to another property of this agent. No increase of extraction was observed at pH 6.5 and 80°C in the presence of chelating agents. However, under those conditions, the buffer extracted more than 60 and 70% of the galacturonic acid for apple and beet, respectively. It is likely that pectins which were not already extracted were linked through stronger linkages, and thus not susceptible to the action of chelating agents. An added difficulty is that precautions must be taken when interpreting results of extraction with such chelating agents as EDTA and CDTA, as it is very difficult to remove them after extraction[51]; in our case, even after extensive dialysis, the freeze-dried extracts still contained up to 40% of chelating agent, which was finally eliminated by stirring in ethanol. This persistence has to be taken into account as it could lead to an artificial increase of the yields.

The DEAE elution patterns showed that there was little contaminating material in the pectin extracts, compared with acid or alkali extracts[18,19,42], which contain much neutral, non-retained material. The apple pectins contained only a very little xylose and glucose, which were mostly found in minor, non-retained fractions containing fucogalactoxyloglucan, i.e., hemicellulosic material. Little neutral arabinan was extracted from sugar beet during the ethanol treatment or with the pectins, indicating that it did not exist as such in the beet cell walls. The intermolecular distributions of dm were not totally homogeneous, but the DEAE patterns were totally different from that obtained after deesterification with plant pectinesterase, where two peaks are observed, one at very low and one at very high ionic strength[52].

The pectins extracted under harsh conditions showed definite degradation compared to those extracted under mild conditions, suggesting that many earlier results on structure of pectins, based on (highly methylated) pectins extracted at high temperatures and pH's, contain undeniable artefacts. There are few reports of intrinsic viscosities and dm for pectins extracted at high pH and temperature values, though such extraction conditions have commonly been used for structural studies of cell walls. Highly methylated pectins are very sensitive to β-elimination: degradation is visible at pH 6.8 and 35°C (ref. 53), and pronounced even for pH 6 at elevated temperatures[54]. There was here a marked diminution of the global size of the extracted material when harsh conditions were used. The extent of degradation seemed lower for sugar-beet pectins, maybe because their lower degrees of methylation make them less sensitive to β-elimination than apple pectins.

The extracted pectins were highly methylated and had a random distribution of the free carboxyl groups. They were not able to bind strongly calcium and form "egg-boxes" in solution. It is therefore highly unlikely that they could have been cross-linked by formation of egg-boxes in the cell walls. Our results do not rule out the possibility of isolated ionic linkages between pectic chains through Ca^{2+}. These bonds can be broken by increasing ionic strength, explaining why buffer alone extracts pectins. Treatments of AIS from grapes by increasing concentrations of NaCl has been shown to extract pectic fractions[55], though less than oxalate at the same pH (4.5) and temperature (20°C).

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