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Extraction and characterisation of very highly methylated pectins from lemon cell walls †

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Abstract

Pectins were extracted either by water from extruded lemon fibre or by hot acid from the raw lemon fibre. The amount of water-soluble polysaccharides from lemon plant cell walls was greatly increased after extrusion-cooking. The pectins obtained by extraction with water from the extruded fibre and the pectins extracted from the raw material by hot acid were studied. The water-soluble pectins obtained after extrusion-cooking had the distinctive feature of being very highly (92%) methylated; they were also particularly rich in arabinose side-chains. High molecular weight material coming from the "hairy" regions was isolated after digestion by an endo-polygalacturonase. Methylation analysis revealed the presence in both pectins of fairly branched (1 → 5)-linked arabinans and arabinogalactans of type I and II side-chains.

1. Introduction

Citrus wastes and apple pomace have a very high content of cell wall polysaccharides. They are sources of dietary fibre and they constitute up to now the only raw material for the production of pectins used by the food industry as stabilisers or gelling agents. Usually, pectins are extracted [1] by a mineral acid at pH 1–2.5 and at 60–80°C. This acid extraction presents several disadvantages, such as water-pollution and corrosion. Furthermore, the residues obtained after the extraction of pectins are not suitable for human nutrition although they are very rich

† Pectins of Lemon Cell Walls.

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in dietary fibre. Enzymes such as "protopectinase", endo-polygalacturonase, endo-arabinanase, or endo-galactanase have been tentatively used to extract pectin from the cell walls [2–5]. Pectins obtained by enzymic degradation often have a low degree of polymerisation and only a few enzymes lead to the extraction of appreciable quantities of pectins with a high degree of polymerisation.

Various types of heat treatment applied to products rich in plant cell wall polysaccharides were investigated. It has been shown that extrusion-cooking, autoclaving, roasting, popping, blanching, freezing, and canning result in an increased amount of soluble polysaccharides in cereals, vegetables, and fruits [6–10]. We have previously shown that extrusion-cooking led to a large solubilisation of polysaccharides of various types of plant cell-wall-rich products without extensive degradation of the polymeric structure [11–13].

In this study, commercial lemon fibres were extruded under controlled conditions at different levels of severity. Water-insoluble residues were characterised by their composition, whereas solubilised pectic substances were characterised both by composition and molecular weight. The structure of purified pectins solubilised by extrusion-cooking was compared to that of pectins obtained by acid extraction from the raw material.

2. Materials and methods

Materials.—Commercial lemon dietary fibres (LF0) were from Sofalia (Ennezat, France); they were obtained from the residue left from the lemon juice industry. LF0 was ground with a hammer mill (linear velocity of 100 m/s) to pass a 2-mm screen.

Endo-polygalacturonase (EC 3.2.1.15) was purified from a commercial enzyme preparation produced by *Aspergillus niger* as previously described [14].

Extrusion-cooking.—A twin-screw extruder Clextral BC 45 was used. The barrel was 1 m long, the ratio of the screw length to screw diameter was 18. It included four heating and cooling zones. The screw configuration featured positive displacement pitches and a reverse pitch element before the die head; the die was made of two cylindrical tubes (diameter, 4 mm; length, 30 mm). Screw configuration 1 stands for the classical arrangement, i.e., with a reverse screw element (length, 50 mm; pitch, 15 mm) in the terminal position; screw configuration 2 included a reverse screw element (length, 100 mm; pitch, 15 mm) located 50 mm before the end. Details on extrusion-cooking can be found in ref. 15. The electrical power of the screw motor drive was measured in order to control the steady state of the extruder during sampling and to compute [15] the specific mechanical energy (S.M.E.). Product temperature was measured just before the die head and the average residence time was < 30 s. Extrusion conditions and the nomenclature of the products are given in Table 1. Extruded products were air-dried in an oven at 70°C for 10 min.

Aqueous extraction.—Samples of initial and extruded fibre (5 g) were stirred with distilled water (150 mL) for 30 min at 25°C. The suspension was centrifuged

Table 1
Nomenclature and processing conditions for lemon fibre samples

Sample	Temperature of the product (°C)	Screw speed (rev/min)	Screw configuration	Water added (% of dry matter)	Feed rate (kg/h)	Specific mechanical energy (kWh/t)
LF0				Initial product		
LF1	92	150	2	30	32	170
LF2	103	240	2	37.5	32	215
LF3	90	240	2	50	24	245
LF4	94	240	2	30	32	210
LF5	110	240	1	30	32	182
LF6	96	240	2	25	32	228
LF7	110	240	1	25	32	220
LF8	112	240	1	20	32	250
LF9	99	240	2	20	32	236
LFH				Extraction of LF0 by 0.05 M HCl		

at 3000*g* at room temperature for 15 min and the supernatant solution collected. This extraction was carried out four times. The supernatant solutions were filtered through G4 sintered glass and concentrated to 250 mL, 200 of which were extensively dialysed against distilled water and freeze-dried. The residue was washed successively with EtOH and acetone, dried overnight at 40°C, and weighed.

Acid extraction.—Acid-treated lemon fibre (LFH) was obtained by heating LF0 (5 g) with 0.05 M HCl (150 mL) for 30 min at 85°C. The slurry was cooled and centrifuged as above. The extraction was carried out 3 times. The supernatant solution was brought to pH 4.5 with 1 M NaOH, filtered, and extensively dialysed against distilled water before being freeze-dried. The residue was washed and dried as described above.

Analytical methods.—Moisture content was determined by drying at 120°C for 2 h. All the compositions are given on moisture-free basis. Uronic acids (as galacturonic acid) were determined by the automated *m*-phenylphenol method [16] and by the method of Ahmed and Labavitch [17] in the soluble and in the insoluble fractions, respectively. Total neutral sugars were quantified by the automated orcinol method [18]. Soluble fractions were hydrolysed by 1 M CF_3CO_2H (2 h, 121°C), and insoluble fractions were prehydrolysed [19] by 72% H_2SO_4 (30 min, 20°C), diluted to 1 M, and heated (2 h, 100°C). The individual sugars were reduced, acetylated, and analysed [20] by GLC. Methanol and AcOH were determined [21] by HPLC (Aminex HPX 87 H). Degree of methylation (dm, mol of MeOH per 100 mol of galacturonic acid) and degree of acetylation (da, mol of AcOH per 100 mol of galacturonic acid) were calculated from the amount of MeOH and AcOH, respectively, and from the amount of galacturonic acid.

Viscosity measurements.—Intrinsic viscosities of the pectins ([η], mL/g) were measured at 25°C in 0.155 M NaCl with an automatic Amtec viscosimeter as

described elsewhere [22]. The viscosity-average molecular weights were calculated according to Owens et al. [23].

Enzymic degradation.—Solutions of pectins (2 mg/mL) were de-esterified at pH 13 (by addition of 1 M NaOH) for 1 h at 2°C. Each solution was brought to pH 4.5 with 0.1 M HCl, dialysed against distilled water, and freeze-dried. Solutions of de-esterified pectins (30 mg) in 0.05 M NaOAc buffer (15 mL, pH 4.5) were incubated for 24 h at 30°C with an endo-polygalacturonase (1 nkat of enzyme/mg of galacturonic acid residues). The liberation of galacturonic acid was followed by the Nelson procedure [24].

Chromatography.—Ion-exchange chromatography was performed on a column (21 × 1.6 cm) of DEAE-Sepharose equilibrated with 0.05 M NaOAc (pH 4.8). Samples (50 mL of a solution at 2 mg/mL) were loaded onto the column and the gel was washed with 200 mL of 0.05 M NaOAc (pH 4.8). The bound material was eluted with a linear NaOAc buffer gradient at pH 4.8 (0.05 to 1 M; 450 mL); 8-mL fractions were collected.

Gel permeation chromatography was carried out on a column (50 × 1.6 cm) of Sepharose CL-6B. Solutions (3 mL at 4 mg/mL) were loaded onto the column and eluted by upward elution at 12.5 mL/h with 0.1 M NaOAc buffer (pH 4). Chromatography on a column (80 × 2.6 cm) of Sephacryl S-500 was carried out at a flow rate of 25 mL/h with 0.1 M NaOAc buffer (pH 4). Chromatography on a column (70 × 2.1 cm) of Sephacryl S-200 was carried out at a flow rate of 25 mL/h with 0.1 M NaOAc buffer (pH 4). Fractions (5 mL) were assayed for uronic acid and neutral sugars, and appropriate fractions were pooled and analysed for individual neutral sugars.

Methylation analysis.—Methylation was carried out as described by Hakomori [25]. A time of contact with the dimethylsulfinyl anion of 1 h was used. Methylated polysaccharides were extracted with 2:1 $CHCl_3$–MeOH, washed three times with distilled water, and air-dried at 40°C. The methylated polysaccharides were then hydrolysed with 90% formic acid (1 h, 100°C) and 2 M CF_3CO_2H (90 min, 120°C), converted into their alditol acetates, and analysed by GLC on (*a*) a fused-silica capillary column (30 m × 0.32 mm) bonded with OV-1; 150°C for 10 min, then 2°C/min to 190°C; injector temperature, 210°C; detector temperature, 240°C; split 60–80 mL/min; H_2 as carrier gas at 0.7 bar; and (*b*) a fused-silica capillary column (30 m × 0.32 mm) bonded with OV-225; 175°C for 15 min, then 5°C/min to 200°C; injector temperature, 240°C; detector temperature, 240°C; split 60–80 mL/min; H_2 as carrier gas at 0.7 bar. Peak identification was based on retention times, using *myo*-inositol as internal standard. Peak areas were corrected by response factors as described by Sweet et al. [26].

3. Results and discussion

Carbohydrate composition of the lemon fibres.—Initial lemon fibres contained 67.4% of carbohydrates (Table 2), mainly galacturonic acid (26.0%), glucose (23.5%), glucose (23.5%), and arabinose (6.8%), in agreement with values previ-

Table 2
Yield and carbohydrate composition (% of dry matter) of initial lemon fibres before and after aqueous and acid extractions, and of extruded samples after aqueous extraction

Sample	Treatment	Yield (%)	GalA	Rha	Fuc	Ara	Xyl	Man	Gal	Glc
LF0	None	100.0	26.0	1.4	0.4	6.8	2.6	3.2	3.5	23.5
LF0	Aqueous extraction	76.5	32.0	1.1	0.4	8.1	3.0	2.2	4.1	22.2
LF1		68.1	26.2	0.9	0.3	7.1	3.1	2.2	3.8	21.6
LF2		65.1	26.5	0.9	0.4	7.5	3.5	2.3	4.1	23.3
LF3		64.3	28.1	0.8	0.4	7.6	3.7	2.5	4.0	25.6
LF4		63.8	27.8	1.0	0.4	6.9	3.6	2.4	4.0	25.5
LF5		62.0	26.9	0.8	0.4	7.5	3.8	2.6	4.1	26.1
LF6		58.6	25.0	1.0	0.4	6.1	3.8	2.3	3.8	26.3
LF7		53.9	21.5	0.9	0.6	6.3	4.7	2.8	4.1	30.2
LF8		50.6	23.2	0.7	0.5	4.4	4.5	2.7	3.8	31.0
LF9		49.6	19.3	0.9	0.5	3.6	4.4	2.9	3.7	31.1
LFH	Acid extraction	42.5	23.4	1.7	0.6	1.8	5.4	4.0	4.6	36.2

ously found [27] for citrus pulp cell walls. An aqueous extraction removed 23.5% of the material; the residue was essentially enriched in galacturonic acid and arabinose, and slightly impoverished in glucose. From the carbohydrate compositions before and after aqueous extraction, it can be calculated that 6% of the galacturonic acid present in the lemon fibres was solubilised, which was comparable [28] to other results. The other sugars were solubilised in larger amounts since 47% of the mannose, 40% of the rhamnose, 28% of the glucose, 12% of the xylose, 10% of the galactose, and 9% of the arabinose initially present were recovered in the water-soluble fraction.

Both extrusion-cooking and acid treatment led to a marked increase in the amounts of soluble material. There is a general trend to increased water-solubility when the S.M.E. of the extrusion process increases, as previously reported for wheat bran and sugar-beet pulp [12,13] and the maximum solubility ($\sim$ 50%) was observed for LF8 and LF9. The water-insoluble residues had higher proportions of glucose, mannose, and xylose while the proportion of the other sugars slightly decreased or remained roughly constant, indicating an increase in the solubilisation of all sugars except glucose, mannose, and xylose. It can be calculated that 31 to 63% of the galacturonic acid, 54 to 75% of the rhamnose, 28 to 74% of the arabinose, and 26 to 48% of the galactose initially present in the lemon fibres were water-soluble after extrusion-cooking. The highest solubility (57.5%) was observed for the fibre after acid treatment. This treatment solubilised 62% of the galacturonic acid, 48% of the rhamnose, 89% of the arabinose, and 44% of the galactose. In the residue obtained after extraction with acid, the arabinose content was drastically diminished. The solubilisation of glucose, mannose, and xylose remained unchanged after extrusion-cooking or acid treatment.

Table 3
Composition (% dry matter), intrinsic viscosity, and yields of the water-soluble and acid extracted polysaccharides

Sample	Yield (%)	Itrinsic viscosity (mL/g)	GalA	Rha	Ara	Xyl	Man	Gal	Glc	Methanol (dm)	Acetic acid (da)
LF0	4.0	762	42.4	1.4	16.9	1.7	2.2	4.7	4.9	7.0 (91)	0.7 (5)
LF1	12.5	460	45.3	1.5	13.3	1.5	2.1	3.4	8.5		
LF2	13.8	413	45.5	2.0	13.9	1.5	2.0	3.6	7.6		
LF3	14.2	450	49.1	2.0	12.9	1.6	2.4	3.7	6.8	8.0 (91)	0.9 (5)
LF4	16.1	504	44.9	1.9	20.7	1.9	1.6	4.0	8.5		
LF5	15.1	305	45.1	1.3	13.3	1.3	1.7	3.0	5.3		
LF6	21.1	314	45.3	1.5	15.4	1.2	1.6	3.1	8.8		
LF7	23.3	243	50.6	2.3	19.9	1.3	1.9	4.1	3.0		
LF8	25.4	244	47.2	1.7	16.1	1.2	1.6	4.5	3.9		
LF9	29.4	191	45.3	1.7	15.9	1.1	1.5	3.8	5.1	8.2 (92)	0.9 (5)
LFH	34.1	588	51.6	1.1	13.4	0.3	0.5	3.0	4.3	7.6 (81)	0.4 (3)

Yields and characterisation of pectins extracted from lemon fibres.—Almost a quarter (23.5%) of the initial lemon fibre was water-soluble. However, the material retained after dialysis represented only 4.0% of the initial material (Table 3), a value close to that (4.9%) found [5] for pectins extracted from citrus wastes by 0.3 M NaOAc buffer (pH 5) at 30°C. From the composition of the water-soluble material after (Table 3) and before dialysis (data not shown), it was calculated that glucose (probably from residual sucrose in the fibre) was the only sugar lost during dialysis.

The water-soluble polysaccharides from the initial fibre were rich in galacturonic acid (42.4%) and arabinose (16.9%), with minor amounts of galactose (4.7%) and glucose (4.9%), and traces of rhamnose (1.4%), xylose (1.7%), and mannose (2.2%). The pectins extracted had a high dm (91 mol%) and a low da (5%) (Table 3). They had a very high intrinsic viscosity corresponding to a viscosity-average molecular weight of 106500. Some water-soluble pectins have been reported [29,30] to have a high molecular weight and a high dm.

The yield of pectins ($\sim 34\%$) obtained after extraction with acid was higher than that previously obtained [5] from citrus pulp (24.1%). It was calculated that more than 90% of the glucose together with 25% of the arabinose were lost during dialysis. The extracted pectin has a composition close to published values. A dm of 81, a da of 3, and a viscosity-average molecular weight (87600) were found for these pectins, in agreement with previously published data for citrus pectins [5,22,31–33].

After extrusion-cooking, yields of water-soluble fractions increased after dialysis by 312 to 735%. It was calculated (data for nondialysed material not shown) that more than 90% of the glucose present in the water-soluble fractions was lost during dialysis, in contrast to the other sugars which were entirely recovered in the dialysed fractions. These results indicated that the material solubilised was mainly

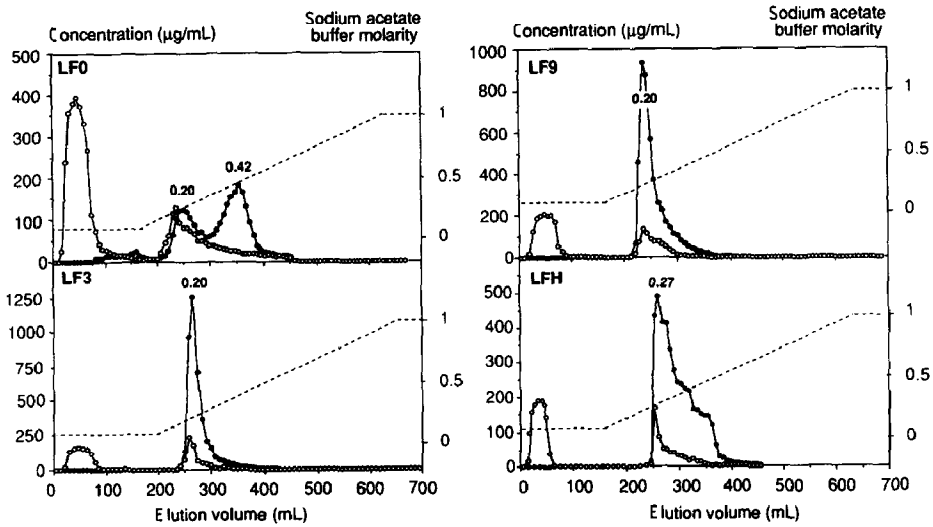

Fig. 1. Ion-exchange chromatography on DEAE-Sepharose CL-6B eluted by NaOAc buffer (pH 4.8) of dialysed water- and acid-soluble fractions. •, Galacturonic acid; ○, neutral sugars; — — —, buffer molarity.

of pectic origin and polymeric. It also was concluded that extrusion-cooking had a less degradative effect on the arabinan side-chains than the acid treatment. The amount of galacturonic acid was in the range 45–50% whatever the severity of the extrusion-cooking. Arabinose was the major neutral sugar (13–20%), followed by glucose (3–9%) and galactose (3–5%); minor amounts of xylose, rhamnose, and mannose were present (1–2%). This pectic material had a very high dm (92%) and a da of 5%, showing that extrusion-cooking has a very limited effect on the esterification of pectins. The viscosity-average molecular weights of pectins extracted after extrusion-cooking were lower than for pectins extracted with acid. The molecular weight decreased with increasing yields of soluble crude pectins, indicating that extrusion-cooking treatments of high energy input led to some depolymerisation.

Purification and structure of the pectic substances.—The pectins studied further were those extracted from LF3, because their molecular weight was similar to that of pectins extracted classically by acid treatment, and those extracted from LF9, because they were extracted with a yield close to the acid extraction. The results were compared to the water-soluble and the acid-extracted pectins from LF0.

The ion-exchange fractionations of crude pectins are shown in Fig. 1. The recoveries were close to 100% for all the samples. Neutral polysaccharides, which were not bound to the column, were present in all samples. This fraction represented 17, 20, and 20% of the injected total (neutral + acidic) sugars, and 55, 62, and 70% of the injected neutral sugars for the water-soluble pectins from LF3, LF9, and the acid-extracted pectins from LF0, respectively. This fraction was

Table 4
Carbohydrate composition (mol%) of the neutral and acidic fractions obtained after ion-exchange chromatography

Sample	Yield (% of injected sugars	GalA	Rha	Fuc	Ara	Xyl	Man	Gal	Glc
Neutral fractions									
LF0	37	0	0	0	70.1	3.5	8.4	9.0	9.0
LF3	17	0	tr. [a]	tr.	52.2	3.5	14.9	9.1	20.4
LF9	20	0	tr.	tr.	65.7	2.2	7.9	6.0	18.3
LFH	20	0	0.2	tr.	75.7	0.5	2.0	6.5	15.0
Acidic fractions									
LF0(a) [b]	28	33.4	4.2	0	34.0	10.1	0	14.7	3.5
LF0(b)	29	61.9	4.3	0	19.7	1.8	0	7.6	4.7
LF3	83	79.9	2.1	0	12.0	2.1	0	3.1	0.8
LF9	80	76.4	2.9	0	13.0	1.3	0	5.4	1.1
LFH	80	81.0	2.2	0.2	8.7	0.8	0.3	5.1	1.7

[a] Trace. [b] (a) Fraction eluted by 0.2 M NaOAc, (b) fraction eluted by 0.4 M NaOAc.

particularly important in the water-soluble pectins from LF0, where it represented 37% of the total injected sugars.

The pectins were eluted by the buffer gradient, and 1 M NaOAc buffer did not elute any additional material. The water-soluble pectins from LF0 were characterised by two peaks: one rich in neutral sugars eluted with 0.20 M NaOAc buffer; the other, richer in galacturonic acid, eluted with 0.42 M NaOAc buffer. Water-soluble pectins from LF3 and LF9 were eluted as narrow and homogeneous peaks with 0.20 M NaOAc buffer, in agreement with their very high dm. The galacturonic acid/neutral sugars ratio (5.0 and 5.3 for pectins from LF3 and LF9, respectively) appeared constant in these populations. Acid-extracted pectins from LF0 were eluted as a wider peak with a higher NaOAc buffer molarity (0.27 M), in agreement with the lower dm of these pectins. The galacturonic acid/neutral sugars ratio (6.6) was higher than the ratio found for the pectins from LF3 and LF9.

The carbohydrate compositions of the neutral and bound fractions are shown in Table 4. The unbound fractions contained mainly arabinose, amounting to 47, 56, and 68% of the arabinose in the pectins from LF3, LF9, and LFH, respectively. Neutral fractions from water extracts of LF3 and LF9 showed higher contents of glucose, mannose, and xylose than the neutral fractions from LF0 or LFH. As shown in Fig. 2, these neutral fractions were eluted as a single narrow peak at $K_{av} \sim 0.8$ on Sepharose CL-6B and represented essentially some arabinan and arabinogalactan molecules.

The uronic acid content amounted to 33.4 mol% in the acidic fraction from LF0 eluting at 0.20 M, and to 61.9 mol% in the fraction eluting at 0.42 M. Arabinose, the main neutral sugar, represented 51–51.6 mol% of the neutral sugars in these fractions. The amount of galactose was similar (20–22 mol%) in the two fractions.

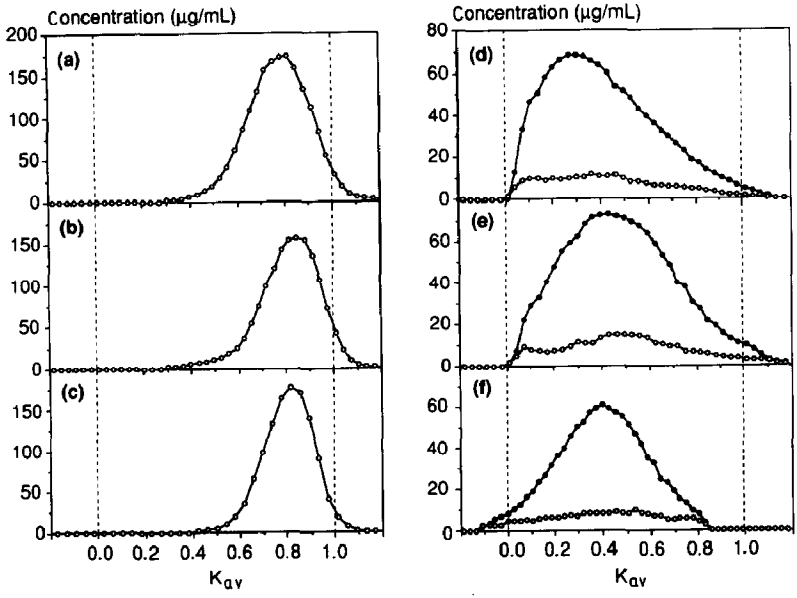

Fig. 2. Elution of neutral and acidic fractions obtained after ion-exchange chromatography on Sepharose CL-6B (**a**, **b**, **c**, **d**, **e**) and Sephacryl S-500 (**f**): **a**, LF3 neutral fraction; **b**, LF9 neutral fraction; **c**, LFH neutral fraction; **d**, LF3 purified pectins; **e**, LF9 purified pectins; **f**, LFH purified pectins. •, Galacturonic acid; ○, neutral sugars.

In contrast, a higher content of xylose and a lower content of glucose and rhamnose were found in the fractions eluted at 0.2 and 0.42 M, respectively.

The galacturonic acid contents of pectins from LF3, LF9, and LFH were in the range 76–81 mol%. Arabinose and galactose were the main neutral sugars and minor amounts of rhamnose, xylose, and glucose were also detected. Although arabinose and galactose were the predominent neutral sugars in all pectins, the molar ratio arabinose/galactose varied between 4 (LF3) and 1.7 (LFH). The pectins from LFH were characterised by a lower amount of arabinose than for the other pectins.

The bound materials were recovered and submitted to gel-permeation chromatography (Fig. 2). As most of the pectins of LFH were eluted from Sepharose CL-6B at the void volume, a gel with larger pores (Sephacryl S-500) was used for this pectin. Purified pectins, water-extracted after extrusion-cooking, showed a wide distribution of molecular weight. The galacturonic acid/neutral sugars ratios (4.5 and 4.6 for pectins from LF3 and LF9, respectively) appeared constant along the fractionation. They were both eluted at K_{av} values (0.3 and 0.4 for pectins from LF3 and LF9, respectively) consistent with the intrinsic viscosities. The acid-extracted pectins from LF0 were eluted as a large peak at K_{av} 0.4 on Sephacryl S-500. Galacturonic acid and neutral sugars (molar ratio 6.6) were distributed similarly in the fraction.

The enzymic degradations of the purified pectins from LF3, LF9, and LFH after de-esterification led to similar final percentages of hydrolysis (~ 40%) with a rapid

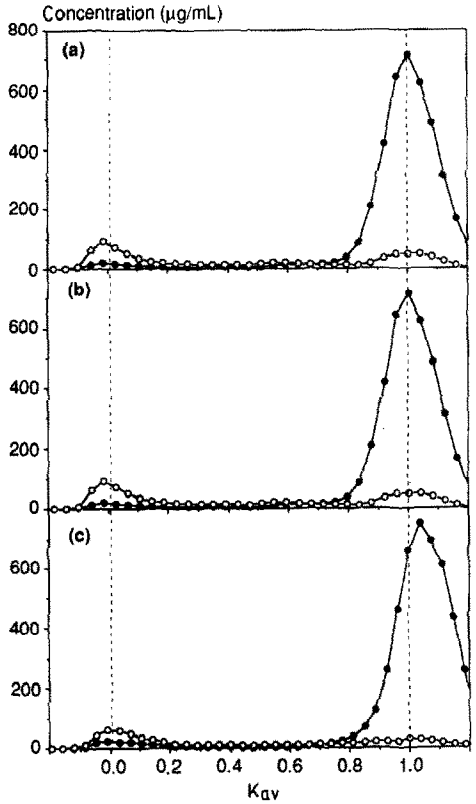

Fig. 3. Gel-permeation chromatography on Sephacryl S-200 of the products obtained after enzymic degradation of LF3 (a), LF9 (b), and LFH (c) de-esterified purified pectins. •, Galacturonic acid; ○, neutral sugars.

phase during the first 2 h (data not shown), which was comparable to previous results [14,34]. The gel-permeation chromatograms of the products (Fig. 3) showed the presence of two distinct populations: a high molecular weight fraction eluted at the void volume, and containing ~ 62% of the neutral sugars and ~ 3–4% of total galacturonic acid; and a low molecular weight fraction eluted at the void volume, and containing almost pure oligogalacturonides accounting for ~ 96–97% of total galacturonic acid. It was established [5,34–36] for various pectins extracted by chemicals (including water) that neutral sugars were grouped in "hairy" regions containing few galacturonic acid residues, while the bulk of the galacturonic acid residues were recovered in "smooth" homogalacturonic regions. The same block-like structure consisting of long homogalacturonan regions interrupted by "hairy" regions in which neutral sugars form side-chains is also present in the pectins solubilised from the cell wall by a physical process such as extrusion-cooking.

The yield in "hairy" regions was higher for the pectins obtained from LF3 or LF9 than for the acid-extracted pectins from LF0 (Table 5). The neutral sugar

Table 5
Yield (% dry matter) and composition (mol%) of the "hairy" regions of pectins from LF3, LF9, and LFH

	LF3	LF9	LFH
Yield	13.3	12.5	10.1
Component			
GalA	11.8	14.7	17.5
Rha	6.8	11.8	9.4
Ara	57.1	49.6	38.7
Xyl	6.8	4.2	3.6
Man	1.4	1.0	1.2
Gal	13.4	14.8	24.3
Glc	2.7	4.0	4.3

distribution (Table 5) and the molar ratios arabinose/galactose were close to those obtained for the initial pectins; the GalA/Rha molar ratios were in the range 1.2–1.9, the highest value being in the "hairy" regions from the acid-extracted pectins from LF0. The "hairy" regions were submitted to methylation analysis, and the results are reported in Table 6. The distribution of the linkages within each sugar was very similar in the three "hairy" regions.

Arabinofuranose was mainly 5-substituted (36–40% of total arabinose) and terminal (24–32% of total arabinose). The fair proportion of 3,5-substituted arabinose (11–13%) indicates a relatively low degree of branching compared to apple or sugar-beet pectin arabinans [37,38]. Low amounts of 3- and 2-substituted arabinofuranose and terminal arabinopyranosyl residues were also detected. Thus, the structural features of the arabinose-containing side-chains are similar in the pectins from LF3, LF9, and LFH, and involve arabinan-like structures with a central core of (1 → 5)-linked arabinofuranosyl residues carrying essentially single substituents on position 3. Such arabinans have been reported in grape berries and apple [39,40]. The differences between the proportions of terminal and 3,5-disubstituted arabinose indicate that terminal arabinose is not only involved in arabinan side-chains but must also be present in other structures.

Some minor differences were observed among galactose methyl ethers for the "hairy" regions. Compared to those from LFH, the "hairy" regions of the pectins from LF3 presented lower amounts of 4-substituted galactose while LF9 contained lower amounts of 3,6-linked galactose. In LFH, 4-, 3-, 6-, and 3,6-substituted galactose were present in equivalent amounts and represented ~ 90% of total galactose. Terminal (3.4%) galactose was also present. This suggests the presence of both types of galactans: (1 → 4)-linked (arabino)galactans and (1 → 3),(1 → 6)-linked branched (arabino)galactans, probably substituted by terminal and (1 → 5)-linked arabinose [41–46]. In contrast to type I (arabino)galactans, type II (arabino)galactans are not widely distributed in pectins. However, they were detected as side-chains in pectins from grape berries, apple, and lemon [39,47,48].

Rhamnose was present as 2-substituted units, ~ 26% also being substituted at position 4. Although higher proportions (~ 50%) of substituted rhamnose are

Table 6
Glycosidic linkages of the "hairy" regions of the pectins from LF3, LF9, and LFH

Methyl ether	LF3	LF9	LFH
2,3,4-Me_3-Rha [a]	0.2 [b]		0.2
3,4-Me_2-Rha	5.3	8.3	5.9
3-Me-Rha	1.9	3.2	2.4
Total	7.4	11.5	8.6
2,3,5-Me_3-Ara	19.9	16.4	11.3
2,3,4-Me_3-Ara	1.7	1.1	0.8
2,5-Me_2-Ara	1.5	1.4	1.3
3,5-Me_2-Ara	0.4	0.6	0.8
2,3-Me_2-Ara	24.9	20.2	18.8
2-Me-Ara	6.6	6.1	6.0
Ara	7.3	9.6	9.9
Total	62.2	55.3	47.1
2,3,4-Me_3-Xyl	0.3	0.5	0.5
2,3-Me_2-Xyl	5.5	3.6	4.1
Total	5.8	4.1	4.6
2,3,4,6-Me_4-Gal	0.7	1.3	1.1
2,4,6-Me_3-Gal	7.8	8.4	10.3
2,3,4-Me_3-Gal	1.8	1.5	3.3
2,3,6-Me_3-Gal	3.8	9.0	8.9
2,3-Me_2-Gal		0.5	
2,4-Me_2-Gal	6.6	3.7	9.7
2-Me-Gal	0.3	0.3	
Total	21.1	24.7	31.8
2,3,4,6-Me_4-Glc	0.3	0.2	0.6
2,3,6-Me_3-Glc	0.7	1.7	1.9
2,3,4-Me_3-Glc	2.3	2.0	1.6
2,3-Me_2-Glc	0.3	0.5	
Total	3.7	4.5	4.0

[a] 2,3,4-Me_3-Rha denotes 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methylrhamnitol, etc. [b] Relative molar ratio.

commonly found in pectic substances, similar values have been reported for pectins from grape berries, sugar-beet, or tobacco [39,49,50].

Terminal xylose, terminal glucose, 6-substituted glucose, 4-substituted glucose, and 4,6-substituted glucose were minor components in the three pectins while 4-substituted xylose was found in significant amounts. The occurrence of xyloglucans in soluble pectins has been previously [51,52] observed. Xylose residues may arise from short side-chains directly attached to O-2 or O-3 of some galacturonic acid residues, as found in some pectins from lemon [28,53].

4. Conclusions

An appreciable amount of pectins with a high degree of polymerisation can be extracted by water from lemon fibres after extrusion-cooking. This treatment

seems to degrade the rhamnogalacturonic backbone more than the side-chains, unlike extraction with acid which had a significant degradative effect on the arabinan pectic side-chains. The acid extraction also led to more demethylation than the extrusion process which produced pectins with unusual dm. The location of these pectins in the cell wall was still unknown. Pectins water-extracted from lemon fibres after extrusion-cooking show, however, the same block-like structure as acid-extracted pectins, consisting of long homogalacturonan regions interrupted by "hairy" regions. This structural analogy could lead to similar functional properties, and water-extracted pectins after extrusion-cooking could offer gelation capabilities. The very high dm of these pectins could also lead to some specific properties.

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