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Extraction and characterisation of water-soluble pectic substances from guava (*Psidium guajava* L.)*

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ABSTRACT

Water-soluble pectic substances (CP-endo and CP-meso) were extracted from the alcohol-insoluble residues of fresh endocarp and mesocarp of white guavas (cv. Suprême) and fractionated by ion-exchange chromatography into minor neutral (NP-endo and NP-meso) and major acidic pectic substances (AP-endo and AP-meso). The neutral fractions contained mainly arabinose, galactose, and glucose, and methylation analysis revealed xyloglucans, highly branched arabinans, and type I arabinogalactans, together with arabino-3,6-galactan–proteins that interacted with the Yariv antigen. The APs contained 72–80% of galacturonic acid together with 16–19% of arabinose, galactose, and rhamnose, were heterogeneous in size-exclusion chromatography, differed greatly in their degrees of methyl esterification (21.9 and 88.0% for AP-endo and AP-meso, respectively) and acetylation (16.0 and 2.0%), and had typical rhamnogalacturonan structures with neutral side chains of densely substituted arabinans and arabinogalactans of both types. Weak intrinsic viscosities [η] (13 and 26 mL/g for AP-endo and AP-meso, respectively) reflected low hydrodynamic volumes for guava pectins.

INTRODUCTION

Guava (*Psidium guajava* L.), which is an important tropical and subtropical fruit, is eaten fresh or made into preserves, jam, jelly, paste, juice, and nectar after the removal of the seeds.

Pectins are complex polysaccharides[1,2] which are involved in the evolution of firmness and cohesiveness of the tissues of fruit and vegetables during maturation and ripening[1] as well as in the texture and consistency of processed products[3]. Until now, studies of guava pectic substances have been focused on changes that occur during maturation[4,5] and after treatment of the purees with enzymes[6,7], but no data on structure are available. We now report on the isolation and characterisation of water-soluble pectic substances from guava fruit.

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EXPERIMENTAL

Plant material. — Sound white guavas (cv. Suprême) were hand-picked at the "turning" stage (mature/ripe transition) in the experimental orchards of the Institut de Recherches Agronomiques (Institut de Recherche sur les Fruits et Agrumes, Nyombé, Cameroon), and a batch of 440 was sorted visually for the colour of the epicarp and size. The fruit was stored and transported in refrigerated boxes at 4° (the time between harvest and processing was two days at 4°) and was fairly homogeneous with regard to weight, density, firmness, and colour of the epicarp.

Preparation of the alcohol-insoluble residues (AIR). — The cold guavas (60) were peeled rapidly, the mesocarp and endocarp (including the central core and the seeds) (Fig. 1) were hand-separated, cut into small pieces, dipped in boiling aqueous 96% ethanol (solid/liquid ratio 1:5 w/w), and homogenised with a polytron. The mixture was boiled for 10 min and the endocarp was freed from seeds by flotation. The insoluble residues were collected by filtration and washed with aqueous 96% ethanol until the filtrate was colourless, then with acetone and ether. The air-dried residues were re-ground in a refrigerated IKA grinder, washed with acetone and ether, air-dried, and sifted through a 0.5-mm screen to give AIR-endo and AIR-meso.

Extraction of water-soluble pectic substances. — Each AIR (15 g) was treated[8] with pronase in 0.2M sodium acetate buffer (pH 5) at 25°, then centrifuged. The supernatant solution was dialysed extensively against distilled water, concentrated under reduced pressure (40°), and freeze-dried, to give the crude pectic substance (CP-endo and CP-meso).

Ion-exchange chromatography. — A solution of each CP (1 mg) in 0.05M acetate buffer (1 mL, pH 4.8) was applied to a column (10 × 1 cm) of DEAE-Sephacel equilibrated with the same buffer, and eluted at 10 mL/h until fractions gave a negative response for carbohydrate (orcinol). The acidic pectic substances ("pectins", AP) were then eluted by a linear gradient (0.05→1M) of sodium acetate (50 mL, pH 4.8), and fractions (2 mL) were assayed for galacturonic acid and neutral sugars as described below (see Fig. 2).

Substantial amounts of each AP were obtained by fractionation of each CP (900 mg) on a column (40 × 5 cm) of DEAE-Sephacel by elution with M sodium acetate

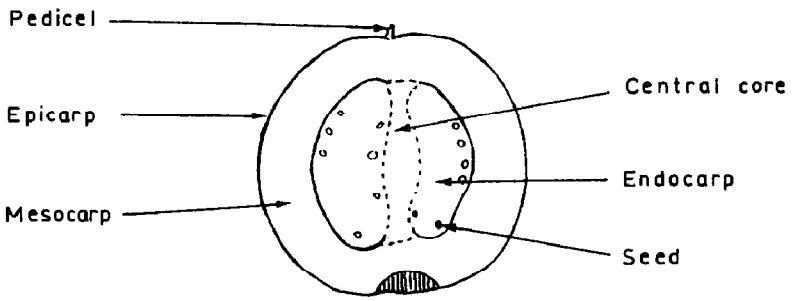

Fig. 1. Longitudinal section of guava fruit.

buffer (650 mL, pH 4.8). Each fraction was dialysed extensively, then concentrated in vacuum, and freeze-dried to give AP-endo and AP-meso.

Size-exclusion chromatography. — A solution of each AP (60 mg) in 0.05M sodium acetate buffer (5 mL, pH 4.8) that contained 0.2M NaCl was applied to a column (100 × 1.5 cm; V_o 88 mL, V_t 202 mL) of Sephacryl S400 equilibrated with the same buffer and eluted at 36 mL/h. Fractions (4.2 mL) were analysed as described below, and the appropriate fractions were combined, dialysed, concentrated, and freeze-dried to give AP_1 and AP_2 (see Fig. 3).

Viscosity measurements. — Intrinsic viscosities ([η]) of AP-endo (18 mg/mL) and AP-meso (9 mg/mL) in 0.2M sodium chloride were determined at 25° with an Automatic Schott Gerate AVS 400 viscosimeter (flow-time of the solvent was 96.309 s).

Enzymic degradation. — Solutions (2 mg/mL) of AP_1 and AP_2 in 0.01M citrate buffer (pH 5.2) were each treated with endopectinlyase (EC 4.2.2.10) from *Aspergillus niger*[9] (type 2, 0.2 U/mL) for 24 h at 30° and the absorbance at 235 nm was monitored (the molar extinction coefficient[10] of the product was 5500).

Analytical methods. — Moisture contents were determined by drying at 55° for 24 h under diminished pressure; all data are given on a moisture-free basis.

Uronic acids and neutral sugars were determined by the automated *m*-phenylphenol[11,12] and orcinol[13] methods, respectively; the results are expressed as "anhydro sugars" with galacturonic acid and arabinose as the respective standards. Neutral sugars were released from the polysaccharides by hydrolysis with 2M trifluoroacetic acid (0.5 mL; 1.25 h at 120°). Each hydrolysate was concentrated in a stream of air at 40°, the liberated monosaccharides were reduced with $NaBH_4$ (0.5 mL, 20 mg/mL) in M ammonia, each mixture was concentrated, and the alditols were acetylated with acetic acid (0.1 mL), ethyl acetate (0.5 mL), acetic anhydride (1.5 mL), and perchloric acid (0.05 mL)[14].

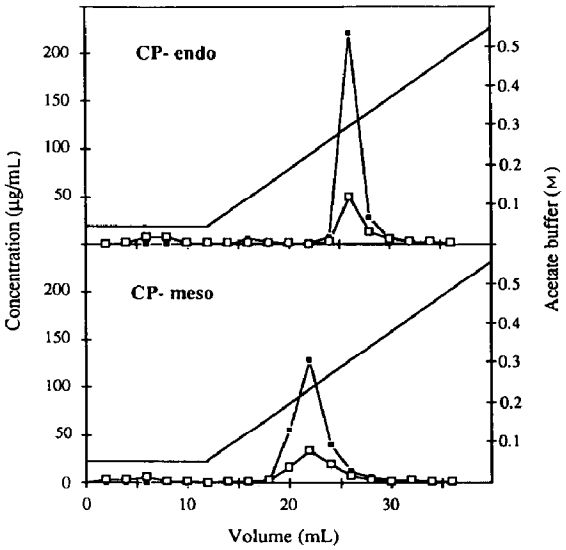

Fig. 2. Ion-exchange chromatography on DEAE-Sephacel of crude pectic substances from guava endocarp (CP-endo) and mesocarp (CP-meso): -■-, uronic acids; -□-, neutral sugars.

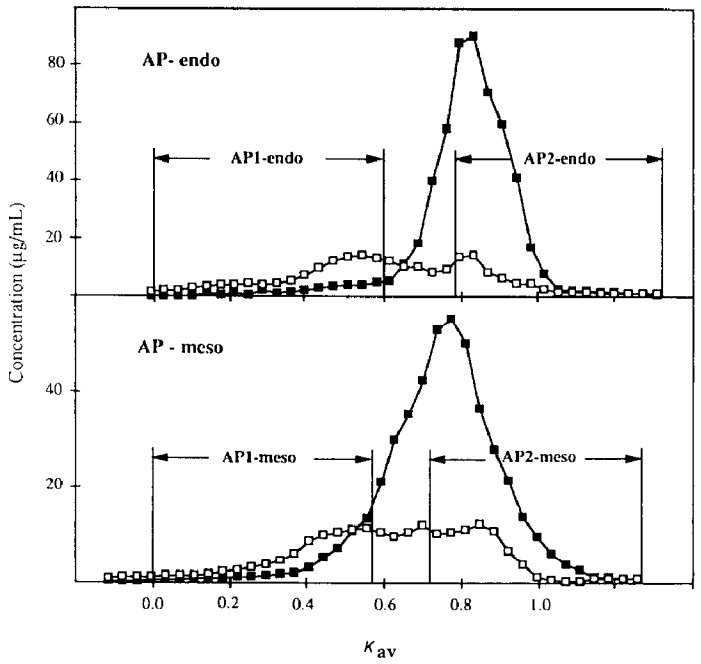

Fig. 3. Size-exclusion chromatography on Sephacryl S400 of acidic pectic substances from guava endocarp (AP-endo) and mesocarp (AP-meso): -■-, uronic acids; -□-, neutral sugars.

The resulting alditol acetates were analysed by g.l.c. at 210° on a DB-225 capillary column (30 m × 0.32 mm i.d., 0.25-μm film; J & W Scientific) with H_2 as the carrier gas. The polysaccharides were methylated with methyl iodide according to Hakomori[15] as described[16]. Prior to methylation, acidic pectic substances (AP) were converted into their H^+ form[17] in order to ensure solubility in methyl sulfoxide. After hydrolysis, the partially methylated sugars were converted into alditol acetates and analysed on DB-1 and DB-225 capillary columns[16]. Identifications were based on retention times and confirmed by g.l.c.–m.s., using the DB-225 column (on-column injection at 50°, injector 50°→220° at 60°/min, oven 90°→170° at 10°/min then 5°/min to 210°; He as carrier gas at 2 mL/min) coupled to a Finnigan Mat ITD 400 mass spectrometer. Methanol and acetic acid were determined by h.p.l.c.[18], after treatment of the polysaccharides with 0.4M NaOH in water–2-propanol (1:1) for 2.5 h at 30°. A column (30 × 0.78 cm i.d.) of Aminex HPX 87H (Bio-Rad) was used and eluted with 0.03M H_2SO_4 at 0.7 mL/min (refractive index detection). Degrees of methyl esterification (d.m.) and acetylation (d.a.) are expressed relative to 100 mol of galacturonic acid.

Protein was determined by the method of Lowry *et al.*[19] as modified by Potty[20].

RESULTS AND DISCUSSION

Extraction and purification of water-soluble pectic substances. — The alcohol-insoluble residues (AIR-endo and AIR-meso) represented 1.6 and 6.3% (w/w), respectively, of fresh endocarp and mesocarp, of which 25.9 and 11.6% respectively, were

solubilised by the combined action of acetate buffer and pronase[21] (Table I). The proportion of water-soluble crude pectic substances in the alcohol-insoluble residue from the endocarp of guava was similar to those observed for carrot[21] and grape pulp[22]. Of the uronic acids and neutral sugars in AIR-endo, 53.2 and 13.4%, respectively, were recovered in CP-endo, whereas the corresponding figures were 36.7 and 4.3% for CP-meso.

Each crude pectic substance (CP) was submitted to ion-exchange chromatography on DEAE-Sephacel (Fig. 2). The recoveries of uronic acid were 88 and 68% for CP-endo and CP-meso, respectively, but the neutral sugars were recovered completely. Apart from a minor proportion of unbound material (NP-endo and NP-meso) that was essentially neutral, most of the material injected was bound, and was eluted as sharp single peaks by 0.29 (AP-endo) and 0.19M (AP-meso) acetate buffer. Each component consisted mainly of uronic acids. Substantial amounts of the NP and AP fractions were obtained by preparative chromatography on DEAE-Sephacel, with an ~80% yield of uronic acid.

Composition and physicochemical properties of water-soluble pectic substances. The compositions of the crude and purified pectic substances are given in Table I. The CPs had similar compositions, ~60% being acidic (supposedly mainly galacturonic acid) and ~15% being neutral, with decreasing proportions of arabinose, galactose, and rhamnose, and minor proportions of glucose, xylose, mannose, and fucose.

TABLE I

Yield[a] and composition[b] of water-soluble crude pectic substances (CP) and their corresponding neutral (NP) and acidic (AP) fractions

	Endocarp			*Mesocarp*		
	CP	*NP*	*AP*	*CP*	*NP*	*AP*
Yield	25.9			11.6		
Uronic acids	59.2	4.4	72.7	60.4	3.3	77.1
Total neutral sugars[c]	16.0	42.2	19.0	14.6	67.5	16.2
Protein (Lowry)	5.2	13.1	0.9	8.8	3.4	0.6
Methanol	2.9	0.0	2.7(21.9)[d]	9.8	0.0	11.5(88.0)
Acetic acid	4.1	11.1	3.7(16.0)[d]	0.7	10.8	0.5(2.0)
Rhamnose[e]	9.5	1.6	13.9	7.4	0.5	9.4
Fucose	1.6	1.7	0.8	1.1	2.3	0.7
Arabinose	53.2	43.5	59.9	63.3	45.1	73.4
Xylose	5.9	5.3	1.9	4.2	8.3	1.6
Mannose	1.4	2.9	0.7	1.5	4.1	0.3
Galactose	21.1	26.4	21.9	17.8	26.2	14.1
Glucose	7.4	18.6	1.0	4.8	13.5	0.4
(η)(mL/g)			13.0			26.0

[a] % (w/w) of AIR. [b] % (w/w). [c] Neutral sugars determined by g.l.c. of the alditol acetates, and expressed as "anhydro sugars". [d] Values in parentheses are the degrees of methylation (d.m.) and acetylation (d.a.), respectively. [e] Mole% of the constituent monosaccharides.

Ion-exchange chromatography gave the almost neutral pectic substances (NP) that contained mainly arabinose and galactose. Each NP contained 3–4% of uronic acids and was enriched (*cf.* CP) in glucose and galactose at the expense of arabinose and rhamnose. The xylose and fucose were increased only in the NP from mesocarp. Each NP was highly acetylated.

The acidic pectic substances (AP) were enriched in galacturonic acid, and arabinose, galactose, and rhamnose were the major neutral constituents of AP-endo and AP-meso. Traces of protein were also detected. The d.m. was high for AP-meso and low for AP-endo, which corroborated their order of elution on DEAE-Sephacel[23,24]. The opposite trend was found for d.a. Hence, hand-separation of the endocarp and mesocarp had fractionated the water-soluble pectic substances with regards to d.m. and d.a., as opposed to studies dealing with the whole fruit, *e.g.*, d.m. ~73% for crude pectins from Indian guava cultivars[25].

Intrinsic viscosities of purified pectins (13 and 26 mL/g for AP-endo and AP-meso, respectively) were smaller than for those of carrot[21], grape berry[22], or sugar beet[26], and reflected low hydrodynamic volumes of guava pectins. Size-exclusion chromatography of each AP on Sephacryl S 400 (Fig. 3) showed, at least, two populations that differed in their K_{av} and (acidic/neutral) sugar ratio. In both cases, a population of high hydrodynamic volume (AP_1) rich in neutral sugars (acidic/neutral sugar ratio 0.2–0.3 and 0.4–0.5 for AP_1-endo and AP_1-meso, respectively) was eluted at K_{av} ~0.55, and a fraction of lower hydrodynamic volume (AP_2) rich in uronic acid (acid/neutral sugar ratio 6.4 and 5.3) was eluted at K_{av} 0.85 for AP_2-endo and 0.78 for AP_2-meso. The lower hydrodynamic volume of AP_2-endo (*cf.* AP_2-meso), indicated by its later elution on Sephacryl S400, is in accordance with its lower intrinsic viscosity.

Structure of the water-soluble pectic substances. — Methylation analysis data for NP, AP, and the sub-fractions AP_1 and AP_2 from the endocarp and mesocarp are reported in Table II. The relative proportions of the sugars calculated either from analyses of the alditol acetates or partially methylated alditol acetates were generally in good agreement and the methylation was assumed to be complete. Each fraction gave a complex mixture of methyl ethers. Arabinofuranose, the preponderant sugar, was found mainly as non-reducing terminal units and also as 2-, 3-, 5-, 3,5-, and 2,3,5-linked units. Although arabinose might be distributed amongst several classes of polysaccharides, the simultaneous occurrence of terminal, 5-, 3,5-, and 2,3,5-linked residues suggested the presence in each fraction of arabinans of a common type that carried 3-substituents[27], the highest degree of branching being observed in AP_1-meso. Galactose was mainly 3-, 6-, and 3,6-linked in most fractions. Thus, type II arabinogalactans occurred as polymers and as side chains attached to the acidic backbone of the guava pectins, as observed for grape berry[16], apple[28], lemon[29], and kiwi[30]. The occurrence of arabino-3,6-galactan–proteins was confirmed by the positive reaction of each NP fraction with the Yariv antigen[31]. 4-Linked galactose was also detected in the neutral pectic substances and indicated the presence of type I arabinogalactans.

Each NP fraction contained large proportions of 4- and 4,6-linked glucose residues, in association with terminal xylose, which suggests the occurrence of xyloglu-

TABLE II

Methylation analysis of neutral (NP) and acidic (AP) water-soluble pectic substances and their corresponding acidic sub-fractions (AP1, AP2)

Methyl ether	*Endocarp*				*Mesocarp*			
	NP	*AP*	*AP1*	*AP2*	*NP*	*AP*	*AP1*	*AP2*
2,3,4-Me_3-Rha[a]	0.9[b]	3.0	1.1	8.1	0.4	1.9	0.4	5.4
3,4-Me_2-Rha	–	8.3	6.5	10.6	–	4.7	3.4	7.4
3-Me-Rha	–	3.9	4.4	2.5	–	2.2	1.9	2.0
4-Me-Rha	–	0.5	0.4	1.4	–	0.4	–	1.0
Rha	–	1.0	0.5	3.0	–	0.9	0.5	3.0
Total	0.9	16.8	12.8	25.5	0.4	10.0	6.1	18.8
	(1.6)[d]	(13.9)	(11.0)	(20.3)	(0.5)	(9.4)	(5.7)	(12.7)
2,3,4-Me_3-Fuc	2.3	1.7	0.8	3.2	2.5	1.7	1.3	2.4
	(1.7)	(0.8)	(0.2)	(2.0)	(2.3)	(0.7)	(0.2)	(0.02)
2,3,5-Me_3-Ara	16.6	18.7	14.4	16.6	19.7	25.0	18.5	11.3
2,3,4-Me_3-Ara	1.2	1.0	0.7	1.6	1.5	0.6	0.2	3.5
2,5-Me_2-Ara	5.3	7.4	9.3	4.3	5.5	9.3	9.0	4.9
3,5-Me_2-Ara	3.0	1.8	1.5	1.3	0.9	1.0	–	1.7
2,3-Me_2-Ara	6.0	16.1	17.7	11.7	10.8	16.8	14.6	13.4
2-Me-Ara	5.1	7.0	8.3	3.8	7.4	11.8	11.8	5.1
Ara	1.3	3.2	3.9	2.3	3.1	6.7	6.4	3.9
Total	38.4	55.1	55.8	41.6	48.9	71.2	60.5	43.9
	(43.5)	(59.9)	(59.4)	(45.0)	(45.1)	(73.4)	(65.5)	(49.1)
2,3,4-Me_3-Xyl	2.2	0.7	0.4	2.9	3.6	0.6	0.4	0.7
2,3-Me_2-Xyl	2.4	–	–	–	3.3	–	–	–
Total	4.6	0.7	0.4	2.9	6.9	0.6	0.4	0.7
	(5.3)	(1.9)	(1.2)	(3.3)	(8.3)	(1.6)	(0.9)	(0.6)
2,3,4,6-Me_4-Gal	1.8	1.4	1.1	2.7	1.6	0.8	0.5	2.4
2,4,6-Me_3-Gal	4.8	7.5	8.0	5.6	7.4	6.4	4.6	6.4
2,3,4-Me_3-Gal	3.0	3.2	3.1	4.5	2.2	0.7	tr[c]	4.4
2,3,6-Me_3-Gal	3.0	0.2	0.9	1.7	4.1	–	–	–
2,6-Me_2-Gal	0.9	1.1	0.5	3.0	1.6	0.6	2.8	3.0
2,3-Me_2-Gal	0.4	–	–	–	0.3	–	–	–
2,4-Me_2-Gal	10.8	9.3	7.7	7.3	3.5	6.4	2.8	8.9
2-Me-Gal	2.3	0.6	0.6	–	1.5	0.3	–	–
Total	27.1	23.2	22.0	24.8	22.2	15.2	10.6	25.0
	(26.4)	(21.9)	(20.0)	(26.4)	(26.2)	(14.1)	(7.3)	(30.1)
2,3,4,6-Me_4-Glc	1.4	0.3	1.1	–	0.5	tr	2.2	1.6
2,3,6-Me_3-Glc	18.5	0.7	1.1	tr	6.0	tr	1.2	–
2,3,4-Me_3-Glc	–	0.5	5.0	2.0	–	0.5	16.9	4.4
2,3-Me_2-Glc	5.5	–	–	–	11.3	–	–	–
Glc	0.8	0.6	0.6	–	0.3	0.8	0.8	3.2
Total	26.1	2.1	7.9	2.0	18.1	1.3	21.1	9.2
	(18.6)	(1.0)	(7.2)	(3.0)	(13.5)	(0.4)	(19.9)	(5.5)
2,3,4,6-Me_4-Man	0.4	0.2	0.3	–	0.5	tr	–	–
2,3-Me_2-Man	0.2	–	–	–	0.6	–	–	–
Total	0.6	0.2	0.3	–	1.1	tr	–	–
	(2.9)	(0.7)	(1.1)		(4.1)	(0.3)	(0.3)	

[a] 2,3,4-Me_3-Rha denotes 1,5-di-1-*O*-acetyl-2,3,4-tri-*O*-methylrhamnitol, *etc.* [b] Relative mole ratio. [c] Traces. [d] Values in parentheses are based on analysis of alditol acetates.

cans as found in association with soluble pectic substances in apple[32] and carrot[21], NP-meso being more branched than NP-endo. Terminal rhamnose, fucose, galactose, and mannose and 4,6-linked mannose were minor structural components of the neutral polysaccharides.

AP, AP_1, and AP_2 had the same types of rhamnose methyl ethers in the endocarp and mesocarp, which indicated the occurrence of rhamnogalacturonan I structures. However, the terminal, 2, 2,4- and 2,3,4-linked rhamnose represented ~7, 50, 30, and 5%, respectively, of the total rhamnose in each AP_1, whereas the corresponding proportions were ~30, 40, 10, and 15% in each AP_2. Furthermore, the content of rhamnose in AP_2 was twice that of AP_1. Therefore, on the assumption that the neutral side chains were branched mainly on the rhamnose residues[1], the arabinan and type II arabinogalactan side chains had higher d.p. in AP_1 than in AP_2. Glucose, which was almost absent from AP, was detected in appreciable proportions in AP_1 and AP_2, was mainly 6-linked, and could have been derived from the Sephacryl.

Enzymic degradation. — A purified endopectinlyase, which catalyses a β-elimination reaction between methyl-esterified galacturonic acid residues, was almost inactive on AP_1-endo (0%) and AP_2-endo (1.1% degradation), whereas AP_1-meso and AP_2-meso were degraded 6.9 and 3.8%, respectively. Thus, the acidic pectic substances from the mesocarp must exhibit some "smooth" homogalacturonic areas[33] where the methyl-esterified residues are arranged in blocks, a prerequisite for endopectinlyase action[34,35]. The weakly methyl-esterified "pectins" from the endocarp (Table I) were resistant to the lyase, which indicated, in accordance with viscosity and size-exclusion chromatography data, that they might be forms of "native pectins" degraded by endogenous endopolygalacturonase and pectin-methyl-esterase, which increase strongly at the end of maturation or "turning" stage[36].

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REFERENCES

1 M. C. Jarvis, *Plant Cell Environ.*, 7 (1984) 153–164.
2 P. M. Dey and K. Brinson, *Adv. Carbohydr. Chem. Biochem.*, 42 (1984) 265–382.
3 W. Pilnik and. F. M. Rombouts, *Carbohydr. Res.*, 142 (1985) 93–105.

4 M. Y. Sastry, *Indian Food Packer*, 19 (1965) 5–10.
5 S. Yusof and S. Mohamed, *J. Food Agric.*, 38 (1987) 31–39.
6 K. R. Sreekantiah, S. A. Jaleel, and T. N. Ramachandra Rao, *J. Food Sci. Technol.*, 5 (1968) 129–132.
7 I. A. G. Weinert and P. J. van Wik, *J. Food. Sci.*, 23 (1988) 501–510.
8 P. Massiot, X. Rouau, and J.-F. Thibault, *Carbohydr. Res.*, 172 (1988) 217–227.
9 F. E. A. van Houdenhoven, Doctoral Thesis, Agricultural University of Wageningen, The Netherlands, 1975.
10 R. D. Edstrom and H. J. Phaff, *J. Biol. Chem.*, 239 (1964) 2403.
11 N. Blumenkrantz and G. Asboe-Hansen, *Anal. Biochem.*, 54 (1973) 484–489.
12 J.-F. Thibault, *Lebensm.-Wiss. Technol.*, 12 (1979) 247–251.
13 M. T. Tollier and J. P. Robin, *Ann. Technol. Agric.*, 28 (1979) 1–15.
14 P. J. Harris, R. J. Henry, A. B. Blakeney, and B. A. Stone, *Carbohydr. Res.*, 127 (1984) 59–73.
15 S. Hakomori, *J. Biochem.*, 55 (1964) 205–208.
16 L. Saulnier, J.-M. Brillouet, and J.-P. Joseleau, *Carbohydr. Res.*, 182 (1988) 63–78.
17 T. J. Waeghe, A. G. Darvill, M. McNeil, and P. Albersheim, *Carbohydr. Res.*, 123 (1983) 281–304.
18 A. G. J. Voragen, H. A. Schols, and W. Pilnik, *Food Hydrocolloids*, 1 (1986) 65–70.
19 O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, 193 (1951) 265–274.
20 V. H. Potty, *Anal. Biochem.*, 29 (1969) 534–539.
21 P. Massiot, X. Rouau, and J.-F. Thibault, *Carbohydr. Res.*, 172 (1988) 229–242.
22 L. Saulnier and J.-F. Thibault, *Carbohydr. Polym.*, 7 (1987) 329–343.
23 J. A. de Vries, F. M. Rombouts, A. G. J. Voragen, and W. Pilnik, *Carbohydr. Polym.*, 3 (1983) 245–258.
24 W. H. Deventer-Schriemer and W. Pilnik, *Lebensm.-Wiss. Technol.*, 9 (1976) 42–44.
25 M. K. Dhingra, O. P. Gupta, and B. S. Chundawat, *J. Food. Sci. Technol.*, 20 (1983) 10–13.
26 F. M. Rombouts and J.-F. Thibault, *Carbohydr. Res.*, 154 (1986) 177–187.
27 I. R. Siddiqui and J. P. Emery, *J. Agric. Food Chem.*, 38 (1990) 387–389.
28 J. A. de Vries, C. H. den Uijl, A. G. J. Voragen, F. M. Rombouts, and W. Pilnik, *Carbohydr. Polym.*, 3 (1983) 193–205.
29 G. O. Aspinall and I. W. Cottrell, *Can. J. Chem.*, 48 (1970) 1283–1289.
30 R. J. Redgwell, *Carbohydr. Res.*, 182 (1988) 241–258.
31 G. B. Fincher, B. A. Stone, and A. E. Clarke, *Annu. Rev. Plant Physiol.*, 34 (1983) 47–70.
32 M. Knee, *Phytochemistry*, 12 (1973) 637–653.
33 J. A. de Vries, F. M. Rombouts, A. G. J. Voragen, and W. Pilnik, *Carbohydr. Polym.*, 2 (1982) 25–33.
34 L. Rexova-Benkova and O. Markovic, *Adv. Carbohydr. Chem. Biochem.*, 33 (1976) 323–385.
35 W. Pilnik and F. M. Rombouts, in G. G. Birch, N. Blake, and K. J. Parker (Eds.), *Enzymes and Food Processing*, Applied Science Publishers, London, 1981, pp. 105–128.
36 G. Mowlah and S. Itoo, *Nippon Shokuhin Gakkaishi*, 30 (1983) 454–461.