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Note

Reexamination of composition and physico-chemical characteristics of water-soluble pectic substances from guava (*Psidium guajava* L.)

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We recently described the extraction and characterisation of water-soluble pectic substances from guava (*Psidium guajava* L.)[1]. We have since begun technological studies of the enzymatic treatment of guava purees and found that the intrinsic viscosities and degrees of methyl (dm) and acetyl (da) esterifications of native pectic substances found in whey from untreated puree (i.e., total water-soluble pectic substances) were inconsistent with the previously published values. The origin of the guavas was not in question since all the studies were conducted on the same homogeneous collection of guavas[1] harvested in Cameroon in 1989. We report here a complete reexamination of the composition and physico-chemical characteristics of water-soluble pectic substances from guava.

Water-soluble pectic substances were extracted from formerly[1] and newly prepared alcohol-insoluble residues (AIR) from endocarp and mesocarp; no differences were observed between the preparations. The compositions of crude and acidic pectic substances are given in Table I. Neutral pectic substances (NP), which are very minor components by weight, were not considered in this study. The yield of crude pectic substances from endocarp (CP-endo) was almost identical to the previously published value, while a slightly higher yield was obtained from CP-meso. Acidic pectic substances (AP-endo and AP-meso) represented 67 and 62% of the dry matter of the corresponding CP, while recoveries of uronic acids after ion-exchange chromatography were 82 and 79%, respectively. The monosaccharide compositions of CP were close to the previously published data[1], while differences were noted in AP. Although the uronic acid contents were similar, AP-endo and AP-meso contained much less neutral sugar than formerly observed[1], and the (arabinose–galactose) ratios were lower.

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TABLE I

Yield [a] and composition [b] of water-soluble crude pectic substances (CP), of their corresponding acidic (AP) fractions, and of total water-soluble pectic substances (TPS) from guava pulp

	Endocarp		Mesocarp		Pulp
	CP	AP	CP	AP	TPS
Yield	25.0		14.0		
Uronic acids [c]	57.4	70.4	56.1	71.1	57.5
Total neutral sugars [d]	14.2	10.3	13.3	10.0	13.3
Protein (amino acids)	6.1	2.3	7.7	2.3	
Protein (Lowry)		1.7		2.6	
Protein (N×6.25)	5.6	4.3	6.7	3.1	
Methanol	7.2	8.2(64.2) [e]	7.1	8.8(68.1)	6.2
Acetic acid	1.4	1.3 (5.4)[e]	1.0	1.6 (6.8)	0.8
Rhamnose [f]	8.9	11.6	8.0	10.8	9.3
Fucose [f]	2.1	1.1	1.5	1.2	0.7
Arabinose [f]	52.3	52.5	60.1	63.1	65.4
Xylose [f]	7.2	2.1	5.3	2.3	4.2
Mannose [f]	1.5	0.7	1.6	0.6	0.6
Galactose [f]	19.6	25.0	17.3	19.0	15.6
Glucose [f]	8.4	7.0	6.2	3.0	4.2
$[\eta]$ (mL/g)		442		246	284

[a] % (w/w) of AIR. [b] % (w/w). [c] Expressed as "anhydrogalacturonic acid". [d] Neutral sugars determined by GLC of the alditol acetates, and expressed as "anhydro sugars". [e] Values in parentheses are the degrees of methylation (dm) and acetylation (da), respectively, calculated as the molar ratios of methanol and acetic acid vs. "anhydrogalacturonic acid"×100. [f] Mole % of constituent monosaccharides.

Methanol and acetic acid were previously determined by HPLC[2] after treatment of polysaccharides with 0.4 M NaOH in 1 : 1 water–2-propanol (1 mL) for 2.5 h at ambient temperature. For comparison purposes, we have determined methyl and acetyl contents of CP and AP by other techniques[3–5] and also by HPLC. A good agreement was observed between the different techniques. Methanol and acetic acid contents were found to be totally different from the previously published values[1]. Both AP had similar dm values, while AP-meso was a little more acetylated than AP-endo.

The acidic pectic substances had a similar low content of protein, a reasonably good agreement being noted between Kjeldahl, Lowry, and amino acid determinations. Their amino acid compositions (Table II) were similar, serine, glutamic acid, glycine, and ornithine being enriched relative to crude pectic substances which were contaminated by cytoplasmic proteins. Their hydroxyproline content was low.

The intrinsic viscosities of purified pectins (AP) were found to be totally different from the previously published values[1], and AP-endo was approximately twice as viscous as AP-meso. We have checked that the viscosity measurements were not modified by the presence in the solvent of 0.05 M EDTA. The intrinsic viscosity of total crude water-soluble pectic substances (TPS) from guava pulp was

TABLE II

Amino acid composition of water-soluble crude pectic substances (CP) and their corresponding acidic (AP) fractions

	Endocarp		Mesocarp	
	CP	AP	CP	AP
Aspartic acid [a]	11.6	8.0	12.5	6.1
Hydroxyproline	2.4	4.6	1.9	2.6
Threonine	8.8	6.0	9.7	8.1
Serine	9.5	17.7	9.8	19.5
Glutamic acid	8.9	12.5	8.1	13.3
Proline	4.0	4.1	4.0	3.5
Glycine	9.5	13.2	12.9	14.5
Alanine	12.2	9.9	11.7	11.1
Valine	5.6	3.6	5.4	4.0
Cysteine	0.5			
Methionine	0.6			0.3
Isoleucine	3.5	4.4	3.0	2.2
Leucine	7.0	3.4	6.1	3.7
Tyrosine	2.4	1.4	2.1	1.6
Phenylalanine	2.9	1.9	2.7	1.8
Lysine	5.1	2.7	4.8	2.2
Histidine	1.9	1.9	2.0	1.6
Arginine	3.1	1.3	3.1	1.2
Ornithine	0.5	3.4	0.2	2.7
Ammonia [b]	16.6	18.7	12.3	10.0

[a] Relative mole ratio. [b] % of total nitrogen calculated from amino acid and ammonia content.

close to that of AP-meso, as expected from the relative amounts of fresh mesocarp and endocarp in guava fruit[5] and the predominance of mesocarp pectins (80% w/w of total water-soluble pectins). Thus, the viscosity of guava juice is mostly determined by the proportion of mesocarp in guava.

Size-exclusion chromatography of each AP on Sephacryl S400 HR (Fig. 1) showed, contrary to previously published chromatograms[1], highly polydisperse material eluting at a far lower K_{av} than former equivalent fractions. Uronic acids and neutral sugars were distributed without noticeable separation between the void volume (partial exclusion) and K_{av} 0.8. Similar chromatograms were observed in HPSEC (Fig. 2), both pectins exhibiting molecular heterogeneity, and AP-endo displaying higher hydrodynamic volume than AP-meso in accordance with its higher intrinsic viscosity.

Since no particular separation occurred in size-exclusion chromatography, methylation structural analyses of neutral side-chains were performed on whole AP-endo and AP-meso (Table III). The relative proportions of the sugars calculated either from analyses of the alditol acetates or partially methylated alditol acetates were in excellent agreement, and the terminal/branched sugar ratios were close to 1. Distribution of methyl ethers was basically similar to that observed

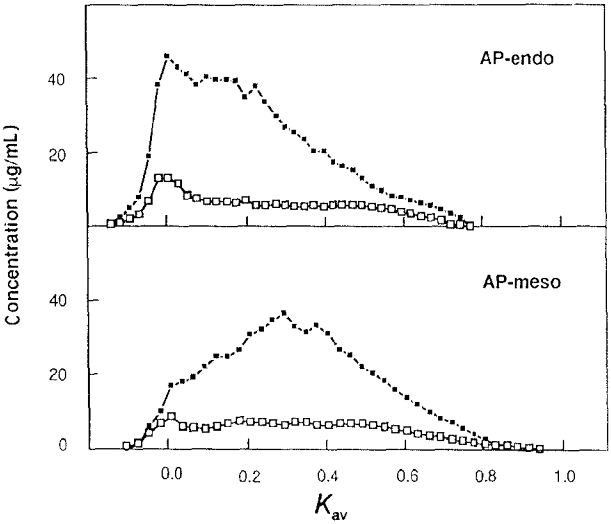

Fig. 1. Size-exclusion chromatography on Sephacryl S400 HR of acidic pectic substances from guava endocarp (AP-endo) and mesocarp (AP-meso): ■, uronic acids; □, neutral sugars.

in the formerly described AP[1], showing the presence of 5-linked arabinans, with some 3- and 2,3-branch points, and of type II arabinogalactans.

We have searched for a plausible cause of such discrepancies with regard to previously published data. Considering that major differences involved dm, da, [η], and size-exclusion chromatograms (hydrodynamic volumes), limited saponification–β-elimination could have occurred if the 1 M acetate buffer (pH 4.8) used for recovery of AP from DEAE-Sephacel had not been adjusted to pH 4.8 with acetic acid. Having re-purified AP accordingly, we did not find any differences with the present results, and other attempts to explain former erroneous data were also

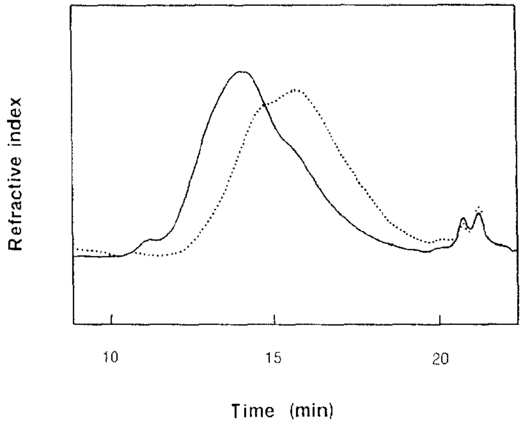

Fig. 2. HPSEC of acidic pectic substances from guava endocarp (AP-endo) (———) and mesocarp (AP-meso) (······).

TABLE III

Methylation analysis of acidic water-soluble pectic substances (AP) from guava endocarp and mesocarp

Methyl ether	Endocarp	Mesocarp
2,3,4-Me_3-Rha[a]	4.3[b]	4.2
3,4-Me_2-Rha	4.3	3.7
3-Me-Rha	2.3	1.6
Rha	0.4	0.6
Total	11.3	10.1
	(11.6)[c]	(10.8)
2,3,4-Me_3-Fuc	0.9	1.0
	(1.1)	(1.2)
2,3,5-Me_3-Ara	23.2	24.0
2,3,4-Me_3-Ara	0.6	1.9
2,5-Me_2-Ara	4.0	6.0
3,5-Me_2-Ara	1.1	2.6
2,3-Me_2-Ara	13.7	16.6
2-Me-Ara	8.2	8.3
Ara	3.1	3.6
Total	53.9	63.0
	(52.5)	(63.1)
2,3,4-Me_3-Xyl	1.4	1.1
2,3-Me_2-Xyl	0.4	0.9
Total	1.8	2.0
	(2.1)	(2.3)
2,3,4,6-Me_4-Gal	1.6	1.3
2,4,6-Me_3-Gal	5.9	4.6
2,3,4-Me_3-Gal	1.9	3.2
2,3,6-Me_3-Gal	1.1	1.1
2,6-Me_2-Gal	1.3	1.0
2,3-Me_2-Gal	0.8	1.0
2,4-Me_2-Gal	11.1	7.1
2-Me-Gal	1.3	0.7
Total	25.0	19.8
	(25.0)	(19.0)
2,3,4,6-Me_4-Glc	2.6	0.8
2,3,6-Me_3-Glc	0.7	1.1
2,3,4-Me_3-Glc	1.0	0.4
2,3-Me_2-Glc	0.7	0.8
Total	5.0	3.1
	(7.0)	(3.0)
2,3,4,6-Me_4-Man	1.4	-
2,3,6-Me_3-Man	0.7	0.8
Total	2.1	0.8
	(0.7)	(0.6)
Terminal–branched ratio	1.00	1.16

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

unsuccessful. Data published here are in accordance with figures we obtained on guava puree, and could be considered as more representative of guava pectic substances. The hypothesis[1] that low molecular weight pectins could have arisen during the maturation process from precursors through degradation by endogenous endopolygalacturonase and pectinmethylesterase is no longer valid.

We have shown that water-soluble acidic pectic substances from guava endocarp and mesocarp (at the "turning stage"), although being rather similar in composition, have different hydrodynamic volumes and intrinsic viscosities. Thus, the relative weight proportion of endocarp and mesocarp in guava fruits might influence the viscosity of the final processed juice.

EXPERIMENTAL

Plant material and alcohol-insoluble residues — Alcohol-insoluble residues from guava (cv. Suprême) endocarp and mesocarp were prepared as previously described[1]. Guava pulp (corresponding to edible portions of guava, i.e., endocarp + mesocarp) was prepared as follows: liquid nitrogen-frozen fruits were allowed to thaw up to 4°C, cut into pieces, gently homogenised for 1 min in a Waring Blendor, then exhaustively sieved on a 2-mm screen (elimination of seeds and skin fragments).

Water-soluble pectic substances. — Crude water-soluble pectic substances were re-extracted from alcohol-insoluble residues (AIR) of guava endocarp and mesocarp (CP-endo and CP-meso) as already described[1]. Total water-soluble pectic substances (TPS) were also obtained from cold guava pulp after centrifugation (10 000g, 15 min, 4°C) by treatment with trichloroacetic acid (7.5% final concentration, 4°C), centrifugation, neutralisation by 5 M KOH, dialysis, and freeze-drying.

Ion-exchange chromatography. — Substantial amounts of acidic pectic substances from guava endocarp and mesocarp (AP-endo and AP-meso) were prepared by submitting crude water-soluble pectic substances (CP) to ion-exchange chromatography on DEAE-Sephacel as previously described[1].

Size-exclusion chromatography. — A solution of each AP (3 mg) in 0.05 M sodium acetate buffer (3 mL, pH 4.8) that contained 0.2 M NaCl was applied to a column (90 × 2.2 cm; V_0 80 mL, V_t 186 mL) of Sephacryl S400 HR equilibrated with the same buffer and eluted at 95 mL/h. Fractions (4 mL) were analysed for uronic acids and neutral sugars as already described[1].

Acidic pectic substances were also analysed (2.5 mg/mL; 20 μL injected) on a HPSEC system constituted of 2 Shodex OH-pack columns (KB-803, KB-805), eluted with 0.1 M $LiNO_3$ at 1 mL/min from a Waters 510 HPLC pump, with on-line refractive index detection (ERMA-ERC 7512 detector thermostated at 40°C).

Viscosity measurements. — Intrinsic viscosities ($[\eta]$) of AP-endo (3 mg/mL), AP-meso (4 mg/mL), and TPS (4 mg/mL) in 0.05 M acetate buffer (pH 4.8) containing 0.2 M NaCl were determined at 24.8°C with an automatic Schott Geräte AVS 400 viscometer (flow time of the solvent was 93.54 s).

Analytical methods. — All methods have been previously detailed[1,5]. Nitrogen was measured by the Kjeldahl procedure and amino acids were analysed according to ref 6.

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