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PII: S0144-8617(97)00240-3

Carbohydrate Polymers **35** (1998) 169–177

0144-8617/98/$19.00+0.00

Characterisation of the cell walls of loquat (*Eriobotrya japonica* L.) fruit tissues

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(Received 14 August 1997; revised 18 October 1997; accepted 29 October 1997)

Loquat fruit (*Eriobotrya japonica* L. cv. Algor) was dissected to give the following tissue zones: epidermis or epicarp, flesh or mesocarp, integument (a thin layer surrounding the seed cotyledons), seed testa, kernel and hairy receptacle. The alcohol insoluble residues (AIRs) from all these tissues were proved to be free of starch, except loquat kernel which on a fresh weight basis contained about 34% of starch. AIRs were analysed for moisture, ashes, protein, lignin and the component sugars were released by two hydrolytic procedures which helped to distinguish the sugars from non-cellulosic polysaccharides and cellulose. Their major component polysaccharides were inferred to be pectic polysaccharides since all AIRs were very rich in sugars such as uronic acids, arabinose and galactose. Pectic polysaccharides contributed up to 70% of total cell wall polysaccharides in the edible flesh of the loquat fruit. Important differences in the degree of branching, degree of esterification and in the amounts of Ca and Mg associated with pectic polysaccharides were detected among pectic polymers depending on the loquat tissue zone. These compositional and structural differences may be related to the role that these pectic polymers play within the tissues which form the loquat fruit.

INTRODUCTION

The structure and textural properties of plant tissues are dependent, to a large extent, on the cell wall (Klockeman, Pressey & Jen, 1991). The loquat fruit is composed of different tissue zones and there is a paucity of information on the cell walls of loquat. Loquat is the fruit of *Eriobotrya japonica* Lindl., a tree native to Japan where it is cultivated as well as in various other tropical and subtropical countries. The golden fruits are round or oval in shape and have a sweet taste. This subtropical fruit is eaten fresh or made into preserves, jam, jellie, juice and nectar after removal of the seeds (Ibarz, Garvin & Costa, 1995). The epicarp, epidermis or coarse skin is slightly harder than that of a peach but about as thick. The colour of the juicy flesh or mesocarp ranges from almost white to deep orange and its taste has been described as mild, subacid and apple-like (Fröehlich & Schreier, 1990). From one to four seeds are commonly found in each fruit. These are separated from the flesh by a very thin tegument. The kernels are covered by a smooth fibrous brown skin.

Different aspects of the chemical composition of loquats have been studied such as the presence of vitamins, amino acids, minerals, fatty acids and soluble sugars (Hall, Smoot, Knight & Nagy, 1980; Shaw & Wilson, 1981; Herrmann, 1995; Lee, Park, Kim, Choi, Kim & Hwang, 1996). However, no information is available on the cell wall components of the different parts which form the loquat fruit. The information on loquat cell walls is crucial for a better understanding of the biochemical changes that occur in loquat during growth, maturation and processing. This paper reports on the composition and structural characteristics of the cell wall polymers of loquat fruit tissues.

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EXPERIMENTAL

Plant material and tissue separation

Fresh loquat fruits (*Eriobotrya japonica* L. cv. Algor) were purchased at a local supermarket. Whole fruits, 5–6 cm in length and 4–5 cm in diameter, were dissected to give various tissues (Fig. 1), namely epidermis or epicarp, flesh or mesocarp (edible portion of the fruit), integument (very thin layer covering the seed), seed (3–4 cm in length and 2–3 cm in diameter) and a hairy receptacle. In addition, the seed testa or seed skin was separated, using a sharp-nosed scalpel, from the kernel. Purchased fruits were stored for not more than 24 h at 1°C prior to tissue separation.

All separated tissues were frozen in liquid nitrogen and then stored at 20°C until required.

Preparation of alcohol insoluble residues (AIRS) and removal of starch from kernel AIR preparation

AIRs from loquat tissues were obtained by immersing the frozen samples in boiling ethanol [final concentration 85% (v/v)] as described by Waldron and Selvendran (1990). Prior to further analysis, the AIR was milled using a laboratory type grain mill and passed through a 0.5-mm aperture sieve. The occurrence of starch in the preparations was tested for by staining AIR with I_2/KI solution, and examining by light microscopy. All samples except kernel preparation were free of starch and they were used in subsequent analyses.

Starch from kernel AIR preparation was removed after dispersing initially the sample in dimethyl sulphoxide (DMSO) and treating the resultant suspension with Termamyl in acetate buffer, followed by Pancrex and pullulanase. The final stage of the digestion involved incubation in phosphate buffer at 100°C for 30 min. (Englyst, Quigley, Hudson & Cummings, 1992). The buffer-insoluble material and the buffer soluble polymeric material were recovered and combined to give a starch-free cell wall preparation of kernel tissue. This material being used for the kernel tissue in subsequent analyses.

Analytical methods

To measure the moisture content, the AIRs were weighed, dried overnight at 60°C in the presence of a dessicant (silica gel), and re-weighed. The nitrogen content of AIR was measured using a Tecator Kjeltec autosampler system 1035 analyser. Protein content was estimated by multiplying the nitrogen value by 6.25. Lignin was gravimetrically determined as Klason lignin. Samples were dispersed in 72% H_2SO_4 at room temperature for 3 h then diluted to 1 M H_2SO_4 and heated to 100°C for 2.5 h. Insoluble material was recovered by filtration (sinter no. 2) and washed thoroughly with hot water (90°C) until acid free before drying at 105°C overnight. The residue weight was recorded as Klason lignin. Ash contents were determined by overnight heating at 550°C (AOAC, 1990). Simultaneous determination of Ca, Mg, K, Na, Fe, P, Mn, Zn and Cu was carried out by inductively coupled plasma atomic emission spectroscopy (ICP/AES) by means of a calibration curve (Boss & Freeden, 1989).

Analysis of carbohydrate composition

Carbohydrate analysis was performed according to Femenia, Lefebvre, Thebaudin, Robertson and Bourgeois (in press) for neutral sugars. Sugars were released from residues by acidic hydrolysis. AIRs were dispersed in 72% H_2SO_4 for 3 h followed by dilution to 1 M and hydrolysed at 100°C for 2.5 h. A 1 M H_2SO_4 hydrolysis (100°C for 2.5 h) was also included to estimate the cellulose content by difference. Neutral sugars were derivatised as their alditol acetates and isothermally separated by gas–liquid chromatography (GLC) (Selvendran, March & Ring, 1979) at 220°C on a 3% OV225 Chromosorb WHP 100/120 mesh column. Uronic acids (UA) were colorimetrically determined, as total uronic acid (Blumenkrantz & Asboe-Hansen, 1973), using a sample hydrolysed for 1 h at 100°C in 1 M H_2SO_4.

Methylation analysis

The polysaccharides were methylated by a modified sequential method using sodium hydroxide and methyl iodide (Ciucanu & Kerek, 1984; Needs & Selvendran, 1993). Some modifications were introduced to improve the overall methylation procedure which are briefly described.

Samples (1–3 mg) were dried overnight in a vacuum oven at 40°C prior to suspension or solubilisation in DMSO (2 ml) under argon. NaOH-DMSO reagent (1 ml), prepared as described by Anumula and Taylor (1992), was added to each sample tube. The tubes were evacuated and flushed several times with argon. The samples were sonicated for 90 min, and left at room temperature for another 90 min. Next, methyl iodide was carefully added and the solution was left for 1 h at room temperature and occasionally sonicated. Methylated polysaccharides were extracted as described by Harris, Henry, Blakeney and Stone (1984) with 2:1 (v/v) chloroform/methanol (3 ml) followed by distilled H_2O (3 ml). The mixture was centrifuged (200*g*, 30 s) and the upper phase was removed by aspiration. The extracts were roto-evaporated to dryness, re-dissolved in 1 ml $CHCl_3$/MeOH and dried before hydrolysis. The sample containing methylated sugars was hydrolysed as described by McNeil, Darvill, Fry, Franzén and Albersheim (1982) using 2 M TFA (0.3 ml) for 2 h at 121°C. After cooling in ice the sample was dried by roto-evaporation. The hydrolysed sample was dissolved in 2 M ammonia and 0.5 M $NaBD_4$ (20 mg $NaBD_4$) was added. Next, the solution was incubated for 1 h at 60°C and 6 ml Dowex resin-H^+ were added to the mixture. The partially methylated alditols were dissolved in 0.5 ml H_2O and transferred to a clean soviril tube. Pyridine (0.1 ml) and acetic anhydride (0.1 ml) were added and the sample was heated at 120°C

for 20 min. Afterwards, it was cooled and roto-evaporated with toluene three times. Dichloromethane (1 ml) was added and the solution back-washed against distilled water, dried down and dissolved in 70 ml of dichloromethane prior analysis by GLC. Partially methylated aldito acetates (PMAAs) were separated by injection onto a capillary column (30 m × 0.32 mm, i.d., OV225, Restek) in a Carlo Erba Model 5160 chromatograph equipped with a flame ionization detector. High-purity helium was used as the carrier gas at a head pressure of 0.96 kg/m^2. Ten seconds after injection, the cooling system was switched off, and the oven temperature maintained at 55°C for 1 min, raised at 45°C/min to 140°C and immediately after, at 2.5°C/min to 218°C. This temperature was maintained for 37 min. The detector temperature was 220°C. Integration was performed using a Spectra-Physics SP4400 integrator. The identity of the peaks was confirmed using a Vectographic Trio 1S mass spectrometer linked to a Hewlett-Packard model 5890 series 2 chromatograph fitted with a second OV225 column.

Methyl esterification of pectic substances

The degree of esterification (DE) of pectic substances was determined after reducing the samples with sodium borohydride (10 mg/ml) in 50% ethanol overnight. In this way, the esterified, but not the de-esterified, groups were reduced. The latter were colorimetrically quantified (Lurie, Levin, Greve & Labavitch, 1994).

Fourier transformed infrared (FTIR) spectroscopy analysis

FTIR spectra were obtained on a Bruker IFS 66 instrument, at a resolution of 3 cm^{-1}, after preparing a KBr disc containing approximately 2 mg of AIR. The single beam traversing each sample was ratioed with the single beam of the corresponding background. Equivalent samples from different experimental runs gave the same spectra in all cases.

RESULTS AND DISCUSSION

Fruit description

Loquat fruit tissues were separated as described in the 'Materials and methods' section (Fig. 1). The fruit flesh or mesocarp was the major tissue zone of the fruit and it represented approximately 75% of the whole fruit, whereas the epidermis or epicarp and the kernel accounted for 10% each. The integument, the seed testa and the hairy receptacle accounted for approximately 1% of the fresh fruit each.

The mesocarp exhibited the greatest tissue homogeneity, containing mainly parenchymatous tissue. The epidermis contained a large amount of waxes. The integument and seed testa were formed of fibrous material, although its textural characteristics were different. Kernels mainly contained parenchyma, although small zones with sclerenchymatous tissue were also present (see Fig. 2(A)).

AIRS overall composition

On fresh weight basis, the amounts of cell wall material recovered, expressed as AIR, were very different depending on the tissue zone. The AIR yields were 1.5, 7.5, 11.6, 11.7 and 32.7% of fresh tissues in the flesh, integument, epidermis, hairy receptacle and seed testa, respectively, suggesting that the concentration of cell-wall polysaccharides was the greatest in the seed testa and the least in the edible flesh tissue. Initially, the AIR recovery from kernel tissue accounted for 42.4% of the fresh material, this high content being owed to the presence of large amounts of starch which was detected after staining the preparation with I_2/KI solution. Such inference was confirmed by light microscopy examination of kernel tissue (see Fig. 2(B)). Starch was removed after dispersing the AIR in DMSO and digesting the sample with a combination of different enzymes

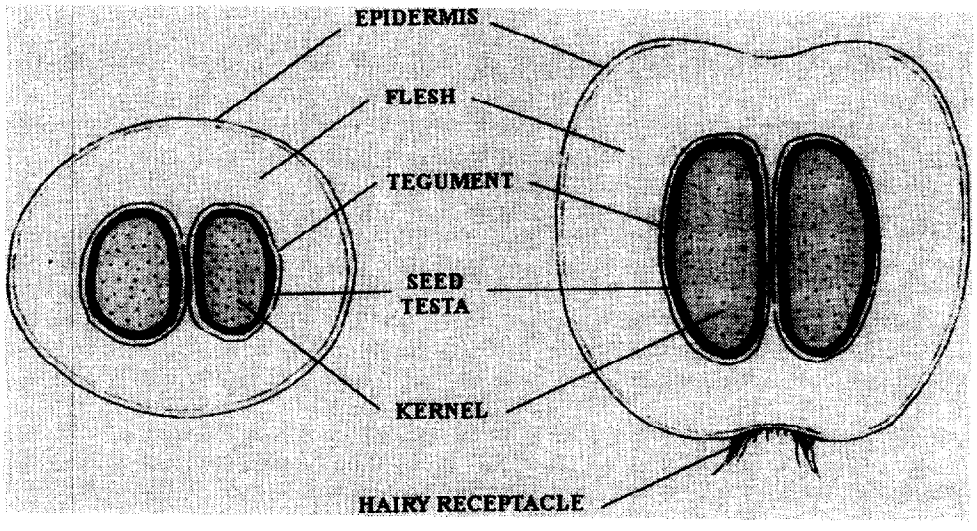

Fig. 1. Diagram of transverse and longitudinal sections of loquat fruit showing the tissue zones excised for analysis.

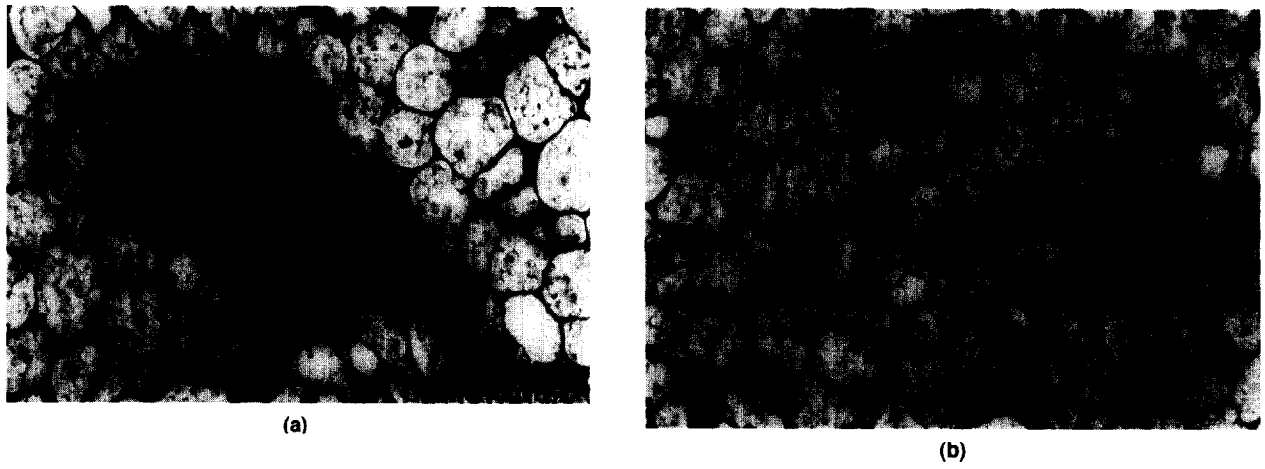

Fig. 2. Light micrograph showing the cell types detected in loquat kernel: (a) sclerenchymatous tissue; (b) parenchyma cells containing large amounts of starch granules.

(Englyst et al., 1992). Next, the cell wall material (virtually free of starch) accounted, on fresh weight basis, for 7.8% of the kernel. The kernel happened to be a good source of starch since it was estimated (by difference) that starch accounted for approximately 34% of the kernel. All subsequent analyses of this sample were carried out on the starch-free material.

The moisture, protein, ashes, total carbohydrate and lignin content of the AIRs are shown in Table 1. The moisture content of the AIRs ranged from 7.8% for the seed testa to 10.1% for the fruit flesh. Protein accounted for 5–15% of the weight of AIR in all the samples. The epidermis, tegument, seed testa and the hairy receptacle exhibited the lowest content of protein in its AIRs, whereas the flesh and kernel contained 10 and 15% of protein, respectively. The bulk of the protein in the AIR of the latter tissues would be of intracellular origin as cell wall protein would contribute only up to 5% of the dry weight of the cell walls (Waldron & Selvendran, 1990). However, in the case of seed testa, tegument and hairy receptacle tissues, where secondary thickened cells are present, the contribution of cell wall protein to the total amount would be higher since these cells exhibited relatively little metabolic activity. The ash content of AIRs ranged from 2.7% for the kernel to 5.8% for the epidermis. The pulp and tegument tissues contained a high percentage of ashes (approximately 5%). Klason lignin accounted for 10% of the AIRs in epidermis and seed testa. The lowest values were detected in the flesh and kernel AIRs indicating the relatively small presence of secondary thickened walls in these tissues. Total carbohydrate accounted for 68–71% of the AIRs, being the predominant fraction in all samples.

Carbohydrate composition of AIRS

The values given in Table 1 are the means of duplicate determinations, and the variation between duplicates was less than 3%. Generally, all AIRs contained high levels of arabinose, glucose, uronic acids and galactose which indicates the presence of large amounts of pectic polysaccharides. However, the relative amounts of the latter sugars detected in the different AIRs preparations suggested

Table 1. Sugars and others components present in loquat tissue AIR [μg anhidrosugar (mg AIR)$^{-1}$]

Component	Epidermis	Flesh	Integument	Seed testa	Kernel[a]	Hairy receptacle
Water	63.4	101.2	90.4	78.4	93.9	85.9
Protein	52.7	103.8	46.5	52.4	146.1	63.3
Ashes	57.8	46.1	49.0	28.7	26.8	36.1
Rhamnose	11.8	18.2	12.1	9.4	17.2	7.2
Fucose	4.3	7.2	1.9	5.0	6.7	4.2
Arabinose	201.9	194.7	174.4	138.8	223.0	84.4
Xylose	32.4	29.6	81.5	81.6	33.5	67.4
Mannose	11.3	11.3	6.6	25.7	32.8	16.7
Galactose	53.4	82.4	113.7	136.4	92.2	156.7
Glucose	172.9	163.5	130.0	118.2	199.0	151.1
Glc (1M)	14.5	19.7	13.4	10.8	34.7	15.4
Uronic acid	221.2	197.6	168.3	164.8	83.4	225.6
% Ester	58	47	17	16	3	51
Total sugar	709.1	704.4	688.5	679.9	687.9	713.1
Lignin	100.2	13.9	91.7	107.1	25.7	97.7

[a]All measurements in the kernel tissue were carried out after enzimatically removing the starch from the AIR.

a great heterogeneity among the pectic polysaccharides. For example, from the ratio between sugars characteristic of pectin side chains (arabinose and galactose) on the one hand, and sugars characteristic of the backbone of rhamnogalacturonans (rhamnose and galacturonic acid) on the other hand, which can give a first indication of the degree of branching exhibited by the pectic polymers it was inferred that the epidermis and the hairy receptacle, with a ratio of approximately 1, exhibited a lower degree of branching than in the case of the tegument and seed testa, with ratios of 1.5 and 1.6, respectively. A ratio of 3 was obtained in the case of the kernel sample which would indicate an even higher degree of branching, although, it should be borne in mind that the cell wall material of the kernel was obtained after enzymatically removing the starch and that during digestion at 100°C a certain amount of galacturonic acid residues may have been degraded, which would partially contribute to the high ratio observed. Moreover, significant differences were found between the relative amounts of arabinose and galactose present in each tissue preparation. In the case of epidermis, flesh, tegument and seed testa, arabinose was the predominant sugar. In the kernel both sugars were detected in similar amounts, whilst in the hairy receptacle the galactose content was higher than that of arabinose.

In addition, important differences were found in the degree of esterification (DE) of pectic polysaccharides depending on the tissue from which AIR was extracted. The epidermis contained pectins with a relatively high DE (58%), whereas the kernel, tegument and seed testa preparations mainly presented non-methyl esterified pectins. Overall, these results suggested that pectic polysaccharides from the various tissues exhibited important differences not only in composition but also in their structural arrangement within the wall.

To obtain an estimate of cellulosic glucose, hydrolysis of AIRs was performed using 1 M sulphuric acid and also by the Saeman method (Table 1). With the exception of the kernel tissue, the glucose released on 1 M sulphuric acid hydrolysis was about 10% of that released by Saeman hydrolysis. A previous work has shown that about 5–10% of the cellulose can be hydrolysed by 1M sulphuric acid hydrolysis (Selvendran et al., 1979) and hence it can be inferred that the bulk of the glucose in the AIRs was derived from cellulose, with some from xyloglucans. It is likely that in the kernel an increased release of glucose by 1 M acid was due to the remaining starch which resisted enzymatic digestion. The AIRs of tegument and seed testa contained higher levels of xylose and lower levels of glucose compared with other AIRs. This data suggested the occurrence of secondary thickened walls in these tissues with the likely presence of acidic xylans which are deposited during secondary wall formation.

Mineral elements associated with airs

The amounts of Mg, Ca, Na, K, P, Fe, Cu, Mn and Zn detected in the different loquat tissue AIRs are shown in Table 2. The amount of individual mineral elements associated with cell wall components may provide useful information related to the structural arrangement of polysaccharides within the wall.

Important amounts of Ca and to a lesser extent of Mg were detected in all AIR preparations except in that of the kernel which contained a lower amount of Ca. However, the ratio of Ca (mol Ca/mg AIR) to uronic acid (mol U.A./mg AIR) was different depending on the tissue zone which could be an indication of the different structural features that pectic polysaccharides present within the walls. Since Ca and Mg have the ability to form cross-links between pectic carboxyl groups, the occurrence of such cross-links may be responsible for the regulation of the mechanical properties of the wall. Whilst high levels of Ca and Mg may be related to high values of firmness, a high level of Na and K has a double effect, it improves texture by reducing the electrostatic repulsion of acidic groups, but it has the opposite effect on texture by competing with calcium (Van Buren, 1979). The fact that K was the predominant mineral element in the kernel preparation could be related to the control of wall porosity of this tissue (Baron-Epel, Paramjit & Schindler, 1988). The interaction of monovalent cations with the carboxyl groups inhibits the ability of pectic polysaccharides to form cross-links, thus overall wall porosity may be increased in this tissue. This could be related to the presence of starch, as the metabolisable carbohydrate of the loquat fruit within the parenchymatic cells of the kernel.

Flesh, tegument and kernel AIRs contained important amounts of P, whereas only traces of this element were

Table 2. Mineral elements present in loquat tissue AIR [μg mineral element (mg AIR)$^{-1}$]

Mineral Element	Epidermis	Flesh	Integument	Seed Testa	Kernel[a]	Hairy receptacle
Mg	1.61	4.42	5.43	1.44	3.00	2.26
Ca	28.04	20.51	18.50	12.29	4.34	20.25
Na	1.13	0.51	0.96	0.17	1.34	0.34
K	1.32	0.94	1.40	0.35	13.52	0.71
P	0.13	1.99	1.67	0.00	1.88	0.11
Fe	0.20	0.38	0.34	0.17	0.16	0.15
Cu	0.15	0.16	0.35	0.09	0.03	0.17
Mn	0.02	0.03	0.05	0.02	0.01	0.03
Zn	0.06	0.10	0.25	0.08	0.06	0.16

[a]Determination of mineral elements in the kernel tissue were carried out after enzimatically removing the starch from the AIR.

detected in the remaining tissues. Minor differences were found in the levels of other elements which were present in small amounts such as Fe, Cu, Mn and Zn. Although, these elements happened to be present in small amounts they may have an important influence on the textural properties derived from pectic polysaccharides since complexes of Fe^{3+} with three carboxylic groups of pectins have been identified in vitro (Deiana, Gessa, Solinas, Piu & Seeber, 1989).

Linkage analysis

Good separation of partially methylated alditol acetates (PMAA) was obtained by GLC and estimates of the PMAAs which co-chromatographed were obtained from the relative abundance of the diagnostic ions. The results of methylation analysis (Table 3) show that the recoveries of neutral sugars obtained from methylation analysis and Saeman hydrolysis were in broad agreement. It seems that cell wall of AIR preparations of tissues are more readily and more completely methylated compared with extracted polysaccharides (Waldron & Selvendran, 1990). The presence of only a few types of methylated ethers from each sugar, and the virtual absence of unmethylated monomers in the hydrolysates of the methylated AIRs were indications of a complete methylation of the cell wall preparations. The presence of small amounts of pentitol acetates would not necessarily imply undermethylation since some pectic arabinans and acidic arabinoxylans have doubly-branched arabinofuranosyl (Stevens & Selvendran, 1980) and xylanopyranosyl residues, respectively (DuPont & Selvendran, 1987).

Table 3. Glycosyl linkage composition of polysaccharidesfrom loquat fruit tissues. Values expressed as relative mol%

Linkage types	Relative mol%											
	Epidermis		Flesh		Integument		Seed testa		Kernel		Receptacle	
Rhamnose												
1,2	1.4		2.3		2.8		2.1		2.3		1.9	
1,2,4	0.3	(2.4)	0.5	(3.6)	0.8	(2.3)	0.4	(1.8)	0.4	(2.9)	0.9	(1.5)
Fucose												
Terminal	1.3	(0.9)	1.7	(1.4)	2.0	(0.4)	0.9	(1.0)	0.6	(1.1)	2.0	(0.9)
Arabinose												
Terminal-f	20.1		15.6		9.8		7.7		8.4		6.3	
1,2	0.7		1.0		2.3		2.6		0.8		1.2	
1,3	1.2		1.2		2.1		1.7		3.2		2.2	
1,5	20.2	(45.3)	17.4	(42.3)	13.5	(36.7)	7.3	(29.8)	14.9	(40.8)	8.0	(19.6)
1,3,5	4.0		9.2		4.9		4.2		10.1		2.2	
1,2,3,5	1.7		2.4		5.4		4.6		3.0		2.8	
Xylose												
Terminal	0.9		0.8		1.0		1.9		1.2		1.5	
1,2	1.7		1.2		1.2		2.6		1.9		2.6	
1,4	4.5		2.7		10.2		10.7		2.2		8.0	
1,2,4	0.5	(7.3)	2.0	(6.4)	1.5	(17.1)	1.9	(17.5)	1.7	(6.1)	2.3	(15.6)
1,3,4	0.4		0.7		0.6		1.5		1.3		1.9	
1,2,3,4	0.1		0.3		0.2		0.4		0.7		1.0	
Mannose												
1,4	0.3		0.3		0.4		0.6		4.9		1.1	
1,4,6	1.6	(2.1)	1.7	(2.0)	1.5	(1.2)	3.0	(4.6)	1.5	(5.0)	3.2	(3.2)
Galactose												
Terminal	2.4		3.3		4.6		5.3		2.8		6.9	
1,4	1.9		2.5		1.9		2.3		1.5		3.0	
1,6	4.9		6.1		9.0		13.6		3.6		12.9	
1,3,6	0.7	(9.9)	1.6	(14.8)	0.4	(19.8)	1.4	(24.3)	1.7	(14.0)	0.5	(30.1)
1,4,6	1.5		1.9		3.5		3.8		3.7		3.5	
Galactitol	0.1		0.5		0.2		0.4		2.1		0.6	
Glucose												
1,4	23.2		18.9		13.6		12.5		14.3		15.2	
1,4,6	2.5		2.0		2.1		2.5		3.6		4.4	
1,3,6	0.2	(32.1)	0.4	(29.4)	0.8	(22.6)	1.7	(21.0)	2.7	(30.2)	0.6	(29.0)
1,2,4	1.5		1.1		1.9		1.3		3.1		1.5	
Glucitol	0.2		0.7		0.8		1.6		1.8		1.7	

Numbers in brackets represent the mol% of neutral sugars as determined by Saeman hydrolysis.

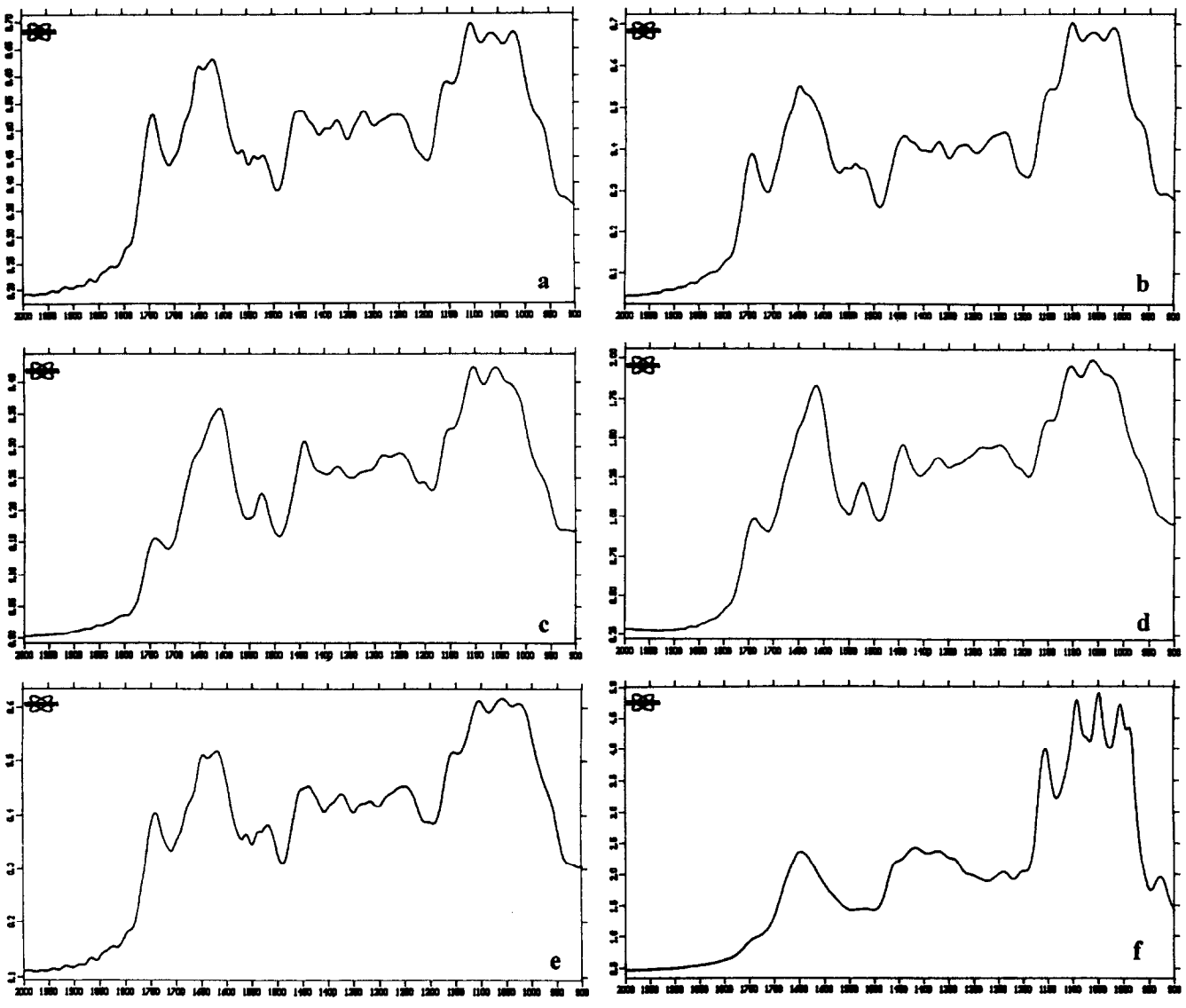

Fig. 3. FTIR spectra from loquat tissue AIRs: (a) epidermis, (b) flesh, (c) integument, (d) seed testa, (e) hairy receptacle and (f) kernel.

Several papers have been published on the types of polysaccharides which can be inferred to be present from overall methylation analysis of cell wall samples (O'Neill & Selvendran, 1980; Lomax, Gordon & Chesson, 1983, 1985; Waldron & Selvendran, 1990). These were used to interpret certain interesting and distinguishing features of the component polysaccharides found in loquat tissues.

Pectic polysaccharides

All the preparations were rich in pectic polysaccharides, indicated by the occurrence of variously linked arabinosyl and galactosyl residues in addition to the presence of (1,2)- and (1,2,4)-linked Rha*p* residues. In different plant tissues it has been observed that C-4 of (1,2)-linked Rha*p* residues is the point of attachment of pectic side chains. However, the position 3 of (1,4)-linked Gal*p*A residues could also be another point of attachment for the side chains (Waldron & Selvendran, 1992). Since the recovery of galacturonopyranosyl residues by methylation analysis is not quantitative (Ryden & Selvendran, 1990; Coimbra, Waldron & Selvendran, 1994; Gooneratne, Needs, Ryden & Selvendran, 1994), carboxyl reduction of the methylated samples to reveal the occurrence of (1,4)- and (1,3,4)-linked Gal*p*A residues was not carried out in this study. The high percentage of (1,3,5), (1,5)- and terminally linked Ara*f* residues present in all the AIRs confirmed the high degree of branching exhibited by the pectic polysaccharides. Residues of (1,4)-, (1,6)- and (1,4,6)-linked Gal*p* residues are also characteristic of these pectic side chains. Methylation analysis confirmed the heterogeneity among the pectic polysaccharides since the percentages of arabinofuranosyl and galactopyranosyl residues were different between samples. For example, epidermis, flesh, integument and kernel AIR preparations contained high percentages of (1,3,5)-, (1,5)- and terminally linked Ara*f* residues, whereas seed testa and the hairy receptacle exhibited higher percentages of (1,6)- and (1,4,6)-linked Gal*p* residues.

Cellulose and hemicellulosic polysaccharides

The presence of significant amounts of cellulose in all the preparations, as inferred from overall carbohydrate analysis using Saeman hydrolysis was confirmed by the occurrence of (1,4)-linked Glc*p* residues. Small amounts of (1,3,6)- and (1,2,4)-linked Glc*p* residues were also detected. Methylation analysis revealed important differences in the cellulose content among samples. The highest percentage was detected in the epidermis and the lowest in the seed testa.

Linkage analysis confirmed the polysaccharide origin of xylosil residues which have been inferred from carbohydrate analysis. The occurrence of important amounts of (1,4)- and (1,2,4)-linked Xyl*p* residues in the tegument, seed testa and hairy receptacle AIRs indicated the presence of acidic xylans which are characteristic of secondary thickened walls.

The presence of small but significant amounts of xyloglucans in all the preparations was inferred from the presence of (1,4)- and (1,4,6)-linked Glc*p* residues, (1,2)-linked Xyl*p* and terminal pentosyl residues which are characteristic glycosidic linkages of xyloglucans (Redgwell & Selvendran, 1986).

FTIR spectroscopy applied on air preparations

Infrared spectroscopy has been only recently applied to biological samples following the advent of rapid Fourier transform data acquisition technology, which permits subtraction of water. FTIR provides a non-destructive chemical assay for wall components and may be used to detect the various chemical and conformational features characteristic of cell wall polysaccharides (McCann, Hammouri, Wilson, Belton & Roberts, 1992). Unfortunately, many polysaccharides present absorbances in the region of the spectrum where carbohydrate absorb (between 1200 and 900 cm^{-1}). Many complex vibrational modes overlap and peaks cannot be assigned uniquely. However, in this region the spectra constitute species-specific fingerprints of cell walls, reflecting even subtle differences in composition.

FTIR spectra (Fig. 3(A–E)) obtained from AIR preparations have peaks in common at 1015, 1070, 1105 cm^{-1} in the carbohydrate region. These are characteristic of pectic polysaccharides (McCann et al., 1992), which confirms the predominance of pectins in loquat tissues. The band at 1015 cm^{-1} was less evident in tegument and seed testa tissues which agrees with the postulated heterogeneity of pectic polysaccharides. Different absorbance intensities detected for the ester band at 1740 cm^{-1} reflects the different degrees of esterification exhibited by pectic polysaccharides from the different tissue zones of loquat fruit. The higher absorbances correspond to the higher degrees of esterification analytically determined (Table 1). In all spectra, the absorption between 1550 and 1650 cm^{-1} may be attributed to amide-stretching bands of contaminating protein in the AIRs. The different features exhibited by the FTIR spectrum of kernel AIR (Fig. 3(F)) were due to the fact that this spectrum was obtained prior to the removal of starch.

CONCLUSIONS

A good indication of the major types and amounts of cell wall polysaccharides present in the various tissue zones of the loquat fruit has been obtained from the sugar and methylation analysis of the different AIR preparations. The analysis has revealed the predominance of highly branched pectic polysaccharides in all tissues; however, these polymers exhibited compositional and structural differences depending on the tissue from which were identified. Such differences were confirmed by determining the mineral elements associated with the AIR preparations and also using FTIR spectroscopy. Significant amounts of cellulose and hemicellulosic xyloglucans were also identified in all preparations. Small but significant amounts of acidic xylans were identified in the integument, the seed testa and the hairy receptacle AIRs indicating the presence of secondary thickened walls in these tissues. Starch was only detected in the kernel AIR preparation and it was enzymatically removed to avoid interferences of glucosyl residues in subsequent analyses. It should be pointed out that the kernel tissue could be considered a good source of starch since, on fresh weight basis, it accounted for 34% of the kernel.

ACKNOWLEDGEMENTS

This research work was supported by the Comisión Interministerial de Ciencia y Tecnología (Project ALI94-0565-CO3) and we thank M. Forcén for her suggestions on the English of the manuscript.

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