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COMPOSITION AND STRUCTURAL FEATURES OF CELL WALL POLYSACCHARIDES FROM TOMATO FRUITS

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Key Word Index—*Lycopersicon esculentum*; Solanaceae; tomato; fruit ripening; cell wall polysaccharides; pectic galactans; ^{13}C NMR spectroscopy.

Abstract—Cell wall material was isolated from the pericarp of unripe and ripe tomatoes, free from intracellular compounds and active wall degrading enzymes. The wall preparations were sequentially extracted with cyclohexane-trans-1,2-diaminetetra-acetate (CDTA) at 20°, 0.05 M Na_2CO_3 at 1°, 0.05 M Na_2CO_3 at 20°, and 0.5, 1 and 4 M KOH at 20° to leave the α-cellulose residue, which contained a significant amount of pectic material. The polysaccharides isolated from the extracts were fractionated by anion-exchange chromatography and selected fractions were subjected to methylation analysis. The CDTA-soluble pectic polysaccharides had slightly-branched rhamnogalacturonan back-bones compared with the sodium carbonate-soluble pectic polysaccharides. The side chains of the pectic polysaccharides were mainly composed of β-(1→4)-linked galactopyranosyl and α-(1→5)-linked arabinofuranosyl residues, and the evidence for this was obtained by both methylation analysis and ^{13}C NMR spectroscopy. The major hemicellulosic polysaccharide was a xyloglucomannan and there was evidence for the occurrence of a small amount of a xylan-pectic complex. The ripe fruit contained much less pectic galactans compared with the unripe fruit, and there was a significant decrease in the content of galactan side-chains of the ripe fruit. The hemicelluloses of the unripe and ripe fruit, however, showed negligible difference in composition.

INTRODUCTION

Changes in the cell wall polysaccharides of tomatoes and many other fruits are thought to play a major role in bringing about the alterations in fruit texture which occur during ripening. At present, however, our understanding of these changes is hampered by limited knowledge of the structure of walls in mature fruit and of the enzymes that modify the wall polysaccharides [1]. In tomato the most widely reported changes in wall structure are an increase in soluble polyuronide and a loss of galactose and arabinose residues [2–4].

The exact mechanism by which the cell wall polyuronides in tomato are solubilized is unclear. Studies have shown that the soluble polyuronides are depolymerized during ripening [5, 6] and that polyuronide weight average $[M]^+$ decreases from *ca* 160 000 to 96 000 [7]. Tomato fruit contains two pectolytic enzymes. Polyuronide degradation *in vivo* appears to require polygalacturonase (PG) (EC 3.2.1.15) activity [8], while the activity of pectinesterase (PE) (EC 3.1.1.11) which can enhance PG action *in vitro* appears to be restricted *in vivo* and its role during ripening may be insignificant [9, 10].

The loss of galactose from tomato cell walls during ripening is well documented [2, 4]. However, the structure and metabolism of the galactose containing polymers is not well understood. Fractionation studies on tomato cell walls showed that galactose was lost from the pectic fractions solubilized by CDTA and dilute sodium carbonate, and from the pectic material associated with the α-cellulose residue [4]. An enzyme has been isolated from tomatoes which can degrade a galactose-rich polysaccharide extracted from unripe tomatoes by the action of PG and PE [11]. ^{13}C NMR of this polysaccharide indicated that it contained relatively long homogeneous blocks of α-(1→5)-linked arabinofuranosyl and β-(1→4)-linked galactopyranosyl residues which were presumed to occur as side chains on a rhamnogalacturonan backbone, thus indicating that the enzyme which hydrolyses the galactan portion of the polysaccharide must be a (1→4)-β-galactanase [12]. Monomeric galactose in tomato extracts may arise from the degradation of the galactose rich polymers by this enzyme [4, 13]. Information on ripening related changes in wall polysaccharides apart from pectins is very sparse, although it has been reported that tomato hemicelluloses show a decrease in M_r during ripening [5], although there are no obvious compositional changes [4]. The aim of the present study was to investigate in more detail the types of cell wall polysaccharides present in tomato and in particular those which undergo changes during ripening.

RESULTS AND DISCUSSION

The method chosen for the isolation of the cell wall material (CWM) was designed to produce walls freed from intracellular components and active wall degrading enzymes, the latter being essential if *in situ* changes in cell

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Table 1. Sugar composition of material solubilized during purification of cell wall material from unripe and ripe tomato fruit (all fractions were dialysed extensively and freeze dried before analysis)

Fraction	Total (mg)	%	Sugars (mol%)							Total sugar (μg/mg)*
			Deoxyhexose	Ara	Xyl	Man	Gal	Glc	Uronic acid	
Acetone										
unripe	118	4	3.8	7.7	3.8	2.6	26.9	29.4	25.6	139
ripe	167	5	2.7	9.1	5.2	2.7	24.6	39.4	16.3	73
PAW										
unripe	34	1	0.8	5.6	2.4	1.6	2.4	78.5	8.7	224
ripe	14	0.4	1.8	34.9	15.3	11.7	13.3	15.1	7.8	914
Ball mill										
unripe	303	11	2.9	3.1	3.6	1.9	11.4	48.9	28.1	791
ripe	245	7	1.7	7.2	3.7	1.3	8.4	3.8	73.8	1042
DMSO ($+H_2O$ wash)										
unripe	28	1	0.8	1.9	5.9	5.7	3.2	77.2	5.3	845
ripe	194	6	0.8	4.7	17.9	13.4	13.0	22.2	27.9	1049
CWM										
unripe	2300	83	1.4	4.8	4.4	4.8	14.7	35.1	34.7	945
ripe	2870	82	0.8	5.3	4.4	5.3	7.8	39.8	36.6	866

*Total sugar as μg/mg of material in each fraction remaining after dialysis and freeze drying and includes the value for uronic acid. This comment applies to the figures in Tables 2 and 3 as well.

wall structure during ripening are to be studied and post-extraction modifications avoided [6]. Polymeric carbohydrate material, the bulk of which is probably of cell wall origin, solubilized during the wall preparation is shown in Table 1. When the pericarp tissue was homogenized in acetone less than 1% of the uronic acids in ripe tomatoes were acetone-soluble and able to pass through the dialysis membrane (nominal cut-off 10 000). Small amounts of ethanol-soluble uronic acid containing material, with relatively low degree of polymerization have been isolated from tomatoes [14], but the bulk are acetone/ethanol-insoluble and have relatively large M_r [6, 7]. During wall preparation small amounts of pectic polysaccharides were solubilized by the solvents used. The yields of CWM and the amounts of polysaccharides solubilized from 400 g fresh tissues and their carbohydrate composition are shown in Table 1. For two of the fractions from the ripe tissue the total sugar content on a μg/mg basis was slightly greater than 1 000. These discrepancies are due to the fact that the uronic acid content of the fractions was determined by colorimetry, whereas the other sugars were estimated as alditol acetates by GC. Of particular interest was the finding that the CWM from the ripe tissue contained much less galactose compared with that from unripe tissue; similar results have been reported previously [3, 4].

Sequential extraction of CWM

The cell wall preparations were sequentially extracted with 0.05 M CDTA at 20°, 0.05 M Na_2CO_3 at 1 and 20°, and 0.5, 1 and 4 M KOH at 20°, essentially as described before [15]. The extraction procedures were designed to minimize β-eliminative degradation of pectins during the initial stages of extraction and solubilize the polymers in as close to their native form as possible [16]. The sugar composition of the various fractions is shown in Table 2. The amounts of material extracted are based on one sequential extraction of CWM from unripe and ripe fruits. The values for sugars in all the Tables are based on single determinations which was considered sufficient from previous work [16]. The CDTA fractions were particularly rich in uronic acid. In CDTA-1 neutral sugars accounted for only 12% of the total sugars present. The majority of the polymers in CDTA-1 were of relatively large M_r as judged by chromatography on Sephacryl S-400 (data not shown) and gave M_r distributions similar to the EDTA-soluble tomato polyuronides reported previously [6]. Both the CDTA-soluble polymers in the present study and the comparable polymers reported in ref. [6] showed some depolymerization during ripening, but the present study shows that this occurs without extensive de-esterification. CDTA-2 had a greater proportion of neutral sugars than CDTA-1 and in the CDTA-2 fraction from the ripe tissue there was *ca* 50% loss in galactose. The small amounts of neutral sugars in these polymers (particularly CDTA-1), their high degree of esterification (CDTA-1 unripe 54%, ripe 50%, CDTA-2 unripe 69%, ripe 55%), and extractability with CDTA at 20° suggests that these polymers probably originate in the middle lamellae [15, 17]. During ripening these pectins are degraded by enzymes, but the activity of PE appears to be limited. These observations are in agreement with previous findings [6, 7].

The sodium carbonate-soluble polymers from CWM of both unripe and ripe fruit contained much larger proportions of neutral sugars compared with those solubilized by CDTA (Table 2) and it is possible that these polymers originated from the primary cell walls [17]. The amounts of pectic polysaccharides solubilized by CDTA-2 and carbonate from CWM of ripe fruit were less than those from unripe fruit. Further, in both sodium carbonate-soluble fractions the most abundant neutral sugar was galactose, and the proportions of this sugar were much less in the fractions from the ripe fruit. Interestingly, the pectic polysaccharides solubilized by

Table 2. Sugar composition of extracts from unripe and ripe tomato cell walls

Fraction	Total* (mg)	%	Sugars (mol %)							Total (μg/mg)
			Deoxyhexose	Ara	Xyl	Man	Gal	Glc	Uronic acid	
CDTA-1										
unripe	282	16	1.3	2.3	0.2	0.1	6.5	1.3	88.2	890
ripe	309	22	3.8	6.4	0.5	0.6	6.6	1.7	80.4	1002
CDTA-2										
unripe	116	7	2.3	6.2	0.8	0.4	15.5	4.2	70.6	321
ripe	69	5	3.7	8.1	0.3	0.6	7.9	3.1	76.3	371
Na_2CO_3(1°)										
unripe	218	13	2.7	7.2	0.3	0.3	21.6	1.9	65.9	705
ripe	165	12	3.8	11.4	0.6	0.2	14.6	1.3	68.1	861
Na_2CO_3(20°)										
unripe	80	5	3.2	11.5	0.2	0.3	39.4	4.9	40.4	755
ripe	11	1	3.1	13.3	0.9	1.0	15.9	4.7	61.1	541
KOH 0.5 M										
unripe	70	4	3.5	12.4	14.7	2.1	25.4	13.0	28.8	365
ripe	86	6	3.8	12.5	9.9	3.6	11.5	25.0	33.8	189
KOH 1.0 M										
unripe	49	3	1.0	6.2	42.0	6.7	10.2	21.3	12.7	634
ripe	45	3	0.8	5.1	35.9	13.3	7.2	27.3	10.4	894
KOH 4.0 M										
unripe	212	12	0.7	6.0	21.7	14.6	16.5	32.5	7.9	865
ripe	160	12	1.0	8.4	22.4	14.6	11.7	30.0	12.1	887
Residue										
unripe	634	37	0.8	4.2	1.1	3.6	9.5	70.4	10.5	804
ripe	515	37	0.9	2.9	0.9	6.6	4.8	77.7	6.3	775

*From *ca* 2 g of purified CWM.

DMSO from ripe tissue during the preparation of the CWM contained a much larger proportion of galactose compared with that from the unripe tissue (Table 1). The significant loss of galactose-rich pectic polysaccharides from the CWM of the ripe fruit shows that these polysaccharides are rapidly metabolized during ripening. These losses in galactose containing polysaccharides is in agreement with earlier work on tomatoes [4] and apples [18]. The galactose content of the potassium hydroxide-soluble fractions and of the α-cellulose residue also showed a decrease in the ripe fruit. Unlike previous reports [19], there was no significant loss of arabinose from the various fractions. The α-cellulose residues from both unripe and ripe tissues contained significant amounts of pectic polysaccharides. Similar observations have been made with α-cellulose residues from a range of soft tissues [15].

Ion-exchange chromatography of selected fractions

Selected fractions were further resolved by ion-exchange chromatography on DEAE-Trisacryl which gives good recoveries of pectic polysaccharides [15], and the results are shown in Table 3. In general, the fractions eluted from the column by buffer and 0.25 M NaCl reflect the trends, particularly the loss of galactose on ripening, shown by the unfractionated materials. The 4 M KOH extracts from CWM of both unripe and ripe fruits contained, in addition to xyloglucomannans, small but significant amounts of xylan-pectic complexes (eluted by 0.25 M NaCl).

Methylation analysis of the CDTA- and sodium carbonate-soluble pectic fractions. Selected fractions were de-esterified with cold dilute alkali (if required) and subjected to methylation analysis as described in the Experimental. Some of the samples either did not completely go into solution in DMSO or formed a gel, although both gave clear solutions on addition of dimsyl anion. This was a particular problem with CDTA-soluble pectins, which resulted in the presence of significant amounts of hexitol and pentitol peaks in the partially methylated alditol acetate (PMAA) chromatograms. This problem was overcome by ensuring that the samples were well dissolved in DMSO, by using a larger volume of DMSO [20]. The problem might also be overcome by freezer-milling the samples. The efficiency of carboxyl-reduction was variable (*ca* 70–80%), although the efficiency of reduction with onion pectins was sometimes as low as 40% [15]. The lower efficiency probably reflects the tendency of pectic polymers to undergo β-eliminative degradation during methylation. In this study the recovery of arabinose residues from the methylated samples as PMAA was generally much higher than the galactose residues and it is possible that segments of pectins carrying galactan side-chains are more susceptible to β-eliminative degradation, and the released fragments are lost during dialysis of the methylated samples.

The results of methylation analysis of the pectic fractions, shown in Table 4, indicated the following: (i) the Na_2CO_3-soluble pectic polymers generally had a more branched rhamnogalacturonan backbone compared with

Table 3. Sugar composition of various cell wall fractions after chromatography on DEAE-Trisacryl column eluted with 50 mM K-Pi buffer pH 6.5 containing 0–0.5 M NaCl

Fraction	Amount (mg)	Sugar (mol%)							Total (μg/mg)
		Deoxyhexose	Ara	Xyl	Man	Gal	Glc	Uronic acid	
CDTA-1									
unripe									
Buffer	58	2.1	1.5	0.3	0.2	7.5	1.4	86.9	812
0.25 M NaCl	18	2.0	2.0	0.6	0.2	4.2	1.2	89.8	662
Na_2CO_3 (1 °)									
unripe									
0.25 M NaCl	103	2.8	7.4	0.3	0.8	18.6	2.6	67.5	732
ripe									
0.25 M NaCl(a)	76	4.3	7.6	0.5	1.1	17.8	3.2	65.4	345
0.25 M NaCl(b)*	21	3.0	3.8	1.9	0.8	3.8	4.2	82.6	501
Na_2CO_3(20 °)									
unripe									
0.25 M NaCl	23	3.8	4.1	0.8	1.8	33.4	6.4	49.7	726
ripe†	11	3.1	13.3	0.9	1.0	15.9	4.7	61.1	541
4 M KOH									
unripe									
Buffer	68	0.6	6.8	18.4	18.1	8.9	45.4	1.8	890
0.25 M NaCl	20	2.6	2.4	37.2	0.9	16.7	5.5	34.6	417
ripe									
Buffer	72	0.4	5.8	19.7	16.3	8.8	45.1	3.9	738
0.25 M NaCl	6	2.2	4.5	39.7	0.8	8.3	5.4	39.1	602

*Not methylated

†Extract not fractionated on DEAE-Trisacryl

the CDTA-soluble pectic polymers, an inference based on the proportions of the (1→2, 1→4)- and (1→2)-linked rhamnose residues. (ii) The Na_2CO_3-(1 °)-soluble pectic polymer of unripe fruit (column 3) had significant amounts of (1→5)-linked arabinofuranosyl (Ara*f*), (1→4)-linked galactopyranosyl (Gal*p*) and terminal-Gal*p* residues. (iii) The Na_2CO_3-(20 °)-soluble polymer from ripe fruit had a significantly lower level of (1→4)-linked Gal*p* residues compared with the corresponding fraction from the unripe fruit (compare columns 6 & 4). This finding is in agreement with the results of sugar analysis (Table 3).

The results of methylation analysis of the Na_2CO_3-(1 °)-soluble pectic polymer from unripe fruit (Table 4, column 3) confirms the results of ^{13}C NMR spectroscopy of the polymer (Fig. 1). Comparable results have been obtained with the pectic polysaccharides of onion [15]. The NMR spectrum of the polymer confirms that the bulk of the galactose and arabinose residues have β-(1→4)- and α-(1→5)-linkages respectively. Assignments for the major peaks in the spectrum of the polymer were as follows: (1→5)-Ara*f* δ108.42 (C-1), 81.74 (C-2), 77.70 (C-3), 83.18 (C-4), 67.85 (C-5); (1→4)-β-D-Gal*p* 105.22 (C-1), 72.76 (C-2), 74.22 (C-3), 78.49 (C-4), 75.38 (C-5), 61.65 (C-6); (1→4)-α-D-Gal*p*A 99.81 (C-1), 69.18 (C-2), 69.88 (C-3), 78.91 (C-4), 72.28 (C-5), 176.0 (C-6) [13, 18, 19]. Several minor peaks were apparent including one for C-6 of rhamnose at δ17.49 (methyl group). It can be seen that the signals associated with the galacturonic acid residues are considerably broader than those arising from the side chain residues; this line broadening is probably caused by motional rigidity of the polygalacturonan backbone. Integration of the anomeric carbon resonances gave the following sugar composition, galacturonic acid (67%), galactose (27%), arabinose (6%). These values are in reasonable agreement with the values for the same polysaccharide as given in Table 3. Of special interest is the finding that the galacturonic acid content, as determined by NMR, corresponds closely to the amount of the acid as determined by chemical analysis. These data indicate that the loss of galactose from the cell walls of tomatoes occurs in several different types of pectic polysaccharides and appears to be mainly due to a removal of β-(1→4)-linked galactose, as suggested by predominance of this linkage in various fractions and the magnitude of galactose loss. These observations provide direct evidence for the action of a (1→4)-β-galactanase *in vivo* during ripening as predicted by the *in vitro* studies of Pressey [11, 12, 19]. Also the loss of galactose would appear to occur mainly from polysaccharides in the primary cell wall.

4 M *Potassium hydroxide-soluble material*

The major component of the 4 M KOH-soluble material from both unripe and ripe fruit was a neutral fraction, rich in glucose, xylose, mannose, galactose and arabinose. Gel-filtration chromatography showed that the M_r of these neutral fractions did not decrease during ripening (Seymour, unpublished results), although it has been reported that tomato hemicelluloses undergo extensive degradation during ripening [5]. The acidic fractions from both unripe and ripe fruits, retained on the columns and eluted with 0.25 M NaCl, were rich in xylose, uronic acid and galactose.

Table 4. Glycosyl linkage composition of cell wall polysaccharides from unripe and ripe tomato fruit (values are expressed as relative mol %)

Sugar and linkage	Unripe				Ripe			
	CDTA-1* Buffer	CDTA-1* 0.25 M NaCl	Na_2CO_3(1°) 0.25 M NaCl	Na_2CO_3(20°)* 0.25 M NaCl	4M KOH Buffer	Na_2CO_3(20°) 0.25 M NaCl	4M KOH Buffer	4M KOH 0.25 M NaCl
Fucose								
Terminal	—	—	1.1	—	t	—	t	—
Rhamnose								
1, 2	1.5	7.4	8.7	5.6	—	6.3	—	0.5
1, 3	—	—	1.6	—	—	—	—	—
1, 2, 4	0.4	1.7	3.9	3.2	—	1.9	—	t
Arabinose								
Terminal	4.4	4.5	4.1	2.8	4.1	2.6	4.2	1.9
1, 5	3.6	6.7	29.9	3.5	0.8	9.4	1.6	4.6
Arabinitol	—	—	2.0	—	—	—	—	1.2
Xylose								
Terminal	t	—	1.1	—	2.6	0.6	3.4	—
1, 2	—	—	—	—	6.1	—	8.5	6.0
1, 4	—	—	—	—	3.3	—	4.6	55.7
1, 2, 4 & 1, 3, 4	t	t	1.6	1.4	—	—	1.0	15.8[a]
Xylitol	—	—	t	—	—	—	—	—
Mannose								
Terminal	—	—	—	—	0.1	—	0.2	—
1,4	—	—	—	—	18.3	—	20.2	—
1, 4, 6	—	—	—	—	4.9	—	5.0	—
Galactose								
Terminal	0.5	3.1	11.1	2.8	6.9	1.3	8.6	—
1, 4	8.5	5.5	25.1	9.7	1.8	2.6	t	9.6
1, 2, 4	—	2.0	2.3	0.6	—	—	—	0.9
1, 3, 4	—	—	1.3	—	—	—	—	—
1, 4, 6	—	—	2.5	—	—	—	—	2.4
Galacturonic acid								
1, 4	75.0	67.7	—	68.0	—	74.7	—	—
Glucose								
Terminal	—	—	—	—	0.3	—	1.8	—
1, 4	6.1	1.4	3.8	2.2	32.6	0.7	27.4	1.0
1, 4, 6	—	—	—	—	18.2	—	13.4	—

t-trace; a-mainly 1,2,4-linkage.
*Methylated and carboxyl reduced.

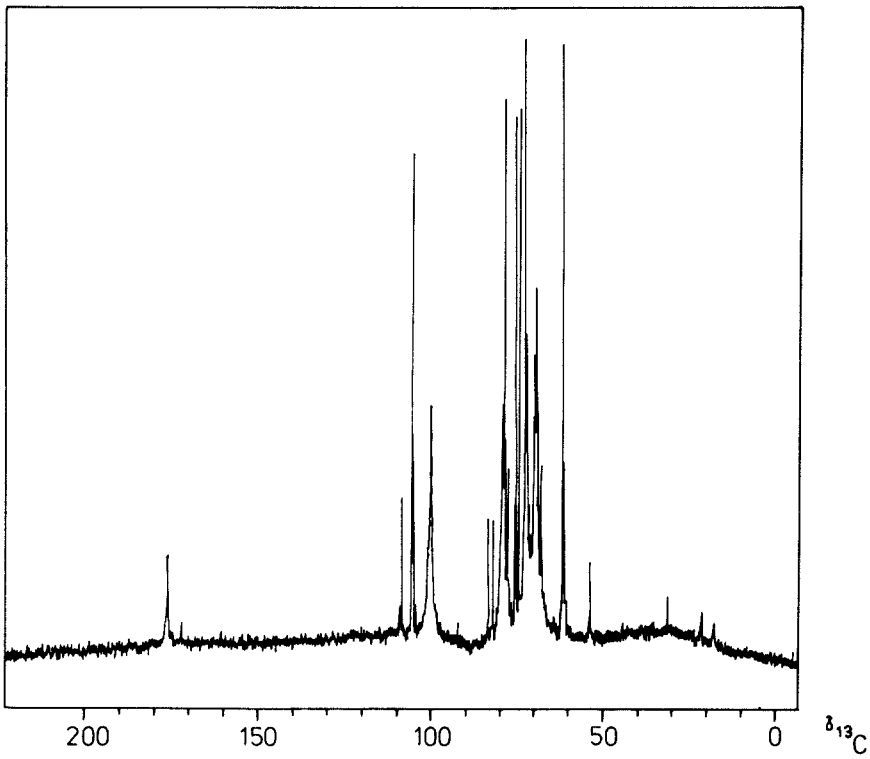

Fig. 1. ^{13}C NMR spectrum of Na_2CO_3-(1°)-soluble pectic polymer from unripe tomato fruit.

The major glycosidic linkages of the neutral fraction from the ripe fruit in decreasing order of amount were (1→4)-linked Glc*p*, (1→4)-linked Man*p*, (1→4, 6)-linked Glc*p*, terminal-Gal*p*, (1→2)-linked Xyl*p*, (1→4, 6)-linked Man*p*, (1→4)-linked Xyl*p*, terminal-Ara*f* and terminal-Xyl*p*. These glycosidic linkages are usually associated with xyloglucomannans (xyloglucans and glucomannans) from parenchymatous tissues of dicotyledons. The major glycosidic linkages of the neutral sugars from the acidic fraction of the ripe fruit in decreasing order of amount were (1→4)-linked Xyl*p*, (1→2,4)-linked Xyl*p*, (1→4)-linked Gal*p*, (1→2)-linked Xyl*p* and (1→5)-linked Ara*f*. These results coupled with the occurrence of small amounts of (1→2)-and (1→2,4)-linked Rha*p* residues and significant amount of uronic acid suggests that the polymer is an acidic-xylan-pectic complex. It is of course possible that the acidic-xylan and pectic-polymer co-chromatograph under the conditions used.

EXPERIMENTAL

Plant material. Tomato plants (*Lycopersicon esculentum* Mill. var Sonato) were grown under glasshouse conditions. Fruit were harvested at the mature green and red ripe stages. The fruits were peeled and the placenta and seeds removed. The pericarp was then frozen in liquid N_2 and stored at −40° until required.

Preparation of cell wall material (CWM). Tomato CWM was prepared by a modification of the method described in ref. [5]. Pericarp tissue 400 g was disintegrated (Polytron, Kinematica GmbH, Luzern, Switzerland) in 1.6 l of cold Me_2CO. The Me_2CO insol. material was collected on Miracloth and washed with 3 l of 80% Me_2CO. The residue was then stirred for 15 min in 1 l of phenol–HOAc–H_2O (2:1:1) at 4° to remove endogenous enzyme activity [6, 21, 22]. Following this treatment, Me_2CO was added to the mixture to a final concn of 80%. The residue was then filtered on glass fibre paper, washed with a further 1 l of 80% Me_2CO and ball milled (Pascall 1 l pot) in a further 500 ml of 80% Me_2CO for 6 hr at 1°. After ball milling the insoluble residue was sedimented at high speed and washed with H_2O by resuspension. The residue was then suspended in 90% DMSO overnight at room temp. The residue was recovered by centrifugation and washed ×5 with H_2O on the centrifuge. The pellet (purified CWM) (2–3 g) was then resuspended in *ca* 130 ml of water and stored at −20°. At each stage of the wall preparation aliquots of extraction medium were dialysed and stored at −20° for analysis at a later date.

Fractionation of the CWM. Sequential extraction of the CWM with aq. solvents was undertaken essentially as described in ref. [16]. To 2 g of an aq. suspension of CWM was added an equal vol. of 0.1 M CDTA, pH 6.5 (CDTA-1). After stirring for 6 hr the residue was recovered by centrifugation and extracted with a further vol. of 0.05 M CDTA pH 6.5 (CDTA-2). The CWM was then washed with H_2O (CDTA-W) on the centrifuge and the 3 extracts filtered and dialysed. The residue which remained was then extracted twice with a 0.05 M Na_2CO_3, once at 1° and then at 20°. The depectinated CWM was then sequentially extracted with 0.5, 1.0 and 4.0 M KOH to leave a residue of essentially α-cellulose. All extracts were neutralized, dialysed and stored from frozen at −20°.

Ion-exchange chromatography. Several selected extracts were further fractionated by chromatography on DEAE-Trisacryl M as decribed in ref. [15].

General carbohydrate and methylation analysis. The presence of carbohydrate in column fractions was determined by the phenol–sulphuric acid method [23]. Neutral sugars were released by a modified Saeman hydrolysis and analysed [24] as

their alditol acetates by GC. Uronic acids were determined colorimetrically, after a modified [25] H_2SO_4 hydrolysis, by the method of ref. [26]. The values for sugars in all the Tables are based on single determinations which, from previous work [24], was considered sufficient. The degree of esterification of the CDTA extracts was calculated from their MeOH contents. MeOH was released as described in ref. [27], but instead of conversion to its nitrite ester, it was analysed directly by GC (carbowax 20M on Diatomite C-AW 100–120 mesh at 190°). Selected fractions from the ion-exchange column (pre-reduced with $NaBH_4$ if necessary) were methylated by a modification [28] of the Hakomori method; for details of carboxyl reduction with $LiAlD_4$ see ref. [16]. The CDTA-fractions were first de-esterified in 0.1 M NaOH for 2 hr at 1°, neutralized, and recovered by dialysis and freeze-drying. The methylated fractions were hydrolysed with aq. 90% HO_2CH at 100° for 2 hr followed by 0.2 M H_2SO_4 at 100° for 12 hr. The products were then converted to PMAA which were separated by GC on OV-225 and ECNSS-M columns and examined by GC-MS (OV-225 column) [28]. The ECNSS-M column was used to separate the derivatives of (1→2)- and (1→4)-linked Xyl*p* from terminal-Gal*p* derivative, which co-elute on OV-225. The PMAA were identified using the mass-spectral data of refs [29, 30]; the values for PMAA were corrected using molar response factors of ref. [31]. For a list of relative retention times on OV-225 and main diagnostic ions of PMAA from some pectins see ref. [31].

^{13}C NMR. The polysaccharide for ^{13}C NMR spectroscopy (50 mg) was dissolved in D_2O (3 ml) and contained in a 10 mm o.d. tube. The spectrum was recorded on a JEOL GX 400 spectrometer at an operating frequency of 100.4 MHz for ^{13}C. The spectrum was obtained at 55°, using a 90° pulse angle and a pulse repetition time of 0.66 sec, with complete proton decoupling. The spectrum shown was the result of 90 000 accumulations and before transformation the FID was multiplied by an exponential function giving an additional line broadening of 5 Hz. Chemical shifts, given relative to TMS, were measured using Me_2CO. as int. ref. (δ31.07).

Gel-filtration. Polymer fractions (2–5 mg) were dissolved in 0.1 M acetate, 0.02 M EDTA pH 6.5, and then applied to a column of Sephacryl S-400 (2.1 × 76.5 cm) and eluted with the same acetate–EDTA buffer.

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