Carbohydrate Research, 209 (1991) 191–202
Elsevier Science Publishers B.V., Amsterdam

Cell-wall polysaccharides of kiwifruit (*Actinidia deliciosa*): effect of ripening on the structural features of cell-wall materials

Robert J. Redgwell*,
Department of Scientific and Industrial Research Fruit and Trees, Mt. Albert Research Centre, Private Bag, Auckland (New Zealand)

Laurence D. Melton, and Donald J. Brasch
University of Otago, P.O. Box 56, Dunedin (New Zealand)

(Received February 15th, 1990; accepted for publication, May 25th, 1990)

ABSTRACT

Cell-wall polysaccharides were solubilised from cell-wall materials (CWMs) isolated from kiwifruit at 2 ripening stages, after treatment for 1 and 7 days with ethylene. The fractions soluble in cyclohexane-*trans*-1,2-diaminetetra-acetate (CDTA), Na_2CO_3, guanidinium thiocyanate (GTC), and KOH were purified by ion-exchange and gel-permeation chromatography, and subjected to methylation analysis. The pectic polymers in the 7-day fractions had increased proportions of 4-linked galactosyl, 2- and 2,4-linked rhamnosyl, and terminal arabinosyl, xylosyl, and galactosyl residues, and decreased proportions of 4-linked galacturonosyl residues. In the major Na_2CO_3-soluble pectic fraction, there was an increase in the proportion of branched polymers of high molecular weight during ripening. Therefore, a large proportion of the polyuronides may have been solubilised without significant structural modification, indicating that the action of endo-polygalacturonase was not involved. There was a decrease in M_w of the xyloglucan fraction during ripening, but no detectable changes in the primary structure or in the 4-*O*-methylglucuronoxylan and galactoglucomannan fractions.

INTRODUCTION

Knowledge of the composition and structure of cell-wall polysaccharides (CWM) of fruit at the commencement of, and during, ripening may provide an insight into the chemical, physical, and enzymological processes involved. We have described[1,2] the composition and structural features of the cell walls of kiwifruit [*Actinidia deliciosa* (A. Chev.) C. F. Liang and A. R. Ferguson, cv. Hayward] at harvest, together with the composition of solubilised cell-wall fractions from different zones of tissue at three stages of ripening, namely, at harvest, and 1 day and 7 days after treatment with ethylene. Between harvest and day 1, there was little apparent change in the CWM fractions but, at day 7, large amounts of pectic polymers were solubilised in the cell wall of the outer pericarp[2]. We now report on changes in the structures of the 1- and 7-day pectic and hemicellulosic polysaccharides in CWMs of the outer pericarp.

* Author for correspondence

0008-6215/91/$ 03.50

RESULTS

The 1- and 7-day fractions soluble in cyclohexane-*trans*-1,2-diaminetetra-acetate (CDTA), sodium carbonate, guanidinium thiocyanate (GTC), and potassium hydroxide previously isolated[2] from the outer pericarp CWMs were fractionated on DEAE Trisacryl M. The compositions of the major fractions are given in Table I.

TABLE I

Data on the CDTA-, Na_2CO_3-, GTC-, and KOH-soluble fractions of cell-wall materials of kiwifruit 1 and 7 days after treatment with ethylene

Fraction[a]		*Day*	*Yield* (%)	*Sugar composition (mol%)*[b]								
				Rha	*Fuc*	*Ara*	*Xyl*	*Man*	*Gal*	*Glc*	*Uronic acid*	*Total (μg/mg)*
CDTA												
Buffer	C1	1	60	2.6	[c]	2.1	0.5		5.7	0.3	88.7	816
	C1	7	52	7.1	[c]	6.2	2.1		21.2	1.6	61.8	683
0.125M	C2	1	9	2.5	[c]	2.2	0.6		6.7	0.3	87.8	771
	C2	7	14	7.2	[c]	6.0	1.6		18.6	0.8	65.9	651
0.25M	C3	1	18	1.4	[c]	1.0	0.4		2.4	0.1	94.7	727
	C3	7	20	4.2	[c]	3.3	0.6		9.3	1.0	81.6	740
Na_2CO_3												
Buffer	N1	1	13	6.8	[c]	6.5	0.8		32.7	0.9	52.3	763
	N1	7	28	7.4	[c]	7.2	1.9		39.0	1.1	43.5	691
0.125M	N2	1	70	3.1	[c]	2.6	0.4		10.8	[c]	83.2	790
	N2	7	55	6.1	[c]	4.9	1.2		19.9	[c]	68.0	736
0.25M	N3	1	5	2.0	[c]	1.7	0.4		5.8	0.5	89.6	683
	N3	7	7	3.3	[c]	2.8	0.8		10.0	0.7	82.3	582
GTC												
Buffer	G1	1	66	[c]	1.2	0.50	36.5	10.5	9.1	37.2	5.0	714
	G1	7	79	[c]	1.2	0.45	34.3	12.2	9.8	36.3	5.8	742
0.25M	G2	1	15	7.3	[c]	6.0	1.7		33.4	1.7	49.8	726
	G2	7	9	7.1	[c]	6.4	3.2		38.4	1.5	43.4	717
KOH												
Buffer	K1	1	63	[c]	1.7	1.7	23.1	5.5	18.6	35.3	14.2	700
	K1	7	74	[c]	0.7	1.1	26.7	7.8	7.5	52.2	3.9	677
0.125M	K2	1	26	3.9	[c]	4.1	17.9	0.1	29.1	1.5	43.4	787
	K2	7	11	2.8	[c]	2.6	62.0	0.4	13.7	2.7	16.0	675
0.25M	K3	1	1	2.7	[c]	2.9	12.2		18.8	2.3	61.0	527
	K3	7	4	4.6	[c]	4.1	23.4		17.0	1.7	49.1	691

[a] Each fraction was recovered from a column of DEAE Trisacryl M in either buffer, or buffer plus 0.125, 0.25, 0.5, or 1.0M NaCl. Fractions not reported represented < 1% of the total polymer. [b] "Anhydro-sugar" values after CF_3CO_2H and Saeman hydrolysis. [c] Trace.

Pectic polymers. — The increase[2] in the proportions of Gal, Rha, and Ara in the CDTA- and Na_2CO_3-soluble pectic polymers during ripening, despite a large decrease in their total proportions, was confirmed. The retention of the polymers on the anion

exchanger indicated that the increased proportions of neutral sugars were components of the pectic molecule and not of neutral polymers which were co-extracted with the acidic polymers.

Selected fractions were subjected in sequence to carboxyl reduction, methylation, and hydrolysis, and the products were converted into partially methylated alditol acetates for g.l.c.–m.s.

The linkages were similar for each pair of pectic fractions (Table II). However, there was a pronounced increase in the proportion of 4-linked Gal, 2,4-linked Rha, terminal Ara, and terminal Gal relative to 4-linked GalA in the CDTA- and Na_2CO_3-soluble pectic fractions, which indicated an increase in the degree of branching of the pectic polymers that remained in the CWM of the 7-day fruit. The proportions of terminal GalA, terminal Rha, and 2,3- and 2,4-linked GalA remained relatively constant, which indicated that these residues may have been in separate side chains that differed from the arabinogalactan type sequences both in composition, distribution along the backbone, and degree of heterogeneity. Even though i.r. spectroscopy indicated negligible proportions of hydroxyl groups in the methylated polymers, glycosyl-*6,6-d*$_2$ residues were detected and were therefore evidence for some undermethylation of the pectic backbone. The 2,3- and 2,4-linked GalA have been established as branch points in pectic polymers of the primary wall[3], but the values reported here are probably higher than their levels *in vivo*.

The pectic polymers of the GTC- and KOH-soluble fractions were more highly branched than the CDTA- and Na_2CO_3-soluble polymers[1], and comparable changes were not defined as clearly. For the KOH-soluble fractions, differences were complicated further by the presence of large proportions of 4-*O*-methylglucuronoxylan which was co-eluted with the pectic polymers. Even so, the ratio of 4-linked Gal and GalA increased with ripening and parallelled the trend in the CDTA- and Na_2CO_3-soluble fractions.

The Na_2CO_3-soluble N2 polymers from the Trisacryl columns (Table I) were the preponderant pectic fractions. The behaviour of each fraction on Sepharose CL-2B is shown in Fig. 1. The $\bar{M}_w$ of fraction 1D was similar to that of blue dextran (2000k). After 7 days, the $\bar{M}_w$ had increased slightly and two components, slightly larger (P1) and smaller (P2) than 1D, were resolved. Analysis of each half of the 1-day peak and of P1 and P2 of the 7-day fraction (Table III) indicated that P1 contained increased proportions of Gal, Rha, and Ara, and decreased proportions of GalA, compared to P2.

Hemicellulosic polymers. — Most of the hemicelluloses were contained in the neutral GTC-soluble (G1) and neutral KOH-soluble (K1) fractions (Table I). The exception was the 4-*O*-methylglucuronoxylan which was retained on the ion exchanger in the KOH fraction. This hemicellulose, in the GTC-soluble fraction, was eluted with the neutral fraction. The acidic compounds in fraction K1 of the 1-day sample were pectic polysaccharides, but elution from DEAE Trisacryl M a second time was only partially successful in removing the pectic material.

Each fraction G1 was subjected to gel filtration on Sephacryl S-300 as was each fraction K1 on Sepharose CL-6B (Fig. 2). Some pectic material still remained in each

TABLE II

Linkage composition of pectic fractions in cell-wall materials prepared from kiwifruit 1 day and 7 days after treatment with ethylene

Residue	*Linkage (s)*[a]	*Fractions (mol% residue)*									
		CDTA-soluble				Na_2CO_3-*soluble*		*GTC-soluble*		*KOH soluble*	
		C1		*C3*		*N2*		*G2*		*K2*	
		1 d	*7 d*	*1 d*	*7 d*	*1 d*	*7 d*	*1 d*	*7 d*	*1 d*	*7 d*
Rha	T	0.2	0.3	0.1	0.2	0.3	0.2	0.2	0.2	0.2	*d*
	2	1.9	4.5	0.8	3.5	1.9	2.8	4.0	3.5	2.1	0.8
	3	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*
	2,4	0.6	2.4	0.3	1.2	0.8	1.7	2.0	2.4	1.8	1.4
	2,3	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*
	2,3,4	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*
Ara	T	1.0	3.3	0.6	2.3	1.5	3.1	3.5	1.9	1.9	1.0
	5	0.5	1.9	0.4	1.0	0.7	0.8	1.4	1.5	0.9	0.6
	3,5	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*
Xyl	T	0.3	1.5	0.3	0.5	0.5	0.9	0.9	1.0	1.8	3.0
	4							0.6	1.3	17.5	52.6
	2,4									2.8	8.7
Fuc	T	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*
Glc	4	0.8	2.2	0.5	1.5	0.4	0.7	0.9	0.9	2.1	2.0
	4,6									0.7	1.2
Gal	T	0.3	1.5	0.1	0.6	0.6	1.3	1.0	1.6	1.1	0.8
	4	5.6	20.9	2.0	7.3	11.1	20.0	30.8	42.2	28.2	10.7
	3	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*
	2,4	0.2	0.5	0.2	0.3	0.4	0.6	0.7	1.4	0.6	0.2
	3,4	1.2	2.0	0.4	0.9	0.8	1.6	2.0	2.7	2.7	0.5
	4,6	0.1	0.9	0.2	0.2	0.4	0.8	0.8	2.0	0.6	0.5
4-*O*-MeGlcA	T[b]							*d*	*d*	3.3	9.7
GalA	T	0.8	0.8	1.1	1.1	1.0	0.9	0.8	0.6	0.4	0.1
	4[b,c]	83.9	53.9	90.4	76.0	75.9	61.7	48.3	34.4	29.4	4.9
	2,4[b]	1.3	1.3	1.7	1.2	1.5	1.2	1.0	1.1	0.5	0.1
	3,4[b]	1.2	2.0	1.7	1.7	2.0	1.7	1.1	1.3	1.4	1.2

[a] T connotes terminal. [b] Analysed as glycosyl-6,6-d_2 residues. [c] Includes proportions (0.2–1.3 mol%) of galactosyl-6,6-d_2 residues undermethylated at O-6. [d] Trace.

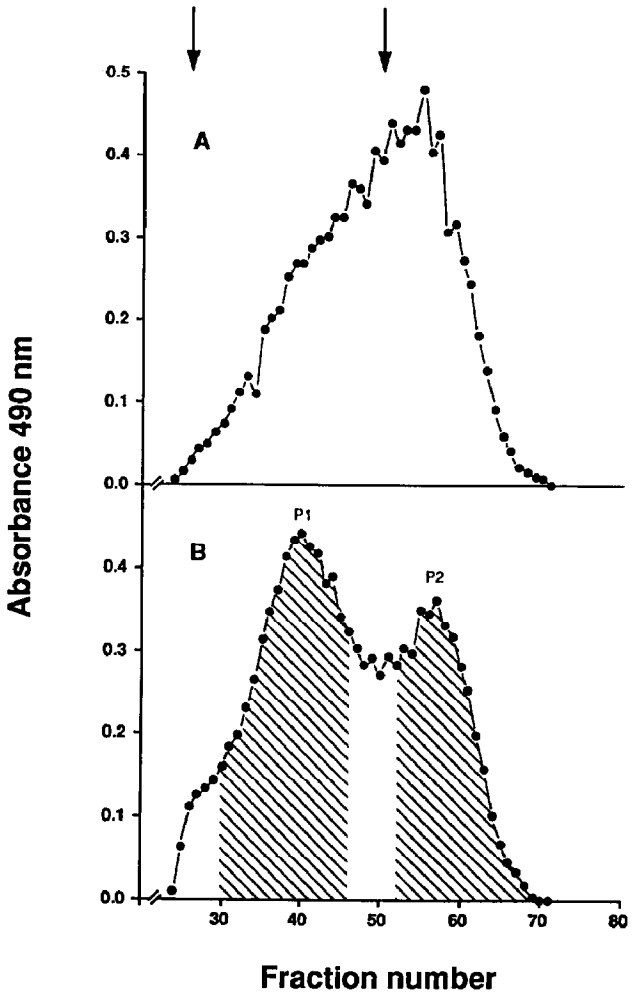

Fig. 1. Profiles on Sepharose CL-2B of the Na_2CO_3-soluble pectic fractions (N2, Table I) prepared from kiwifruit CWMs after treatment with ethylene: *A* 1 day, *B* 7 days. Fractions 30–46 and 52–70 in *B* were combined to give P1 and P2, respectively. Arrows represent the elution position of the void volume and dextran 2000.

fraction K1, particularly in the 1-day samples, and was eluted in the void volume. Small amounts of Xyl, Glc, and Man were also present in the void volume, indicating that the molecular mass of some hemicellulosic molecules may have exceeded 1000 kDa. Appropriate fractions were combined and subjected to composition and methylation analysis (Tables III and IV, Fig. 2). The results indicated that (*a*) the $\bar{M}_w$ of the GTC-soluble hemicelluloses ranged between 185k–22k, whereas those of the KOH-soluble polymers were generally larger (500k–30k), (*b*) the 4-*O*-methylglucuronoxylan and mannan were different polysaccharides and not components of a hemicellulose complex, (*c*) the xyloglucan was the largest polymer (500 kDa) and showed a high degree of molecular heterogeneity (the 4- and 4,6-linked Glc, 2-linked Xyl, 2-linked Gal, and terminal Fuc,

TABLE III

Composition of the pectic and hemicellulosic fractions following gel filtration chromatography

Fraction	*Day*	*Composition (mol%)*[a]								
		Fuc	*Rha*	*Ara*	*Xyl*	*Man*	*Gal*	*Glc*	*Uronic acid*	*Total (μg/mg)*
Na_2CO_3-soluble	*1 P1*	[b]	4.7	3.5	0.7		14.3		76.8	700
	P2	[b]	1.7	1.3	0.5		5.6		90.8	750
	7 P1	[b]	8.0	6.6	2.2		24.4		58.7	800
	P2	[b]	1.8	1.8	0.8		9.2		86.4	790
GTC-soluble	1 P1	2.1	0.2	0.8	33.6	0.3	7.6	51.6	3.8	700
	P2	0.8	0.1	0.5	56.1	1.6	3.6	26.3	11.1	893
	P3	0.2	[b]	1.0	25.2	20.9	11.6	33.7	7.5	777
	7 P1	2.0	0.1	1.9	35.5	0.2	6.6	51.2	2.5	720
	P2	1.1	0.1	0.7	53.2	1.4	4.1	28.4	11.0	657
	P3	0.3	[b]	1.0	22.6	23.7	13.8	32.2	6.5	792
KOH-soluble	1 P1	1.1	0.2	0.6	36.6	0.2	6.9	50.7	3.8	720
	P2	[b]	[b]	0.1	18.0	24.7	17.8	34.2	5.1	777
	7 P1	1.3	0.1	0.5	37.6	0.2	5.1	53.6	1.5	760
	P2	0.5	0.2	0.5	20.8	22.1	13.4	36.8	4.9	700

[a] "Anhydro"-values after CF_3CO_2H and Seaman hydrolysis. [b] Trace.

typically found in dicotyledonous xyloglucans, were present in each fraction), (*d*) the mannan was the smallest polymer (20 kDa) and had a low degree of molecular heterogeneity (the simultaneous occurrence of comparable proportions of 4,6-linked Man and terminal Gal in GTC-soluble P3 suggested that single Gal residues occurred at each branch point and that the polymer was a galactomannan), (*e*) the size (40 kDa) of the 4-*O*-methylglucuronoxylan was intermediate of those of xyloglucan and the galactomannan, but there was also a high degree of molecular heterogeneity, (*f*) there was a consistent decrease in the $\bar{M}_w$ of the xyloglucan fraction in both the KOH- (500 → 300 kDa) and GTC-soluble (185 → 115 kDa) polymers during ripening (no change in the glycosyl linkage composition of the xyloglucan of P1 accompanied this decrease), and (*g*) no changes in either molecular weight or glycosyl linkage composition occurred in the fractions P2 or P3 of the GTC- and KOH-soluble polymers.

As well as molecular heterogeneity, some structural heterogeneity was shown by the hemicelluloses. The ratio of 4- and 2,4-linked Xyl residues varied between 5:1 in the KOH-soluble P2 fraction to 1.3:1 in the GTC-soluble P1 fraction of the 7-day sample, indicating that the latter was a more branched molecule. The GTC-soluble P3 fractions contained increased proportions of 2,4-linked and terminal Man compared to the

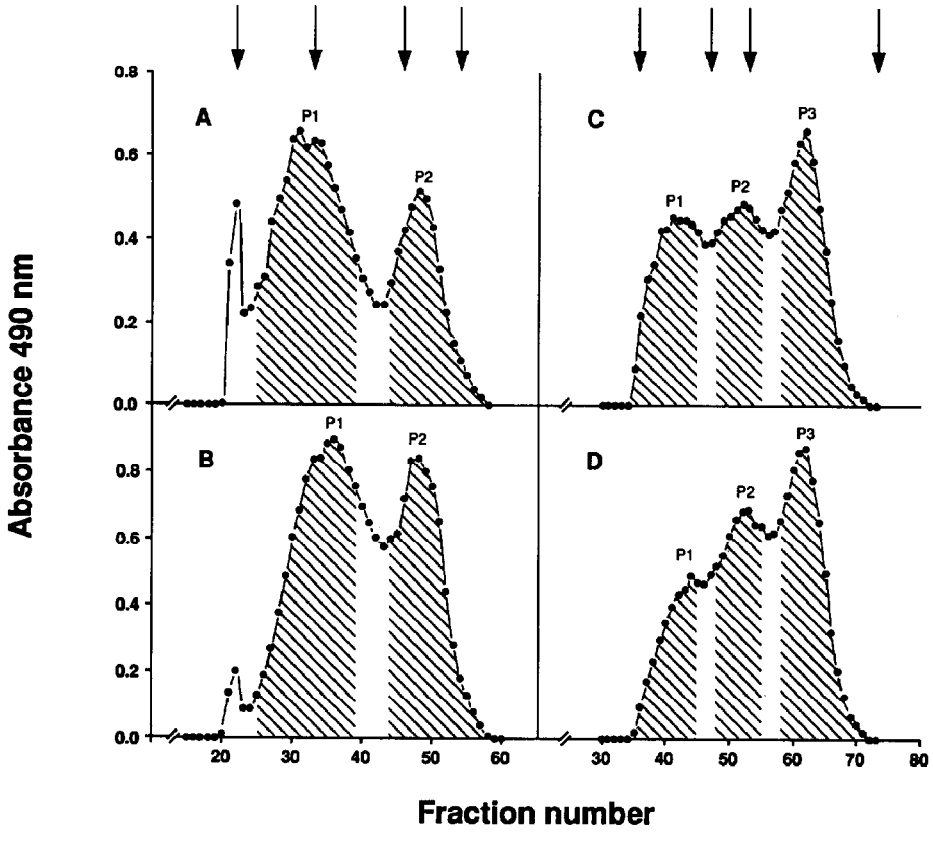

Fig. 2. Profiles on Sepharose CL-6B (*A* and *B*) and Sephacryl S-300 (*C* and *D*) of hemicelluloses solubilised from CWMs of kiwifruit by KOH and GTC 1 and 7 days after treatment with ethylene: *A*, KOH 1 day (K1, Table I); *B*, KOH 7 days (K1); *C*, GTC 1 day (G1); *D*, GTC 7 days (G1). The fractions designated by the shaded areas were combined to give P1–P3. The arrows represent, for *A* and *B*, the positions of elution of the void volume, and dextrans with M_w 500k, 40k, and 5k; and for *C* and *D*, void volume and 70k, 40k, and 5k.

KOH-soluble P2 fractions and 4–5 times as much 6-linked Gal. There were similar variations in the xyloglucan fractions, particularly with regard to the ratio of terminal Xyl, 2-linked Xyl, and 2-linked Gal. The dramatic decrease in the ratio of 4,6- and 4-linked Glc in fractions P2 and P3 indicated the smaller chains of xyloglucan to be less branched. However, the fact that this difference occurred only in those fractions that contained the galactomannan raised the possibility that the increased proportions of 4-linked Glc in these fractions may have been part of the mannan backbone and that the polymer was a galactoglucomannan. The variations in composition of each type of hemicellulose were not a consequence of ripening but of fundamental features related to molecular size.

The 7-day CWM formed a much more viscous slurry with water than did the 1-day CWM. The viscosity (in centipoise) of each CWM as aqueous 0.6 and 1.2% suspensions were, respectively, 6.0 and 12.0 for the 1-day CWM, and 120 and 1000 for the 7-day CWM.

TABLE IV

Linkage analysis of GTC- and KOH-soluble hemicelluloses isolated from kiwifruit 1 day (1 d) and 7 days (7 d) after treatment with ethylene and partially purified by gel filtration chromatography

Polymer type[a]	*Linkage*	*Composition (mol%)*										
		KOH-soluble				*GTC-soluble*						
		P1		*P2*		*P1*		*P2*			*P3*	
		1 d	*7 d*	*1 d*	*7 d*	*1 d*	*7 d*	*1 d*	*7 d*	*7 d*[b]	*1 d*	*7 d*
Xyloglucan												
	T-Ara	0.6	1.0	0.1	0.2	1.1	0.8	0.6	0.9	0.6	0.2	0.6
	T-Fuc	0.7	1.0	0.1	0.3	1.8	1.4	0.9	1.2	0.8	[c]	0.2
	T-Xyl	14.0	16.8	2.1	3.8	12.1	16.3	9.9	9.3	9.4	3.4	3.6
	2-Xyl	12.0	11.4	3.6	4.0	9.2	8.8	5.0	7.1	6.5	2.2	2.4
	2-Gal	1.1	1.3	3.6	4.1	1.9	2.1	0.9	1.2	1.1	4.0	4.2
	4-Glc	23.1	26.2	28.5	30.9	22.5	24.4	12.6	13.2	12.3	29.4	29.3
	4,6-Glc	27.7	30.6	5.2	7.9	31.4	34.4	16.4	18.6	15.7	4.9	5.0
Xylan												
	T-4-*O*-MeGlcA									8.1		
	4-Xyl	9.4	6.1	12.2	12.7	2.8	3.1	33.8	32.8	31.8	12.7	11.8
	2,4-Xyl	2.3	2.5	2.6	2.4	1.7	2.3	14.6	11.2	9.8	4.3	3.7
Galactomannan												
	T-Gal	2.2	2.5	7.5	8.6	3.5	3.6	1.6	2.2	1.9	9.0	8.9
	4-Man	0.3	[c]	17.0	15.9	[c]	0.5	2.0	0.9	0.6	13.8	14.4
	4,6-Man			9.5	9.1						8.3	8.7
	2,4-Man			0.3	[c]						2.1	1.9
	T-Man			[c]	0.3			[c]			0.6	0.6
Others												
	T-Glc	0.3	0.6	0.7	0.8	[c]	[c]	[c]	[c]	[c]	0.8	0.8
	2,4-Glc	0.5	[c]	0.7	[c]	[c]	0.3	[c]	[c]	[c]	0.4	0.4
	4-Gal	4.4	[c]	4.7	[c]	2.4	1.2	1.0	0.8	0.9	0.7	0.6
	6-Gal	0.4	[c]	0.6	0.3	0.6	0.7	0.4	0.5	0.4	3.1	2.8

[a] The grouping of linkages into polymer categories is tentative; T connotes terminal. Where a glycosyl residues occurs in two polymer types, it has been assigned to

DISCUSSION

The pectic polymers of kiwifruit were heterogeneous on the basis of degrees of branching as indicated by the proportions of 4-linked Gal, normally assigned to side chains, and the 4-linked GalA which made up the acidic core. During ripening, considerable proportions of these polymers are lost from the CWM, but those remaining showed not only little structural modification but also, in at least one instance (the Na_2CO_3-soluble N2 fraction), no decrease in $\bar{M}_w$. In fact, $\bar{M}_w$ of the pectic polymers increased as the ratio of the fractions of high (high degree of branching) and low molecular weight (low degree of branching) increased. There are several possible explanations for this phenomenon.

As the fruit ripen and the physical state of the walls changes, some of the more highly-branched pectic polymers (which, after 1 day, were solubilised only in 4M KOH and GTC or remained in the residue) may be solubilised by Na_2CO_3 after 7 days. However, if this phenomenon occurred, the average degree of branching of the polymers remaining in the GTC- and KOH-soluble fractions should decrease and not increase, as shown in Table II. Another possibility is that there is a post-ethylene surge of polysaccharide synthesis in response to the sudden induction of changes in cell-wall ripening. Data on the extent of polymer synthesis during the ripening of kiwifruit are lacking, however, and the present results will be discussed only in relation to breakdown of the cell wall. The increased proportions of Gal found in the pectic polymers during ripening may have occurred[2] as a result of degradation and release of unbranched sequences of the pectic backbone. The lack of any decrease in the $\bar{M}_w$ for the Na_2CO_3-soluble fraction makes this unlikely and the increase in the degree of branching may occur by preferential solubilisation of the less-branched pectic polymers. Alternatively, the pectic polymers may be degraded by depolymerases, but only in some areas of the cell wall.

There is evidence from ultrastructure that, to a limited extent, selective depolymerisation may occur. In kiwifruit, the pectic polymers concentrated in the region of the plasmadesmata showed little if any change during ripening, whereas other regions of the primary wall and the middle lamella showed extensive loss of integrity[4]. However, the plasmadesmatal regions of the cell wall are probably insufficient to account for the proportion of intact pectic material present in the 7-day CWM.

Therefore, the polyuronides may be solubilised in the cell wall by a process that does not involve depolymerisation. Calcium may play a part and, although its role in the softening of fruit is still a matter of debate[5], it is probable that changes in its concentration and binding sites within the wall could exercise some control over the retention or release of pectin[6]. Another factor could be the different rheology of the 7-day CWM in aqueous suspension compared to that of the 1-day CWM; the latter formed a suspension of low viscosity which settled when left. However, the 7-day CWM showed a much greater affinity for water and formed a stable viscous gel. This finding suggested that the polymers of the 7-day CWM had moved apart, allowing penetration and interaction of the water molecules within the cell-wall matrix. The solubilisation of pectin within the

cell wall would leave space that water could replace. However, the removal of the pectin polymers by CDTA and Na_2CO_3 did not confer similar properties on the CWM residue of the 1-day fruit, whereas the 7-day CWM, even after extraction with CDTA and Na_2CO_3, still formed gel-like suspensions in water. Therefore, it is possible that the changes that caused this phenomenon may occur in the hemicellulose polymers and the cellulose fibrils. Xyloglucans are intimately associated with the cellulose fibrils[7], and occur across the thickness of the primary wall and also in the middle lamella[8]. A decrease in their molecular weight, such as that shown in this study, may lessen the degree of hydrogen bonding between the xyloglucan and the cellulose fibrils and contribute towards loosening and increased hydration of the cell wall. Following this process, pectic molecules may be solubilised, and the integrity lost. The fact that smaller molecules with a low degree of branching are preferentially solubilised is logical if physical entanglement plays a major role in polymer–polymer interactions in the cell wall.

Whatever the cause of the viscosity of CWM, it is likely to be an important factor in the softening of kiwifruit. The apparent absence of polygalacturonase in some fruits[9,10] that soften normally has implied alternative mechanisms for cell-wall dissolution. Degradation of polyuronide is insufficient to account for softening in tomato[11], and changes in the molecular size of solubilised polyuronides are not as extensive as thought previously[12]. In the light of these, and the above results, it is appropriate to re-evaluate the processes of softening in relation to the physicochemical interactions of cell-wall polymers.

The differences in composition between kiwifruit at harvest and after 1 day were slight. However, the solubilisation of small amounts of polyuronide, with an $\bar{M}_w$ similar to that of the Na_2CO_3-soluble pectic polymers, occurred within 1 day after treatment with ethylene[13]. The change was masked in the present work because sodium dodecylsulphate (SDS) solubilises moderate amounts of the CWM during its preparation, making it difficult to distinguish small amounts of pectic polymers derived from *in vivo* solubilisation and by SDS. The first steps in cell-wall breakdown during ripening have important implications for controlling the initiation of softening, and current work is focused in this area.

EXPERIMENTAL

Preparation of CWM. — Kiwifruit were harvested in May at Tepuke and exposed to ethylene within 1 day[2]. Fruit, 1 and 7 days after treatment with ethylene, had firmnesses of 2.2 and 0.9 Kgf, and levels of soluble solids of 10.8 and 14.1, respectively. CWM of the outer pericarp was prepared by sequential extraction of cryo-milled (−196°) tissue with aqueous SDS, phenol–acetic acid–water (2:1:1), and aqueous 90% Me_2SO[1].

Fractionation of CWMs. — CWMs were fractionated by extraction with H_2O, 0.05M CDTA (pH 6.5), 0.05M Na_2CO_3 (plus 20mM $NaBH_4$), 6M GTC, and 4M KOH (plus 20mM $NaBH_4$) as described[2].

Ion-exchange chromatography of polymers. — The CDTA-, Na_2CO_3-, GTC-, and KOH-soluble polymers (200–300 mg) were each dissolved in 0.05M phosphate buffer (pH 6.5) and fractionated on a column (2.5 × 20 cm) of DEAE Trisacryl M by sequential elution with 400-mL volumes of buffer and buffer containing 0.125, 0.25, and 0.5M NaCl[1].

Chemical analyses. — The general procedures for determining monosaccharide composition of the polysaccharides were as described[1].

Methylation analysis. — The method of Ciucanu and Kerek[14] was used. Each pectic fraction was carboxyl-reduced with $NaBD_4$ prior to methylation as described[1]. The hemicelluloses were methylated without reduction, except for P2 of the 7-day GTC-soluble fraction which was methylated (CD_3I) with and without reduction in order to establish the presence of 4-*O*-MeGlcA in the hemicellulose. The proportions of hexose and hexose-d_2 were determined by m.s. G.l.c.–m.s. of the partially methylated alditol acetates was done with a VG 70-SE mass spectrometer, using an SP-2330 fused-silica capillary column (30 m × 0.32 mm) programmed for 4 min at 70°, then to 150° at 25°/min, and to 220° at 4°/min. The molar response factors reported by Sweet *et al.*[15] were used.

Gel filtration. — The following columns were used: Sepharose CL-2B (2.5 cm × 70 cm) for the Na_2CO_3-soluble pectic fractions, Sepharose CL-6B (2.0 cm × 90 cm) for the KOH-soluble hemicellulose fractions, and Sephacryl S-300 (2.5 cm × 100 cm) for the GTC-soluble hemicellulose fractions. Gel mediums were equilibrated in 0.05M acetate buffer (pH 6.0) that contained 125mM NaCl. Each polymer (~5 mg) was dissolved in 1.0 mL of buffer and eluted from the column at 10 mL/h; 2.0-mL fractions were collected and assayed for carbohydrate by the phenol–sulphuric method.

Viscosity measurements. — A Brookfield Synchro-lectric Viscometer Model LVF was used. Suspensions (0.6 and 1.2%) of CWMs were prepared in 80 and 40 mL of H_2O, respectively. The freshly made suspensions were homogenised with an Ultra Turrax in order to achieve complete dispersion of the CWMs, then stored for 20 min in order to allow full hydration. All measurements were made at 21°.

ACKNOWLEDGMENTS

We thank E. A. MacRae for useful discussions, and V. J. Paterson and H. Young for help with g.l.c.–m.s. This research was partially funded by the New Zealand Kiwifruit Authority.

REFERENCES

1 R. J. Redgwell, L. D. Melton, and D. J. Brasch, *Carbohydr. Res.*, 182 (1988) 241–258.
2 R. J. Redgwell, L. D. Melton, and D. J. Brasch, *Phytochemistry*, 29 (1990) 399–407.
3 T. T. Stevenson, A. G. Darvill, and P. Albersheim, *Carbohydr. Res.*, 182 (1988) 207–226.
4 I. Hallet and E. A. Macrae, unpublished results.
5 C. J. Brady, W. B. McGlasson, J. A. Pearson, S. K. Meldrum, and E. Kopeliovitch, *J. Am. Soc. Hortic. Sci.*, 110 (1985) 254–258.

6 J. K. Burns and R. Pressey, *J. Am. Soc. Hortic. Sci.*, 112 (1987) 783–787.
7 S. C. Fry, *J. Exp. Bot.*, 210 (1989) 1–11.
8 P. J. Moore, A. G. Darvill, P. Albersheim, and A. L. Staehelin, *Plant Physiol.*, 82 (1986) 787–794.
9 T. G. McCollum, D. J. Huber, and D. J. Cantliffe, *Physiol. Plant.*, 76 (1989) 303–308.
10 D. J. Huber, *J. Food Sci.*, 49 (1984) 1310–1315.
11 J. J. Giovannoni, D. Dellapenna, A. B. Bennett, and R. L. Fischer, *Plant Cell*, 1 (1989) 53–63.
12 G. B. Seymour, S. E. Harding, A. J. Taylor, G. E. Hobson, and G. A. Tucker, *Phytochemistry*, 26 (1987) 1871–1875.
13 R. J. Redgwell, L. D. Melton, and D. J. Brasch, unpublished results.
14 I. Ciucanu and F. Kerek, *Carbohydr. Res.*, 131 (1984) 209–217.
15 D. P. Sweet, R. H. Shapiro, and P. Albersheim, *Carbohydr. Res.*, 40 (1975) 217–225.