Carbohydrate Research, 182 (1988) 241–258
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

CELL-WALL POLYSACCHARIDES OF KIWIFRUIT (*Actinidia deliciosa*): CHEMICAL FEATURES IN DIFFERENT TISSUE ZONES OF THE FRUIT AT HARVEST

ROBERT J. REDGWELL*,
Division of Horticulture and Processing, Department of Scientific and Industrial Research, Private Bag, Auckland (New Zealand)
LAURENCE D. MELTON, AND DONALD J. BRASCH
University of Otago, P.O. Box 56, Dunedin (New Zealand)
(Received January 28th, 1988; accepted for publication, April 18th, 1988)

ABSTRACT

Cell-wall material (CWM) was isolated from cryo-milled (−196°) powders prepared from 4 different tissue zones of kiwifruit (*Actinidia deliciosa*). Polysaccharides were solubilised by stepwise extraction with cyclohexane-*trans*-1,2-diaminetetra-acetate (CDTA), 0.05M Na_2CO_3, 6M guanidinium thiocyanate (GTC), and 4M KOH. A heterogeneous mixture of pectic galactans accounted for 40–50% of the CWMs, while hemicelluloses, the bulk of which were xyloglucans, accounted for 15–25%. Each tissue zone contained similar types of polysaccharide. Variability in their amount and sugar composition are thought to reflect different stages in the physiological development of the fruit at harvest, in the 4 zones. Polymers from the outer pericarp tissue were fractionated by anion-exchange chromatography and subjected to methylation analysis. The CDTA- and Na_2CO_3-soluble polymers were rhamnogalacturonans substituted to varying degrees with galactan and arabinogalactan side-chains containing 4-, 2,4-, 3,4- and 4,6-linked galactose and 5- and 3,5-linked arabinose. Side chains were terminated by galactose and arabinose and lesser amounts of rhamnose, fucose, xylose, and galacturonic acid. The pectic polysaccharides of the GTC- and KOH-soluble fractions had more highly branched rhamnogalacturonan backbones than the CDTA- and Na_2CO_3-soluble polymers and contained hemicellulosic elements. The major hemicellulose was a xyloglucan, but lesser amounts of a 4-*O*-methylglucuronoxylan and a branched mannan were partially characterised. Several polymers were associated with proteins low in hydroxyproline. Evidence is presented that a polysaccharide of the rhamnogalacturonan II type is associated with the pectic polymers of kiwifruit.

*Author for correspondence

0008-6215/88/$03.50

INTRODUCTION

Kiwifruit [*Actinidia deliciosa* (A. Chev.) C. F. Liang and A. R. Ferguson, cv. Haywood] is not eaten at harvest and undergoes dramatic changes in firmness during ripening after harvest[1]. In many fruit, tissue softening is related to the breakdown of middle lamella polysaccharide[2,3] and the primary cell wall[4]. Increased levels of cell-wall degrading enzymes[5] accompany the softening process. However, enzyme studies alone will not reveal the subtle modifications of specific wall polysaccharides that occur during ripening. The structural characterisation of these polymers before, during, and after modification by ripening may provide important additional information on the mechanism of the process. The approach has been attempted with tomato cell walls[6] but, elsewhere, has been limited to reporting gross changes in the sugar composition of total cell-wall fractions[7].

Within kiwifruit, the outer pericarp, inner pericarp, and core appear as characteristic and separate tissue zones. In freshly harvested fruit, concentrations of individual sugars and organic acids are different for each of these zones[8], as are cell size and the physical arrangement of cells within each tissue zone[9]. In order to assess whether these chemical differences extended to the cell-wall polysaccharides, cell-wall materials were prepared from each of the 4 tissue zones.

We now report on the isolation, fractionation, and partial characterisation of the cell walls of kiwifruit at harvest.

RESULTS AND DISCUSSION

Isolation of CWM. — CWM was prepared from each of the 4 tissue zones of kiwifruit, shown in Fig. 1, by the modified procedures of Stevens and Selvendran[10]. It was important to inactivate endogenous enzymes which could alter the structure of cell-wall polymers during their isolation. For this reason, where possible, tissue was frozen in liquid nitrogen and ground to a fine powder in a cryo-mill; this technique replaced the wet ball-milling used in previous work[11]. This treatment allowed the frozen tissues to be suspended more rapidly in aqueous sodium dodecylsulphate (SDS), an advantage if cell-wall enzymes are to be denatured quickly. As a final precaution against adverse effects from residual enzyme activity, the tissues were extracted with phenol–acetic acid–water (2:1:1; PAW) at 4° (ref.12).

In the OP, IP, LW, and C zones, starch comprised 1.3, 1.2, 1.4, and 4.0%, respectively, of the fresh weight of the fruit tissue, and was removed by solubilising in aqueous 90% dimethyl sulphoxide[12]. Only traces of non-starchy carbohydrates were dissolved by this reagent (unpublished results). Some starch (0.5–2.0% of the dry wt.) remained associated with the CWMs but was removed during subsequent fractionation of the CWMs. Alpha-amylase was not used because of the risk of cell-wall modification by impure enzyme preparations.

Composition of SDS-soluble polymers. — Each of the SDS-soluble fractions contained considerable amounts of protein, presumably cytoplasmic in origin (OP,

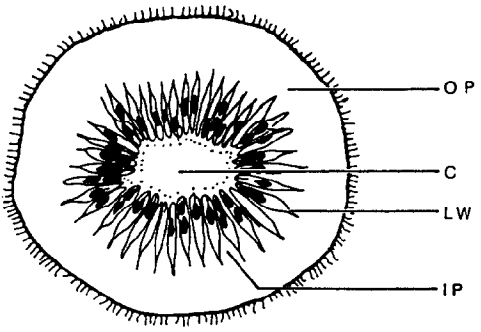

Fig. 1. Cross-section of mature kiwifruit: OP, outer pericarp; IP, inner pericarp; LW, locule wall; C, core.

IP, LW, and C; 28.8, 11.9, 48.8, and 67.8% of protein, respectively). The monosaccharide composition from each tissue zone (Table I) indicated that the carbohydrate polymers were mostly pectic galactans and probably cell-wall derived. Small amounts of hemicellulosic polymers may also be present, as inferred from the levels of xylose and glucose in each of the 4 fractions. The glucose in these polymers was not removed by alpha-amylase and pullulanase, and therefore was unlikely to be from starch. The SDS-soluble fractions (minus protein) represented 9.6, 26.2, 32.1,

TABLE I

MONOSACCHARIDE COMPOSITION OF SDS-SOLUBLE POLYMERS AND PURIFIED CELL-WALL MATERIAL IN DIFFERENT TISSUE ZONES OF KIWIFRUIT

Tissue zone	*Sugar composition (mole%)*[a]										
	Rha	*Fuc*	*Ara*	*Unkn*[b]	*Xyl*	*Man*	*Gal*	*Glc*	*Uronic acid*	*D.e.*[c]	*Total (μg/mg)*
SDS-soluble polymers											
OP	1.2	1.3	6.3	0.4	2.1	1.5	20.7	8.5	57.4		700
IP	0.7	5.4	9.7		4.3	5.2	20.1	6.1	48.6		730
LW	0.7	2.8	9.3	1.6	5.1	3.5	20.2	6.5	50.3		300
C	1.6	4.5	14.9	1.5	5.7	4.9	32.6	5.9	28.6		250
CWMS											
OP	1.8	0.5	2.3		6.0	0.8	13.8	42.0	32.8	71	850
IP	2.0	0.7	3.0		6.8	1.0	9.0	39.6	37.9	63	606
LW	2.0	0.6	2.7		5.6	0.8	13.2	42.1	32.9	68	820
C	2.0	0.6	3.0		4.8	0.8	12.9	40.1	35.8	72	800

[a]Anhydro values after CF_3CO_2H and Saeman hydrolysis. [b]Unknown peak; retention time of the alditol acetate derivative relative to inositol hexa-acetate on column *A*, 0.42. [c]D.e., degree of esterification.

and 18.4% of the total non-starchy polysaccharides in the OP, IP, LW, and C zones, respectively (unpublished results). These results demonstrated that, at harvest, moderate amounts of the cell-wall polymers were solubilished, either *in vivo*, by the processes of fruit ripening, or by the subsequent treatment of the cell walls in SDS solution. However, the relative amounts of the soluble polymers (represented by the SDS-soluble fraction) and the insoluble polymers (represented by the CWMs) were different for each tissue zone.

Variation in composition of the SDS-soluble polysaccharides between tissue zones could be attributed, in part, to soluble intracellular polysaccharides which are not derived from the wall. A water-soluble glucuronomannan has been reported in kiwifruit[13-15]. It is characterised by a backbone of alternating glucuronic acid and mannose residues with galactose side-chains terminated by arabinose and fucose. The presence of this polymer in the inner tissue zones was indicated by levels of mannose, fucose, and glucuronic acid (unpublished results) that were higher than normally found in pectic polymers.

Composition of CWMs. — The monosaccharide compositions of the CWMs

TABLE II

AMINO ACID COMPOSITION OF CELL-WALL MATERIALS FROM DIFFERENT TISSUE ZONES OF KIWIFRUIT AND FROM PECTIC AND HEMICELLULOSIC FRACTIONS OF THE OUTER PERICARP

Amino acid	*Amount (mole%)*										
	CWMS				*CDTA-soluble*		*GTC-soluble*			*KOH-soluble*	
							Na_2CO_3-soluble				
	OP	*IP*	*LW*	*C*	*C1*	*C2*	*N2*	*G1*	*G3*	*K1*	*K3*
Asp	8.7	11.9	9.2	10.0	9.8	9.4	8.3	7.6	9.0	7.7	9.5
Glu	5.8	10.5	8.2	6.8	10.9	10.2	9.1	10.5	10.5	9.4	9.9
Hyp	5.0	3.1	3.5	11.0	0.9	0.7	2.3	1.3	2.2	3.5	2.0
Ser	7.3	6.5	7.3	8.4	12.0	14.3	9.2	7.6	10.5	8.4	9.3
Gly	8.7	11.3	7.9	8.1	13.3	15.1	11.4	11.0	10.1	9.8	11.6
His	3.9	2.4	2.4	2.9	3.3	3.6	5.5	5.8	4.6	3.8	4.7
Arg	5.5	4.5	5.1	3.3	5.3	4.3	5.1	5.2	5.0	6.3	3.6
Thr	5.0	4.8	5.4	3.8	5.0	5.3	5.1	5.2	5.5	4.9	4.9
Ala	6.9	7.9	7.0	6.3	10.5	10.2	9.2	9.9	10.9	10.8	10.4
Pro	9.6	7.1	7.3	8.3	3.0	4.0	4.3	3.4	4.2	3.2	4.0
Tyr	2.7	2.8	2.9	5.0	3.6	2.5	2.5	5.2	4.6	3.8	5.2
Val	10.5	7.1	8.2	10.0	5.1	5.0	9.1	5.8	5.9	5.9	6.1
Ile	4.5	4.2	6.3	3.8	3.2	3.3	3.7	3.9	2.5	3.8	3.7
Leu	7.7	8.2	8.9	6.6	7.1	6.1	8.2	9.9	7.6	7.3	8.0
Phe	4.1	3.7	4.1	2.0	2.5	2.9	3.7	4.2	2.9	3.5	3.4
Lys	4.0	4.0	6.2	4.1	4.2	3.0	3.5	3.5	4.1	4.5	3.7
Total (μg/mg)	14.0	27.0	24.8	22.0	9.1	16.3	5.9	5.4	16.1	8.5	6.5

from each tissue showed only minor variations (Table I). The IP CWM showed greater variation in composition compared to the others; it had a slightly lower galactose content and the degree of esterification of its uronic acid was also lower than for the other 3 zones. In each CWM, the uronic acid was highly esterified. The IP CWM was light brown, evidence that it may contain oxidised polyphenolics. Whether these substances are native to the cell walls of the IP or are contaminants from the seeds is not known. The IP also differed from the other 3 tissues in that it contained a higher non-carbohydrate component. This was accounted for as Klason lignin[16] (7%) and calcium oxalate (12%). Numerous deposits of calcium oxalate, enclosed in raphide cells, are concentrated in the IP of kiwifruit[17].

The protein contents of the OP, IP, LW, and C CWMs were 1.4, 2.7, 2.4, and 2.2%, respectively (Table II). The hydroxyproline content of the C CWM was twice that found in the other tissue zones and was the preponderant amino acid in the protein of this tissue.

Fractionation of the CWMs. The chemical fractionation of the CWMs by sequential extraction with solutions of CDTA, Na_2CO_3, GTC, and KOH was based on published procedures[11,18-20]. The four reagents were chosen for their effectiveness in solubilising polymers under conditions which minimised degradation and because they allowed a nominal distinction to be made between covalent and non-covalent bound fractions within the wall.

Thus, non-covalent bound pectic substances held in the wall by Ca^{2+}, ionic, and steric interactions were solubilised in 0.05M CDTA and are thought to include middle lamella pectic substances. However, the major portions of the pectic substances were covalently bound and were released by 0.05M Na_2CO_3 at 1° and 20°. An interpolymeric ester bond may be broken by this reagent, but the specific nature of the link is unknown.

Hemicelluloses were solubilised by 6M GTC, a strong chaotropic agent, and 4M KOH, the former extracting non-covalent bound polymers with their acyl substituents intact. 4M KOH is used conventionally to release hemicellulose which is extensively hydrogen-bonded to the cellulose fibrils, but will, of course, break any alkali-labile bonds in the process. Moderate amounts of pectic polymers were also released by the GTC and KOH reagents. No selectivity was shown by the GTC or KOH reagents as solvents for different types of hemicellulosic polysaccharides. The use of chaotropic agents (8M urea, 6M GTC) has been reported as ineffective in releasing the bulk of cell-wall hemicellulose[21,22]. In the present study, a moderate amount of cell-wall hemicellulose was solubilised by 6M GTC at room temperature. Its effectiveness here is not completely understood but may relate, in part, to the techniques used to prepare the CWMs. Most other workers have prepared CWM from Waring-Blendor or Polytron-tissue homogenates which can give incomplete disruption of the cell walls. Cryo-milling (and ball-milling) overcomes this problem and may expose more hemicellulosic polymer to chaotropic solvation.

TABLE III

MONOSACCHARIDE COMPOSITION OF SOLUBILISED CELL-WALL POLYSACCHARIDES FROM DIFFERENT TISSUE ZONES OF KIWIFRUIT

Fraction	*%CWM*	*Sugar (mole%)*[a]								
		Rha	*Fuc*	*Ara*	*Xyl*	*Man*	*Gal*	*Glc*	*Uronic acid*	*Total (μg/mg)*
CDTA										
OP	8.9	1.7	0.9	2.0	0.6		14.2	[b]	80.6	750
IP	9.0	1.3	1.1	2.5	1.2	0.7	6.8	[b]	86.5	640
LW	14.9	1.8	0.9	2.8	1.0	0.2	14.6	[b]	79.1	577
C	15.0	1.6	1.0	3.3	0.7	0.1	14.2	[b]	79.3	725
Na_2CO_3										
OP	20.2	2.1	0.6	2.5	0.3		9.6	[b]	84.8	760
IP	16.9	2.7	0.7	3.7	1.2	0.2	8.3	[b]	83.3	805
LW	12.7	3.7	1.1	4.7	1.3	0.2	20.5	[b]	68.6	721
C	17.5	3.2	1.1	4.6	0.9	0.2	20.2	[b]	70.0	760
GTC										
OP	6.9	2.2	1.3	2.9	17.6	6.5	19.2	25.1	25.2	702
IP	10.1	1.2	1.4	2.0	12.5	3.7	7.5	57.8	13.8	726
LW	8.2	1.6	1.5	2.7	18.0	5.9	15.8	37.2	17.1	700
C	8.4	3.3	1.7	4.1	14.7	4.8	24.1	24.3	23.1	706
KOH										
OP	21.2	1.8	1.4	3.0	18.8	3.8	23.9	20.1	27.1	739
IP	18.5	1.7	1.4	4.5	18.7	4.0	18.6	18.8	32.3	664
LW	16.3	1.5	1.8	2.8	27.0	7.5	16.5	27.8	16.5	640
C	15.1	1.4	1.8	2.5	26.9	7.7	15.4	27.8	16.5	700
α-Cellulose										
OP	42.8	2.7		4.0	2.6	2.9	25.0	41.4	21.4	201
IP	45.5	3.6		5.0	6.7	2.1	15.2	38.2	29.1	207
LW	47.9	2.4		4.6	2.3	2.9	18.1	46.9	22.7	199
C	44.0	2.9		4.8	2.5	3.5	18.8	44.9	22.5	210

[a]Anhydro values after hydrolysis with CF_3CO_2H. [b]Trace.

There was considerable variability among the 4 tissues in the amounts and sugar compositions of the polymer fractions solubilised by the 4 reagents (Table III) despite the similarity in monosaccharide composition of the original CWMs. This finding demonstrated that significant modification of specific cell-wall polysaccharides could occur without an apparent change in the monosaccharide composition of the CWM and emphasised the limitation of studies which look only at the net loss of monosaccharide constituents of the cell wall as an indicator of cell-wall changes during ripening.

Some of the compositional differences may be due to differences in tissue morphology. The C tissue, which is composed of smaller more tightly packed cells

than those of the OP[9], could be expected to contain different relative amounts of middle lamella and primary cell-wall pectin. A lower galactose content in pectic polymers during fruit ripening is well documented[23]. Thus, the decreased galactose content of the Na_2CO_3-soluble pectins of the IP and OP zones compared to that in the LW and C zones suggested that ripening was more advanced in the former. It was noticeable that compositional differences were far less between the C and LW fractions. The obvious physical continuity between the two zones together with their chemical similarity suggested that the cell walls of the C and LW zones were more closely related than the other tissue zones.

Pectic substances accounted for the bulk of the polysaccharides in each of the tissue zones (between 40–50% CWMs). Although most were solubilised in CDTA and Na_2CO_3, moderate amounts were found in the GTC, KOH, and α-cellulose fractions.

The α-cellulose contained moderate amounts of CF_3CO_2H-hydrolysable glucose. Normally, cellulose is resistant to hydrolysis by this acid, but the pre-treatment with 6M GTC and 4M KOH may have rendered a proportion of the cellulose fibrils

TABLE IV

MONOSACCHARIDE COMPOSITION OF COMPONENTS OF SOLUBLE FRACTIONS OF THE OUTER PERICARP OF KIWIFRUIT CELL-WALL MATERIAL SEPARATED ON DEAE TRISACRYL

Fraction		*Amount (mg)*	*D.e.,*[a]	*Sugar composition (mole%)*[b]								
				Rha	*Fuc*	*Ara*	*Xyl*	*Man*	*Gal*	*Glc*	*Uronic acid*	*Total (μg/mg)*
CDTA												
Buffer	C1	103	79.0	0.7	1.2	1.2	0.7		34.1	[c]	63.3	680
0.125M	C2	46	82.7	3.2	[c]	3.7	0.7	[c]	12.5		80.0	685
0.25M	C3	35	61.9	2.9	[c]	2.2	0.3	[c]	4.7		89.7	681
0.5M	C4	12		1.0	[c]	0.8	0.4	[c]	2.2		94.8	514
Na_2CO_3												
Buffer	N1	7		2.4	[c]	5.5	1.8	0.5	59.1	[c]	26.9	447
0.125M	N2	9		8.6	[c]	7.7	1.7	0.2	40.4		40.7	702
0.25M	N3	102		3.8	[c]	3.3	0.8	[c]	11.6		80.3	752
0.5M	N4	51		1.3	[c]	0.9	0.2	[c]	3.4		94.2	824
GTC												
Buffer	G1	89			1.2	0.5	37.4	5.9	11.2	39.3	4.5	700
0.125M	G2	3		6.5		6.6	13.5	0.9	39.2	18.0	15.2	400
0.25M	G3	34		6.6		5.0	2.3	[c]	30.3	2.5	53.3	702
0.5M	G4	20		3.8		3.2	1.2	[c]	12.4	1.4	78.0	780
KOH												
Buffer	K1	130			1.7	1.1	29.8	8.0	10.8	45.2	3.7	685
0.125M	K2	23		5.7		6.2	18.9	0.1	40.4	3.8	24.8	700
0.25M	K3	75		6.7		6.7	9.0		39.7	1.1	36.8	701
0.5M	K4	24		4.2		3.6	2.6		15.0	0.7	73.9	837

[a]Degree of esterification. [b]Anhydro values after hydrolysis with CF_3CO_2H. [c]Trace.

susceptible. Alternatively, the glucose could be derived from a hemicellulosic glucan which is either covalently linked to, or so extensively hydrogen-bonded to the cellulose that it resists release by 4M KOH.

Studies of the OP CWM.— A more detailed study was done of the chemical nature of the individual polysaccharides contained in each of the CDTA, Na_2CO_3, GTC, and KOH fractions. Since essentially the same polysaccharides occurred in all tissue zones, the OP CWM was used in this work.

Anion-exchange chromatography of the CDTA-, Na_2CO_3, GTC-, and KOH-soluble fractions. — The polymers were fractionated on the anion exchanger, DEAE-Trisacryl M. Polysaccharide recoveries and the sugar composition of each fraction are given in Table IV.

The CDTA- and Na_2CO_3-soluble polymers were pectic galactans. Galacturonic acid and galactose accounted for >92% of each fraction, but their proportions varied considerably, demonstrating the heterogeneity of the pectic polymers of kiwifruit at harvest. The CDTA-soluble fractions were highly esterified to varying degrees. The Na_2CO_3-soluble polymers were de-esterified by the conditions of their extraction. However, based on the high methoxyl content of the CWM (71%, Table I), they were likely to be highly esterified *in vivo*.

The main component of the CDTA-soluble polymers was not retained on the Trisacryl column despite high levels of uronic acid. This phenomenon has been discussed elsewhere[11].

The GTC- and KOH-soluble polymers each contained a slightly acidic fraction (G1, K1), not retained on the column, which consisted mainly of glucose, xylose, galactose, and mannose. These fractions were subjected to partial hydrolysis with 0.5M CF_3CO_2H, and the acidic moieties were recovered from anion-exchange columns, converted into trimethylsilyl methyl glycosides, and examined by g.l.c. (column *B*). Equivalent amounts of xylose and 4-*O*-methylglucuronic acid were found, implying that the aldobiouronic acid 4-*O*-MeGlcA→Xyl was produced by acid hydrolysis of G1 and K1 (see later discussion on methylation data).

Methylation analysis of the CDTA- and Na_2CO_3-soluble fractions. — Selected fractions were carboxyl-reduced by a modified carbodi-imide method, methylated, converted into partially methylated alditol acetates, and examined by g.l.c.–m.s. The results are given in Table V.

Attempts to methylate the native polysaccharides gave low and inconsistent recoveries of methylated sugars, probably because the native polymers were only partially soluble in Me_2SO even at elevated temperatures. The modified carbodi-imide procedure reduced 90–95% of the uronosyl residues in the pectic polymers. Each of the carboxyl-reduced polysaccharides dissolved readily in Me_2SO at room temperature.

The galactosyl residues in the carboxyl-reduced pectic polysaccharides (Table V) presumably originated from 4-linked galactosyluronic acid residues in linear chains with branch points at infrequent and various intervals. In common with many pectins, the kiwifruit polymers contain 2- and 2,4-linked rhamnosyl residues,

the latter being the probable branch-points in the galacturonan chain. Small amounts of 2,4-, 3,4-, and 4,6-linked dideuterated Gal*p* residues were detected in each fraction. The last residue appears to be a product of undermethylation at O-6[24] and casts doubt on the legitimacy of the 2,4- and 3,4-linked deuterated residues. Although several workers have reported 2,4- and 3,4-linked Gal*p*A residues in methylation analyses of pectic polymers[25,26], there is no additional evidence that they occur as branch points in rhamnogalacturonan backbones.

The more acidic polymers had less highly branced rhamnogalacturonan backbones, an inference based on the ratio of rhamnose and galacturonic acid and their decreased content of 4-linked galactose. The C1 fraction, however, contained very small amounts of rhamnose derivatives and moderately high amounts of 4-linked galactose. This contradiction may be explained by the presence of a pure galactan in C1, which is possible since the anion exchanger did not distinguish between neutral and acidic polymers in this fraction.

The side chains of the rhamnogalacturonans contained primarily 4-linked galactosyl residues with lesser amounts of 4,6-, 2,4- and 3,4-linked galactosyl residues and 5- and 3,5-linked arabinosyl residues. The side chains were terminated by galactose and arabinose, but smaller amounts of the terminal derivatives of xylose, fucose, rhamnose, and GalA residues (inferred from the dideuteration of C-6 of terminal galactose residues) were present.

Methylation analysis of the GTC- and KOH-soluble polymers. — The results of linkage analysis of the carboxyl-reduced polysaccharides of K1, K2, K3, G1, and G3 are given in Table V. They showed that a xyloglucan, a 4-*O*-methylglucuronoxylan, and a branched mannan accounted for ~10, 5, and 2%, respectively, of the OP cell wall.

Fractions K1 and G1 were deuteriomethylated with CD_3I to differentiate between endogenously methylated and unmethylated glycosyl derivatives. Mass-spectral analysis of the terminal glycosyl groups revealed the ions *m/z* 213 (20%), 168 (45%), and 166 (35%), confirming the earlier g.l.c. evidence that 4-*O*-methylglucuronic acid was the acidic moiety in fractions G1 and K1. This result, together with the occurrence of 4- and 2,4-linked xylosyl residues, was evidence that a 4-*O*-methylglucuronoxylan occurred in kiwifruit cell walls similar to that isolated from the primary cell walls of cultured sycamore cells and other plants[27,28]. The e.i.-m.s. fragment ions indicated that >90% of the terminal glucuronic acid was 4-*O*-methylated. The acidic xylan of sycamore cell walls contained much less (38%) of the endogenously methylated derivative.

Fractions K1 and G1 also contained the glycosidic linkages usually associated with xyloglucans from dicotyledonous plants. The xyloglucan accounted for most of the hemicellulose in these fractions. Of the K1 and G1 methylated derivatives, ~8 mole% was accounted for as 4-linked mannose of which 45% was also substituted at O-6. A small amount of terminal mannose was detected. These results suggested that a mannan, a glucomannan, or a xyloglucomannan complex was part of the neutral hemicellulose of kiwifruit.

TABLE V

GLYCOSYL-LINKAGE COMPOSITION OF CDTA-, Na_2CO_3-, GTC-, AND KOH-SOLUBLE POLYSACCHARIDES IN CELL-WALL MATERIAL PREPARED FROM THE OUTER PERICARP OF KIWIFRUIT

Glycosyl residue	*Linkage*	*Mole%*									
		CDTA-soluble fractions			*Na_2CO_3-soluble fractions*		*GTC-soluble fractions*		*KOH-soluble fractions*		
		C1	*C2*	*C3*	*N3*	*N4*	*G1*	*G3*	*K1*	*K2*	*K3*
Rha											
	terminal	0.1	0.2	0.2	0.3	[a]		[a]		[a]	[a]
	2-linked	0.7	2.1	3.5	3.2	1.4		4.2		3.7	2.9
	3-linked	[a]	[a]	[a]	[a]	[a]		[a]		[a]	[a]
	2,3-linked	[a]	[a]	[a]	[a]	[a]		[a]		[a]	[a]
	2,4-linked	0.3	1.2	0.5	0.9	0.4		2.1		1.7	3.3
	2,3,4-linked	[a]	[a]	[a]	[a]	[a]		[a]		[a]	[a]
Ara											
	terminal (*f*)	0.7	2.2	2.0	2.6	0.6	0.2	2.2	0.3	3.2	1.9
	terminal (*p*)										0.1
	3-linked	[a]	[a]	[a]	[a]	[a]		[a]		[a]	[a]
	5-linked	0.1	0.9	0.8	0.9	0.4		1.1		2.1	0.7
	3,5-linked	0.2	0.6	0.3	0.1	0.3		0.3		0.8	0.7
Xyl											
	terminal	0.2	0.2	0.1	0.4	0.1	18.8	0.9	15.5	5.0	1.9
	2-linked						5.8	0.3	7.0	1.4	1.4
	4-linked						11.5	0.4	4.7	7.5	5.4
	2,4-linked						2.8	[a]	0.9	0.4	1.0
Fuc											
	terminal	[a]	0.2	[a]	0.7	[a]	1.0	0.8	1.1		0.1
Glc											
	4-linked	0.4	0.5	0.5	[a]	0.1	11.2	1.8	18.9	0.9	1.0
	4,6-linked						24.6	1.5	25.4	0.5	1.8
Gal											
	terminal	0.3	0.6	0.6	0.7	0.4	3.4	1.3	5.4	1.4	1.0
	4-linked	30.1	6.9	4.2	12.0	3.8	3.7	27.7	3.0	41.8	40.0
	2-linked						3.0		3.2		
	3-linked	[a]	[a]	[a]	[a]	[a]		[a]		[a]	[a]
	2,4-linked	0.6	0.4	0.2	0.3	0.1		0.5		0.6	0.2
	3,4-linked	0.6	1.4	0.7	0.7	0.4		1.6		1.5	1.0
	4,6-linked	0.5	1.3	0.3	1.5	0.3	0.2	2.2	0.2	2.1	1.9
Man											
	terminal						0.2		0.1		
	4-linked						4.4		5.2		
	4,6-linked						3.1		3.6		
GlcA[b]											
	terminal						[a]		[a]	[a]	[a]
	2-linked	[a]	[a]	[a]	[a]	[a]		[a]		[a]	[a]

4-O-MeGlcA[b]										
	terminal					1.4		0.5	0.5	0.9
GalA[b]										
	terminal	[a]	0.5	0.4	0.5	0.5		0.3	0.3	0.2
	4-linked[c]	63.6	76.6	80.0	72.5	87.8		48.9	23.4	31.0
	2,4-linked	0.4	0.8	0.4	0.5	0.5		0.6	0.2	0.2
	3,4-linked	0.8	1.0	1.0	0.8	0.7		1.1	0.5	0.7
Unknown						3.9		4.7		

[a]Trace. [b]Analysed as 6,6-dideuterioglycosyl residues. [c]Includes amounts (0.3–1.2 mole%) of 6,6-dideuteriogalactosyl residues undermethylated at O-6.

The unknown component (retention time of 1.03 on column *D* relative to terminal galactose) did not give an e.i.-mass spectrum expected for an alditol acetate.

The glycosidic and galactosiduronic linkages in G3, K2, and K3 clearly indicated pectic substances similar to those in the CDTA- and Na_2CO_3-soluble fractions. However, the KOH- and GTC-soluble pectic substances generally had higher rhamnose-to-galacturonic acid ratios and contained more 4-linked galactose and 2,4-linked rhamnose, indicating more highly branched backbones than their CDTA- and Na_2CO_3-soluble equivalents.

The acidic hemicellulose fractions were associated with small but possibly significant amounts of the xyloglucan glycosidic derivatives (4- and 4,6-linked glucose, and 2-linked xylose) as well as those of the 4-*O*-methylated glucuronoxylan. Tentative evidence for covalent bonds between the pectic and hemicellulosic polysaccharides, based on their co-chromatography on anion exchangers, has been reported[25,29,30]. However, the co-chromatography of pectic and hemicellulosic elements, particularly in such disproportionate amounts, is not unequivocal evidence of a covalent link between the two. The interaction of native plant polysaccharides with ion-exchange matrices is complex and not always predictable[11,31]. Non-specific absorption, entrapment, partial precipitation, and other phenomena not based on ion-exchange principles could retard small amounts of neutral polymers and allow them to become haphazardly recovered with the acidic polymers when elution buffer conditions are changed. The fragments reported here may well be linkage points between xyloglucan and pectic polymers, but more rigorous purification of these fractions will be necessary to establish the fact.

Rhamnogalacturonan II in kiwifruit. — There have been few reports, other than from Albersheim's laboratory, of the existence of the complex polysaccharide rhamnogalacturonan II (RG II)[32] in plant cell walls, despite the distinctive nature of many of its component monosaccharides. In part, this situation is due to the very small amounts (<0.5%) of the monosaccharide components of RG II in CWM. Also, some components are not detectable as alditol acetates [3-deoxy-D-*manno*-2-octulosonic acid (KDO), aceric acid], and apiose as an alditol acetate is indistinguishable from xylitol penta-acetate in the several g.l.c. systems tested in this labo-

ratory. Therefore, unless these sugars are specifically looked for, they are not easily found. Some evidence for RG II was provided by detecting trace amounts of 2-linked glucuronic acid and 3-linked rhamnose in the linkage analysis of the pectic polymers (Table V). Before the discovery of RG II, these unusual glycosyl constituents had not been found in primary cell wall[32]. In this study, further evidence for the existence of RG II in kiwifruit cell walls was obtained by identifying apiose and KDO in three of the pectic fractions of the OP CWM. These two sugars were chosen because their distinctive chemistry enabled each to be assayed conveniently and because pure standards were available or easily prepared.

Apiose was isolated from partial acid hydrolysates of each pectic fraction by preparative paper chromatography and quantified by g.l.c. of its alditol acetate. The KDO content of each fraction was determined directly using the method of Karkhanis *et al.*[33]. In addition, KDO was released by mild hydrolysis of the OP CWM, purified by anion-exchange chromatography, and examined by g.l.c. of its trimethylsilyated methyl glycosides[34]. This revealed the characteristic peaks of KDO described by York *et al.*[34] and two peaks corresponding to the anomers of rhamnose. These results confirmed the published findings that KDO in RG II is found in the form of the disaccharide rhamose–KDO.

The apiose contents of the CDTA- and Na_2CO_3-soluble polymers and of the α-cellulose were 0.25, 0.42, and 0.03%, respectively. For KDO, the respective values were 0.24, 0.29, and 0.04%. Assuming that KDO and apiose account for ~3 and ~6 mole% of RG II[35], respectively, the total amount of RG II in the OP CWM of kiwifruit would be ~2%. This value is consistent with the estimated amount of RG II in the primary cell wall of cultured sycamore cells[32]. In kiwifruit, RG II is not associated with a specific cell-wall fraction but appears to be a general feature of the pectic polysaccharides.

Acetyl content of polysaccharides. — CWM (1 g) was sequentially extracted with CDTA and 6M GTC. The treatment of partially depectinated CWM with GTC before, instead of after, the Na_2CO_3 extraction ensured that the alkali-labile acetyl substituents of the GTC-soluble polymers remained intact. The sugar composition of the GTC-soluble polymers obtained in this way was very similar to that of the original GTC fraction. The GTC fraction was purified on a Trisacryl column and the acetyl content of selected fractions determined by the procedure of McComb and McCready[36]. Acetyl groups accounted for 3% of the hemicelluloses of the G1 fraction. The G2 and G3 fractions and the CDTA-soluble fraction contained <0.5% of acetyl.

The function of acetyl substituents in wall polysaccharides is unclear, but there is some evidence[37] that they could impede enzyme hydrolysis of polysaccharide chains and hence play a role in regulating the rate rather than the extent of cell-wall degradation.

Protein–polysaccharide associations. — The OP CWM contained ~1% of protein (Table II), most of which was solubilised with the pectic and hemicellulosic polymers. Although the protein contents of selected fractions varied in the range

0.5–1.6%, the amino acid compositions were in close agreement (Table II). The hydroxyproline content was low and, no doubt, the proteins were functionally distinct from the hydroxyproline-rich, or extensin-type, proteins which are intimately associated with the α-cellulose of some plants[38]. Recently, a proteoglycan with very similar amino acid composition was isolated from the M KOH extract of the depectinated cell walls of runner beans (*Phaseolus coccineus*)[39]. In the present study, essentially the same protein/proteins were found to co-elute with a variety of pectic and hemicellulosic polysaccharides. The procedure for cell-wall preparation was originally designed to remove contaminating cytoplasmic proteins from CWMs[12], but there remains a possibility that these associations are artifacts and not native to the wall.

Phenolic content. — CWM (1 g) was saponified and extracted with ethyl acetate as described by Fry[40]. The ethyl acetate-soluble fraction was examined by t.l.c., g.l.c.[41], and u.v. spectroscopy. No phenolic acids were detected and there were negligible amounts of u.v.(375 nm)-absorbing material. However in t.l.c., an origin-bound band fluoresced moderately under u.v. light and gave a positive reaction to alkaline permanganate. Fry[40] has reported a similar, chromatographically immobile, saponifiable fraction in spinach cell walls, and suggested that it consisted of oxidatively coupled phenols which would be ideally suited for cross-linking wall polysaccharides.

There is now considerable evidence that such phenolic acids as ferulic and coumaric are important structural features of the cell walls of many monocotyledons[42,43], but that in dicotyledons they are restricted to the Caryophyllales to which sugar beet and spinach belong[44]. Ferulic and coumaric acid are absent from the cell walls of kiwifruit, but the initial findings of this study provide tentative evidence for the involvement of more complex phenolic compounds in the cell walls of kiwifruit and perhaps dicotyledons generally.

The present study has shown that the cell walls of 4 tissue zones of kiwifruit at harvest consisted of a range of structurally related polymers, with pectic galactans accounting for most of the wall polysaccharide. The latter were present in each of the solubilised cell-wall fractions and within each fraction exhibited even further heterogeneity. In tomato, Gross[6] contends that at least two galactose-containing polymers are involved in the net loss of wall galactose during fruit ripening. Kiwifruit are known to lose galactose and uronic acid from cell-wall polymers during ripening[7]. The extensive heterogeneity of the pectic galactans in kiwifruit at harvest suggests that, as with tomato, more than one and probably several different polymers are involved in the cell-wall release of galactose and uronic acid during ripening. Current work is aimed at relating these changes to specific wall polysaccharides in order to clarify the sequence of events leading to cell-wall breakdown in post-harvested kiwifruit.

EXPERIMENTAL

General. — The polysaccharide fractions and CWMs were hydrolysed with 2M CF_3CO_2H for 1 h at 121°. The sugar mixtures were converted into alditol acetates[45] for g.l.c. analysis, using column *A*. The CF_3CO_2H-resistant material was dissolved in 72% H_2SO_4 and the carbohydrate content was determined by the phenol–sulphuric acid method[46], using D-glucose as standard. Uronic acid was determined colorimetrically on aqueous solutions of the polysaccharides[47]. Insoluble materials were dissolved in 72% H_2SO_4 and diluted. The degree of esterification was calculated from the methanol content as a molar percentage of the uronic acid content[48].

G.l.c. utilised flame-ionisation detection and *A*, a stainless steel column (2 m x 2 mm i.d.) packed with SP2330 on 100/120 Supelcoport; *B*, as in *A* but with OV-101 on 5% Chromosorb WHP; *C*, a stainless steel column (2.5 m x 2 mm i.d.) packed with 3% of OV-225 on 100/120 Chrom Q; and *D*, a SP-2330 fused-silica capillary column (30 m x 0.32 mm) (Supelco Inc.). The column was maintained at 70° for 4 min, raised to 150° at 25°/min, and then to 220° at 4°/min.

Chromatography solvents were *1*, propyl acetate–formic acid–water (11:5:3); *2*, 1-butanol–acetic acid–water (4:1:5); and *3*, toluene–acetic acid (9:1). T.l.c. was performed on Silica Gel G or MN-300 cellulose. Preparative p.c. used Whatman 3MM paper which had been pre-eluted with 2-propanol, aqueous 10% acetic acid, and water. Sugars were detected with *p*-anisidine-KIO_4[49].

Amino acids were released by hydrolysis with 6M HCl containing 1% of phenol and analysed as their phenyl isothiocyanate derivatives by h.p.l.c.[50]. Protein was estimated by summing the amino acid contents and calcium by atomic absorption after digestion with perchloric acid. Oxalate as its Trisyl derivative was estimated by g.l.c. analysis on column *B*. Starch was determined by using a Starch kit (Boehringer, Mannheim)[51] and, where necessary, starch was removed from polysaccharide fractions by incubating the polymers with a mixture of pullulanase and alpha-amylase at 37°.

Preparation of CWM. — Kiwifruit were harvested in May 1985 at Te Puke, the premier kiwifruit-growing district in New Zealand. Maturity parameters (average of 10 fruit) were: weight, 122 g (range 95–144 g); soluble solids, 8.2 g; and firmness, 6.8 kgf[52]. To minimise the time that cut fruit were exposed to ambient temperature, the 32 fruit were processed within 24 h of harvesting in batches of 4.

The ends of each fruit were discarded, the remainder was peeled and cut in halves (transversely), and core tissue (C) and outer pericarp (OP) were immersed in liquid nitrogen. The remainder of the fruit consisted of the locule walls (LW), the inner pericarp (IP), and the seeds. This tissue was cut into 1–2-cm^3 pieces (90–100 g per 4 fruit) and macerated with a Virtis homogeniser in 70 mL of aqueous 0.5% SDS containing 5mM sodium metabisulphite and 2 drops of octanol. A cutting blade was used but the homogeniser speed was kept low to prevent seed maceration. The homogenate was poured through a Buchner funnel which retained the LW fragments and the seeds. The filtrate contained the IP tissue. The LW tissue was separated from

the seeds by flotation in water, blotted dry, and frozen in liquid nitrogen. The IP tissue was separated from the SDS solution by centrifugation. All solutions and glassware were kept below 4°.

The OP, C, and LW tissues (750, 50 and 58 g) were each ground to a fine powder in a cryo-mill (Franz Morat A70) for 1 min at −196°. The 3 frozen tissues and the wetted IP tissue were sequentially extracted with aqueous 1.5% SDS containing 5mM sodium metabisulphite, water (twice), PAW (2:1:1 v/v), water (twice), and aqueous 90% Me_2SO. For the 750 g of OP tissue, the volumes of solution used were 1 L of SDS, 600 mL of water, 600 mL of PAW, and 2.5 L of aqueous 90% Me_2SO. Similar tissue-to-volumes ratios were used for the other 3 tissues. Temperatures were kept below 5° up to and including the PAW extraction. The tissue suspensions in Me_2SO were stirred overnight at ambient temperature and ultrasonicated for 30 min. The CWMs were recovered, dialysed at 4°, freeze-dried (7.2, 2.0, 0.4, and 0.8 g, from OP, IP, LW, and C, respectively), and stored at −20°. Polymers solubilised by SDS and Me_2SO during purification of the CWM were recovered by dialysis and freeze-dried.

Solubilisation of cell-wall polymers. — CWM from each tissue zone (1 g, OP; 0.7 g, IP; 0.2 g, LW; 0.4 g, C) was suspended (ratio 1:100 w/v) in 0.05MCDTA solution, pH 6.5 (potassium salt), and the suspension was stirred for 6 h at ambient temperature. The residue was resuspended in 0.05M Na_2CO_3 containing 20mM $NaBH_4$, and stirred for 20 h at 2° and then for 2 h at room temperature. The residue was then stirred for 18 h at room temperature in 6M GTC, and finally in 4M KOH containing 20mM $NaBH_4$ under nitrogen for 2 h. Each supernatant solution was filtered (those containing alkali were neutralised), concentrated, dialysed, and freeze-dried.

Ion-exchange chromatography of OP polymers. — The CDTA-, Na_2CO_3-, GTC-, and KOH-soluble polymers (205, 213, 220, 328 mg) were each dissolved in 0.05M phosphate buffer (pH 6.5) and fractionated on a column (2.5 x 20 cm) of DEAE Trisacryl M by sequential elution with buffer and buffer containing 0.125M, 0.25M, and 0.5M NaCl[11].

Carboxyl-reduction of the glycosyluronic acid residues. — The carboxyl groups of the uronic acid residues of selected polymers were reduced to the corresponding 6,6-dideuterio-glycosyl residues by a variation of the method of Taylor and Conrad[53].

Polysaccharide (20 mg) was weighed into a beaker containing 4.8 g of urea and a magnetic stirrer, and kept overnight under vacuum over P_2O_5 at 40°. D_2O (8 mL) was added and the contents were stirred untill the polysaccharide dissolved (1–2 h). The pH was adjusted to 4.75 with 0.1M DCl (prepared from HCl and D_2O) and maintained at this pH while 1-cyclohexyl-3-(2-morpholinoethyl)carbodi-imide metho-*p*-toluensulfonate (400 mg) was added during 30–35 min. The solution was stirred for a further 30 min, and a solution (200 μL) containing 0.1 g of $NaBD_4$, 0.8 mL of D_2O, and 0.6 mL of 4M DCl was added. The pH was adjusted to 7.0 and maintained manually (a pH stat was unnecessary) at 7.0–7.5 with 4M DCl while a solution of 500

mg of $NaBD_4$ in 4 mL of D_2O was added during 30–35 min. The $NaBD_4$ and DCl were initially added in 10-μL aliquots and then 100 μL as the buffering capacity of the reagents took effect. After the last of the $NaBD_4$ had been added, the solution was allowed to stand at pH 7.5 for 1–2 h, then acidified with HCl (pH 4), dialysed for 2 days, and freeze-dried. The reduction was repeated for each sample.

The amounts of dideuterio-labelled hexose and unlabelled hexose, resulting from the reduction with deuterated reagents, were determined by mass spectrometry.

Methylation analysis. — Reduced polysaccharides (5 mg) were methylated by a modification of the Hakomori method[54]. G.l.c.-m.s. of the partially methylated alditol acetates was accomplished with a VG 70-SE instrument (70 eV) with direct coupling between the g.l.c. (column *D*) and mass spectrometer. The molar response factors of Sweet *et al.*[55] were used.

Identification of glycosyluronic acids. — A modification of the method of York *et al.*[31] was used.

The polysaccharide (2–20 mg) was heated in 1–2 mL of 0.5M CF_3CO_2H for 1 h at 121°. Solvent was removed by a stream of air, and the residue was dried down twice after addition of 0.5 mL of 2-propanol and 0.3 mL of M NH_4OH. A solution of the residue in 1 mL of H_2O was eluted through a column of QAE Sephadex in the formate form. The neutral sugars were eluted with H_2O and the acidic components recovered in 30 mL of aqueous 5% HCOOH. The acid fraction was dried down from H_2O and M NH_4OH, and the methyl ester methyl glycosides were prepared as described[31]. Silylation was done in either Trisil Z or MSTFA, and both reaction mixtures were used directly for g.l.c. The latter reagent gave a much narrower solvent peak than Trisil Z but increased the anomerisation of the sugars.

Isolation and determination of apiose and KDO in the OP CWM. — (*a*) *Apiose.* The CDTA, Na_2CO_3, and α-cellulose fractions (130, 170, and 500 mg) were each suspended in 30 mL of 0.4M CF_3CO_2H and heated for 30 min at 121°. Each hydrolysate was filtered, dried down from H_2O several times, and chromatographed for 36 h on 2 sheets of 3MM paper with solvent *2*. Standard apiose prepared from di-*O*-isopropylidine-D-apiose was used as a marker along one edge. Apiose was located with a benzidine–trichloroacetic acid spray[56] which reacts with the sugars to give an intense white u.v. fluorescence. It was eluted from the paper with aqueous 10% 2-propanol and re-chromatographed on a single sheet of paper. The recovered apiose was quantified by g.l.c. of its alditol acetate on column *A*.

(*b*) *KDO.* The KDO contents of the CDTA, Na_2CO_3, and α-cellulose fractions were determined colorimetrically by the method of Karkhanis *et al.*[33].

Qualitative evidence for the presence of KDO in the CWM of the OP was obtained by the modified method of York *et al.*[34]. CWM (1 g) was suspended in water for 6 h. The aqueous supernatant solution was discarded and the residue was resuspended in M acetic acid for 18 h at 50°. The viscous supernatant solution was dialysed against 1 L of H_2O for 4 h and then overnight against another 1 L of H_2O. The combined dialysates (2 L) were dried down, and a solution of each residue in H_2O was dried to remove traces of acid. A solution of each residue in M NH_4OH was

dried down and a solution of the residue passed through a column (1 x 5 cm) of QAE Sephadex ($HCOO^-$ form). The acidic fraction was recovered as described above, converted into trimethylsilylated methyl glycosides, and examined by g.l.c. (column *B*).

Phenolic content of OP CWM. — CWM (1 g) was suspended in 100 mL of M NaOH for 18 h at 20° under nitrogen (40). The saponified products were acidified to pH 2 with H_3PO_4 and partitioned against ethyl acetate. The organic phase was analysed for its phenolic content by u.v. spectroscopy, t.l.c. (silica gel, solvent 0), and g.l.c.[41] (column *C*).

ACKNOWLEDGMENTS

We thank E. A. MacRae for supplying the fruit and 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 A. R. Ferguson, *Hortic. Rev.*, 6 (1984) 1–64.
2 P. R. Crookes and D. Grierson, *Plant Physiol.*, 72 (1983) 1088–1093.
3 R. Ben-Arie, N. Kislev, and C. Frenkel, *Plant Physiol.*, 64 (1979) 197–202.
4 A. E. Ahmed and J. M. Labavitch, *Plant Physiol.*, 65 (1980) 1009–1013.
5 A. E. Ahmed and J. M. Labavitch, *Plant Physiol.*, 65 (1980) 1014–1016.
6 K. C. Gross, *Physiol. Plant.*, 62 (1984) 25–32.
7 M. L. Arpaia, J. M. Labavitch, C. Greve, and A. A. Kader, *J. Am. Soc. Hortic. Sci.*, 112 (1987) 474–481.
8 E. A. MacRae, J. H. Bowen, M. G. H. Stec, and J. A. Hodgson, *J. Sci. Food. Agric.*, submitted.
9 M. A. Hopping, *N.Z. J. Bot.*, 14 (1976) 63–68.
10 B. J. H. Stevens and R. R. Selvendran, *Carbohydr. Res.*, 135 (1984) 155–166.
11 R. J. Redgwell and R. R. Selvendran, *Carbohydr. Res.*, 157 (1986) 183–199.
12 R. R. Selvendran and M. S. Dupont, *Cereal Chem.*, 57 (1980) 278–283.
13 R. J. Redgwell, *Phytochemistry*, 22 (1983) 951–956.
14 R. J. Redgwell, M. A. O'Neill, R. R. Selvendran, and K. J. Parsley, *Carbohydr. Res.*, 153 (1986) 97–106.
15 R. J. Redgwell, M. A. O'Neill, R. R. Selvendran, and K. J. Parsley, *Carbohydr. Res.*, 153 (1986) 107–118.
16 I. A. Pearl, *The Chemistry of Lignin*, Dekker, New York, 1967, p. 39.
17 D. Strauss, *Dtsch. Lebensm.-Rundsch.*, 66 (1970) 260–263.
18 M. C. Jarvis, *Planta*, 154 (1982) 344–346.
19 M. C. Jarvis, M. A. Hall, D. R. Threlfall, and J. Friend, *Planta*, 152 (1981) 93–100.
20 J. A. Monroe, R. W. Bailey, and D. Penny, *Phytochemistry*, 15 (1976) 175–181.
21 T. Hayashi, M. P. F. Marsden, and D. P. Delmer, *Plant Physiol.*, 83 (1987) 384–389.
22 S. C. Fry, *Annu. Rev. Plant Physiol.*, 37 (1986) 165–186.
23 Y. Y. Leshem, A. H. Halevy, and C. Frenkel, *Processes and Control of Plant Senescence*, Elsevier, Amsterdam, 1986, pp. 170–178.
24 J. M. Lau, M. McNeil, A. G. Darvill, and P. Albersheim, *Carbohydr. Res.*, 137 (1985) 111–125.
25 K. W. Talmadge, K. Keegstra, W. D. Bauer, and P. Albersheim, *Plant Physiol.*, 51 (1973) 158–173.

26 G. O. ASPINALL, IN W. PIGMAN AND D. HORTON (EDS.), *The Carbohydrates,* Vol. IIB, Academic Press, New York, 1970, pp. 515–536.
27 J. E. DARVILL, M. MCNEIL, A. G. DARVILL, AND P. ALBERSHEIM *Plant Physiol.,* 66 (1980) 1135–1139.
28 N. C. CARPITA AND D. WHITTERN, *Carbohydr. Res.,* 146 (1986) 129–140.
29 B. J. H. STEVENS AND R. R. SELVENDRAN, *Phytochemistry,* 23 (1984) 339–347.
30 B. J. H. STEVENS AND R. R. SELVENDRAN, *Carbohydr. Res.,* 135 (1984) 155–166.
31 W. S. YORK, A. G. DARVILL, M. MCNEIL, T. T. STEVENSON, AND P. ALBERSHEIM, *Methods Enzymol.,* 118 (1985) 3–40.
32 A. G. DARVILL, M. MCNEIL, AND P. ALBERSHEIM, *Plant Physiol.,* 62 (1978) 418–422.
33 Y. D. KARKHANIS, J. Y. ZELTNER, J. J. JACKSON, AND D. J. CARLO, *Anal. Biochem.,* 85 (1978) 595–601.
34 W. S. YORK, A. G. DARVILL, M. MCNEIL, AND P. ALBERSHEIM, *Carbohydr. Res.,* 138 (1985) 109–126.
35 L. D. MELTON, M. MCNEIL, A. G. DARVILL, P. ALBERSHEIM, AND A. DELL, *Carbohydr. Res.,* 146 (1986) 279–305.
36 E. A. MCCOMB AND R. M. MCCREADY, *Anal. Chem.,* 29 (1957) 819–825.
37 T. M. WOOD AND S. I. MACRAE, *Phytochemistry,* 25 (1986) 1053–1055.
38 D. T. A. LAMPORT AND D. H. MILLER, *Plant Physiol.,* 48 (1971) 454–456.
39 M. A. O'NEIL AND R. R. SELVENDRAN, *Biochem. J.,* 227 (1985) 475–481.
40 S. C. FRY, *Phytochemistry,* 23 (1984) 50–64.
41 F. GUBLER AND A. E. ASHFORD, *Aust. J. Plant Physiol.,* 12 (1985) 297–305.
42 P. J. HARRIS AND R. D. HARTLEY, *Biochem. Syst. Ecol.,* 8 (1980) 153–160.
43 I. MULLER-HARVEY AND R. D. HARTLEY, *Carbohydr. Res.,* 148 (1986) 71–85.
44 F. M. ROMBOUTS AND J. F. THIBAULT, *Carbohydr. Res.,* 154 (1986) 177–187.
45 P. ALBERSHEIM, D. J. NEVINS, P. D. ENGLISH, AND A. KARR, *Carbohydr. Res.,* 5 (1967) 340–345.
46 M. DUBOIS, K. O. GILLES, J. K. HAMILTON, P. A. REBERS, AND F. SMITH, *Anal. Chem.,* 28 (1956) 350–356.
47 N. BLUMENKRANTZ AND G. ASBOE-HANSEN, *Anal. Biochem.,* 54 (1973) 484–489.
48 M. P. FILIPPOV AND V. I. KUZMINOV, *Zh. Anal. Khim.,* 26 (1971) 143–146.
49 R. C. BEAN AND G. G. PORTER, *Anal. Chem., 29 (1957) 1927–1931.*
50 R. L. HEINRIKSON AND S. C. MEREDITH, *Anal. Biochem.,* 136 (1984) 65–71.
51 J. H. BOWEN, E. A. MACRAE, AND R. G. LOWE, *Sci. Hortic.,* in press.
52 N. LALLU, A. M. SEARLE, AND E. A. MACRAE, *J. Sci. Food Agric.,* submitted.
53 R. L. TAYLOR AND H. E. CONRAD, *Biochemistry,* 11 (1972) 1383–1388.
54 S. G. RING AND R. R. SELVENDRAN, *Phytochemistry,* 17 (1978) 745–752.
55 D. P. SWEET, R. H. SHAPIRO, AND P. ALBERSHEIM, *Carbohydr. Res.,* 40 (1975) 217–225.
56 J. S. D. BACON AND J. EDLEMAN, *Biochem. J.,* 48 (1951) 114–118.