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CELL WALL SYNTHESIS IN KIWIFRUIT FOLLOWING POSTHARVEST ETHYLENE TREATMENT

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Key Word Index—*Actinidia deliciosa*; Actinidaceae; kiwifruit; fruit discs; cell wall polysaccharides; fruit-ripening; cell wall synthesis; ethylene treatment.

Abstract—Kiwifruits (*Actinidia deliciosa*) labelled by exposure to $^{14}CO_2$ were examined for their capacity to synthesize cell wall polysaccharides immediately after harvest and during ripening. The biosynthetic ability of intact kiwifruit was compared with that of the corresponding fruit discs. Discs were treated in two ways. They were either allowed to soften over 72 hr after excision from $^{14}CO_2$-labelled intact fruit or they were excised from unlabelled fruit and treated with ^{14}C-glucose as an exogenous precursor. Intact fruit showed incorporation of radioactivity into cell wall material during ripening, but over 90% of radioactivity was in cell wall-associated protein. Radioactivity was detected in galactose, mannose and glucose from the cell wall material, but there was no ripening-related enhancement of the labelled components. Similar results were obtained with $^{14}CO_2$-labelled discs. In contrast, discs labelled with ^{14}C-glucose were able to incorporate a greater proportion of the radioactivity into cell wall polysaccharide with extensive labelling of all monosaccharide components of the cell wall. The amount of incorporated label increased over 72 hr, but there was no evidence for incorporation into different polysaccharides between 24 and 72 hr as discs softened. It is concluded that although mature and ripe kiwifruit are able to synthesize cell wall polymers during ripening it does not appear to be related to the softening process.

INTRODUCTION

While cell wall degradation in ripening fruit is regarded as a factor leading to textural changes and fruit-softening, the role of cell wall synthetic processes has rarely been considered. In excised cortical tissue from ripe apple, ^{14}C-methionine was incorporated into methyl ester groups of wall pectin [1], but in strawberry, incorporation of ^{14}C-glucose into cell wall polysaccharides ceased at the onset of ripening [2]. The failure to establish a causal relationship between softening and the degradation of a specific cell wall polysaccharide or the action of any wall degrading enzyme has led to the speculation that *de novo* synthesis of cell wall polysaccharides during ripening may have a role in fruit-softening and has prompted a reassessment of cell wall synthetic processes in ripening fruit.

Mitcham *et al.* [3] reported that ^{14}C-sucrose injected into the pedicel of ripe tomato fruits attached to the plant was incorporated into fractions solubilized from cell wall material (CWM), but did not report whether the label was incorporated into monosaccharide residues of the polysaccharides. Another study [4] used D-[U-^{13}C]glucose to show that label was incorporated into polysaccharides of pericarp discs allowed to ripen in culture, after excision from mature green tomato fruits. There is no evidence that in intact fruit, postharvest ripening and softening is accompained by cell wall synthesis.

In the present study, kiwifruit were harvested, labelled with $^{14}CO_2$ and treated with ethylene to accelerate ripening. The distribution of ^{14}C was determined in the pectic and hemicellulosic polysaccharides and cell wall-associated proteins from outer pericarp (OP) tissue at three ripening stages. Parallel experiments were done with kiwifruit discs prepared from $^{14}CO_2$-labelled fruit and with discs treated with ^{14}C-glucose in order to compare labelling patterns in the *in vivo* and *in vitro* systems.

RESULTS AND DISCUSSION

Distribution of ^{14}C in tissue zones and primary metabolites

Net fixation of $^{14}CO_2$ into kiwifruit occurs by a combination of the C3 pathway of photosynthesis and the C4 CAM pathway (MacRae *et al.*, unpublished data). Radioactivity was found in all tissue zones (Table 1). In unripe fruit immediately after labelling, the highest incorporation appeared in the skin (44.7%) and OP (39.7%), but moderate amounts were located in the inner pericarp (IP) (14.3%) and core tissue (1.2%).

To show that $^{14}CO_2$ was incorporated into primary metabolites suitable for cell wall synthesis, the distribution of radioactivity was determined among the neutral sugar, organic acid and amino acid components of the methanol–chloroform–H_2O (MCW)-soluble fractions in

Table 1. Changes to fruit firmness, SSC and amount of radioactivity among tissue zones of kiwifruit at different stages of ripeness after application of $^{14}CO_2$ to intact fruit

Ripening stage	Fruit firmness(N)	SSC (%)	Radioactivity (cpm × 10^{-4} g^{-1} fr. wt) Skin	OP	IP	Core
Unripe	88.0 ± 5.0	10.8 ± 0.6	279.1 ± 46.1	67.2 ± 12.1	20.7 ± 1.7	10.3 ± 0.9
Control	57.0 ± 9.0	11.9 ± 0.6	136.6 ± 4.2	63.2 ± 2.2	29.7 ± 0.6	19.0 ± 1.9
Ripe	13.0 ± 2.0	15.0 ± 0.7	152.6 ± 5.9	61.8 ± 2.4	23.6 ± 2.4	12.2 ± 2.1

Values are means ± SE of three fruits.

Table 2. Distribution of radioactivity among neutral sugar, organic acid and amino acid fractions and among the sucrose, glucose and fructose of the neutral sugar fraction in the MCW-soluble extract of OP at three ripening stages following $^{14}CO_2$-labelling of kiwifruit

	Percentage total radioactivity		
	Unripe	Control	Ripe
Sugar	79.9	89.1	90.6
Organic acid	15.5	9.3	7.9
Amino acid	3.8	1.2	1.1
Sucrose	82.1	18.0	28.9
Glucose	9.7	42.4	39.5
Fructose	8.2	39.5	35.6

the OP of unripe, ripe and control fruits (Table 2). In unripe fruit, 80% of the radioactivity was in the neutral sugar fraction, 15% in the organic acid and 3.8% in the amino acid fraction. Of the radioactivity in the neutral sugars, 82.1% was in sucrose in unripe fruit, a natural precursor for cell wall synthesis (Table 2).

Distribution of ^{14}C among CWMs and water-soluble polymers of intact fruit

Following a postharvest ethylene treatment, labelled fruit softened (Table 1) and yields of CWM and water-soluble polymers from the OP at each ripening stage (Table 3) were consistent with previous studies which reported a solubilization of pectic polysaccharides during kiwifruit-ripening [5]. Control fruit which softened only slightly showed much less pectin solubilization.

There was a three-fold increase in total radioactivity and specific activity of CWM of ripe fruit compared to levels in unripe fruit. Control fruit showed a two-fold increase for the same measurements (Table 3). CWMs were fractionated into CDTA-, Na_2CO_3-, KOH-soluble and residue fractions, and each fraction was subjected to trifluoroacetic acid (TFA) hydrolysis and ion-exchange chromatography to determine the relative amounts of radioactivity in the neutral sugar, acidic and protein fractions (Table 4). Nearly all radioactivity was located in proteins. It is likely that some of the protein was intracellular protein precipitated on the CWM during its preparation. This was supported by analysis of the ninhydrin-positive products of TFA hydrolysis, which showed an identical composition for each cell wall fraction. The KOH-soluble fraction showed a moderate incorporation of radioactivity into the neutral sugar fraction of unripe fruit (20.5%), but this did not increase during ripening. There was no ripening-related enhancement of radioactivity into non-protein cell wall components.

Water-soluble polymers contained pectic polysaccharides solubilized during ripening [5, 6] and were higher in ripe fruit than unripe fruit both in weight and total radioactivity. The specific activity of the water-soluble polymers decreased during ripening, probably because the newly solubilized pectic polysaccharides were diluting the radioactivity of the original unripe fruit fraction. Following TFA hydrolysis, the distribution of radioactivity in the neutral, acidic and protein fractions was 27, 39 and 34%, respectively. The proportions in the ripe fruit were 17, 17 and 66%, respectively, demonstrating that the increase in radioactivity during ripening could be accounted for by the protein component.

^{14}C among CWMs and water-soluble polymers of fruit discs

Kiwifruit discs showed no browning or outward sign of deterioration during the 72 hr after excision. Studies in our laboratory [7] indicate that cells from freshly harvested kiwifruit rupture when incubated in solutions containing less than 0.4 M mannitol. The solution chosen contained the lowest concentration of mannitol that would maintain cell turgidity without causing rupture.

Discs from $^{14}CO_2$-labelled intact fruit

Discs were excised from $^{14}CO_2$-labelled fruit and allowed to soften in culture at 20°. Over 72 hr, the discs

Table 3. Radioactivity and specific activity of CWMs and water-soluble polymers prepared from $^{14}CO_2$-labelled intact fruit, from excised discs of $^{14}CO_2$ labelled fruit, and from excised discs subsequently labelled with ^{14}C-glucose

Treatment and sampling time	Polymers (mg g^{-1} fresh wt)	Radioactivity (cpm g^{-1} fresh wt)	Specific activity (cpm mg^{-1} polymer)
$^{14}CO_2$-labelled intact fruit			
Water-soluble polymer			
Unripe	0.6 ± 0.1	382 ± 60	602 ± 163
Control	1.0 ± 0.1	671 ± 74	531 ± 54
Ripe	3.3 ± 0.2	545 ± 42	201 ± 19
Cell wall material			
Unripe	18.7 ± 1.9	5848 ± 1137	306 ± 34
Control	15.8 ± 1.4	9802 ± 988	626 ± 73
Ripe	16.6 ± 0.4	15837 ± 900	950 ± 39
$^{14}CO_2$-labelled discs			
Water-soluble polymer			
0	1.0 ± 0.1	166 ± 31	160 ± 30
24 hr	1.7 ± 0.1	279 ± 9	161 ± 8
72 hr	2.6 ± 0.1	416 ± 23	162 ± 6
Cell wall material			
0	14.9 ± 1.7	4826 ± 543	323 ± 1
24 hr	13.3 ± 1.7	5139 ± 495	388 ± 14
72 hr	11.3 ± 1.2	5778 ± 289	516 ± 33
^{14}C-glucose labelled discs			
Water-soluble polymer			
0	1.0 ± 0.1	—	—
24 hr	1.4 ± 0.1	6140 ± 150	4300 ± 54
72 hr	2.0 ± 0.2	7980 ± 620	4200 ± 30
Cell wall material			
0	13.7 ± 1.1	—	—
24 hr	12.9 ± 1.7	29200 ± 2520	2300 ± 170
72 hr	11.2 ± 2.2	52000 ± 2200	4700 ± 240

Values are mean ± SE of three samples.

softened appreciably [48, 13 and 2.3 newtons (N) at time 0, 24 and 72 hr, respectively]. Ripening of kiwifruit discs *in vitro* as it relates to cell wall modification has been shown to closely resemble the pattern in intact fruit [8]. In the present study, changes in distribution of radioactivity into water-soluble and CWM fractions paralleled changes in the intact fruit (Table 3). The pattern of incorporation of radioactivity into the CDTA-, Na_2CO_3-, KOH-soluble and residue fractions was similar to the intact fruit, with between 85 and 95% located in the protein fraction (Table 4).

Discs supplied with ^{14}C-glucose

Discs were excised from kiwifruit and ^{14}C-[U]glucose applied to the surface of each disc. In contrast to the $^{14}CO_2$-labelled intact fruit and discs, ^{14}C-glucose treated discs were able to incorporate a higher proportion of radioactivity into the neutral and acidic fractions of each cell wall fraction (Table 4). In the water-soluble polymers, the distribution of radioactivity among the neutral, acidic and protein fractions was 69, 21 and 21%, respectively.

Incorporation of ^{14}C into monosaccharide residue of cell wall polysaccharides

Between 5 and 25% of the radioactivity solubilized from the CWMs was distributed between the acidic (galacturonosyl residues) and neutral cell wall monosaccharides. To determine whether radioactivity was incorporated into cell-wall derived monosaccharides, selected fractions were subjected to TLC and paper chromatography (PC), and monosaccharides were identified by comparison with standard sugars, following autoradiography and chemical localization using *p*-anisidine.

$^{14}CO_2$-labelled intact fruit and discs

In intact fruit and *in vivo* labelled discs, the low amount of radioactivity in acidic and neutral fractions meant that after extended autoradiography only faint images were detected on the autoradiographs. No radioactivity could be detected in galacturonic acid or its oligomers following TLC of acid hydrolysates of CDTA- and Na_2CO_3-soluble fractions. Radioactive galactose and mannose were detected in the Na_2CO_3-soluble fraction of ripe and control fruit, as was glucose in the KOH-soluble fraction

Table 4. Percentage distribution of radioactivity incorporated into neutral, acidic and protein components of cell wall fractions prepared from $^{14}CO_2$-labelled intact kiwifruit at different ripening stages (unripe, control and ripe) and from kiwifruit discs ($^{14}CO_2$ and ^{14}C-glucose labelled) 72 hr after excision

Treatment and cell wall fraction	Percentage total radioactivity		
	Neutral sugar	Acidic	Protein
CDTA-soluble			
Unripe	1.9	3.6	94.4
Control	0.7	1.8	97.6
Ripe	0.9	4.6	94.5
$^{14}CO_2$ disc	1.8	2.6	95.6
^{14}C-glucose disc	19.4	11.9	68.6
Na_2CO_3-soluble			
Unripe	3.3	4.3	92.3
Control	1.6	2.1	96.3
Ripe	4.1	2.8	93.0
$^{14}CO_2$ disc	5.2	4.5	90.3
^{14}C-Glucose disc	28.0	13.6	58.2
KOH-soluble			
Unripe	20.5	3.5	76.0
Control	20.9	2.4	76.7
Ripe	9.7	3.5	86.8
$^{14}CO_2$ disc	10.5	4.2	85.2
^{14}C-glucose disc	34.7	6.4	58.9
Residue			
Unripe	9.8	6.9	83.3
Control	10.5	6.3	83.1
Ripe	6.8	5.2	88.0
$^{14}CO_2$ disc	5.8	7.3	86.9
^{14}C-glucose disc	37.7	14.0	48.3

of all three ripening stages of intact fruit. Mannose was not detected by *p*-anisidine, indicating that its specific activity was higher than that of other radiolabelled sugars. Mannose was possibly derived from the carbohydrate moiety of glycosylated proteins, which may have a higher specific activity. It cannot be discounted that galactose and glucose were also constituents of glycosylated proteins and were merely co-incident on the TLC plate with the same monosaccharides present in the cell wall polysaccharides. As fruit softened, there was no apparent change in the labelling pattern of the radioactive monosaccharides that would indicate a ripening-related synthesis of cell wall polysaccharides.

^{14}C-glucose-labelled discs

In ^{14}C-glucose labelled discs, incorporation of radioactivity was detected among all monosaccharide residues of the cell wall polysaccharides (Fig. 1). A study with tomato discs labelled with ^{13}C-glucose reported extensive incorporation of label into neutral glycosyl residues of chelator-soluble pectin, but not into galacturonosyl residues of the pectin fraction [4]. In ^{14}C-glucose labelled kiwifruit discs, there was incorporation of radioactivity into galacturonoyl residues of the CDTA-, Na_2CO_3-, KOH-soluble and residue fractions (Fig. 1). Despite the fact that galacturonic acid accounted for 89 and 82% of the CDTA- and Na_2CO_3-soluble fractions, respectively, there was preferential incorporation into the neutral residues (Fig. 1, Table 5).

It could be argued that the altered composition of the radioactive polysaccharides may reflect different specific activities of pools of UDP-sugars derived from a single radioactive precursor. However, none of the UDP-sugar interconversions are significantly remote for this to be a likely factor in the labelling patterns. Although there was a continuous increase in the total amount of radioactivity fixed by ^{14}C-glucose labelled discs between 24 and 72 hr, there was no change in labelling pattern among individual monosaccharide residues (data not given).

In the KOH-soluble fraction, the hemicellulosic sugars, glucose, xylose, mannose and fucose were radioactive, but there were differences between the radioactive composition and the monosaccharide composition as determined by GC of the alditol acetates (Table 5). Amounts of glucose and xylose were present in a ratio of 1.4:1, whereas the ^{14}C-label ratio in each residue was 3.3:1. Rhamnose, arabinose and galactose were all present in greater proportions as radioactive components compared to the proportions found by compositional analysis. These residues were probably associated with pectic polysaccharides, which co-extract with the hemicelluloses in 4 M KOH [6].

The extensive synthesis of cell wall polysaccharides in the glucose-treated discs compared to the intact fruit may be a consequence of two factors—the exogenous precursor and a wound response of freshly cut discs.

Total radioactivity taken up by discs as ^{14}C-glucose was higher than could be assimilated as $^{14}CO_2$ by intact fruit and therefore the specific activity of CWM from the glucose-labelled discs was five-fold higher than in the $^{14}CO_2$-labelled fruit (Table 3). However, this did not explain the different proportions of radioactivity allocated to the protein and polysaccharide fractions in the two systems. Also, the specific activities of CDTA-soluble fractions from intact fruit and glucose labelled discs were similar (intact ripe fruit: 1660 cpm mg^{-1}; glucose discs 72 hr: 2120 cpm mg^{-1}).

A well documented consequence of wounding in plant tissue is enhanced synthesis of callose [9], but it is possible that it may induce a more general synthesis of matrix polysaccharides. In the present study, the wound response should have taken place in discs cut from $^{14}CO_2$-labelled intact fruit and yet there was no enhanced labelling of wall polysaccharides in these discs compared to intact fruit. However, the wound response is probably a surface phenomenon affecting only those cells on the cut disc surface on to which the ^{14}C-glucose was applied. The *in vivo*-labelled discs had the substrate distributed throughout the thickness of the disc and, therefore, cell wall synthesis induced by wounding may have been a small fraction of the total tissue response.

The present study has demonstrated that in ethylene-treated kiwifruit there were low levels of cell wall polysaccharide synthesis during ripening, but no evidence for

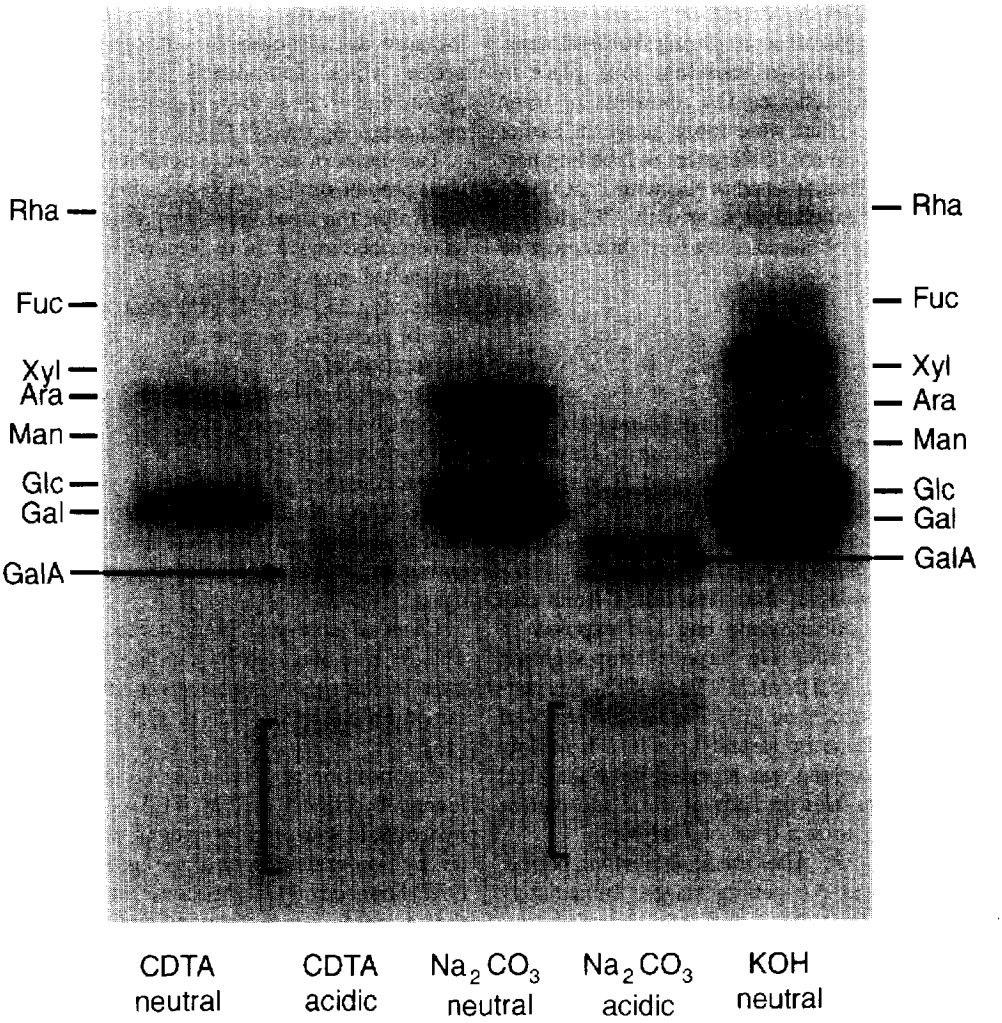

Fig. 1. Autoradiograph of TLC of TFA hydrolysates of cell wall fractions prepared from kiwifruit discs labelled with ^{14}C-glucose. GalA: galacturonic acid. Bracketed bands in the CDTA and Na_2CO_3 acidic fractions are oligomers of galacturonic acid. Unmarked radioactive bands were not identified. Cell wall fractions were hydrolysed in 2 M TFA. Hydrolysates were separated by ion-exchange chromatography into neutral and acidic carbohydrate fractions and aliquots chromatographed by TLC as described in the Experimental.

Table 5. Monosaccharide composition of CDTA-, Na_2CO_3- and KOH-soluble fractions and percentage distribution of radioactivity among each sugar residue. Pericarp discs were labelled with ^{14}C-glucose for 72 hr and CDTA-, Na_2CO_3- and KOH-soluble fractions isolated as described in the Experimental. Quantitation was done by GC and ^{14}C distribution determined by liquid scintillation counting of individual radioactive sugars located by autoradiography following TLC and PC

	Monosaccharide (mol %)							
Fraction	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	Uronic acid
CDTA-soluble	2.0 (4.0)	0.2 (2.9)	1.9 (19.6)	1.0 (1.4)	0.1 (7.3)	5.7 (30.2)	— —	89.2 (33.7)
Na_2CO_3-soluble	3.3 (8.2)	0.1 (2.4)	3.1 (18.4)	0.8 (2.0)	0.1 (8.1)	11.0 (38.4)	— —	81.7 (22.5)
KOH-soluble	1.0 (2.5)	1.4 (6.4)	1.5 (6.5)	27.0 (13.0)	6.4 (5.1)	9.8 (20.9)	38.5 (43.1)	15.3 (2.4)

Values in parentheses are the percentage distribution of radioactivity among individual residues.

any ripening-related enhancement or differential synthesis of cell wall polysaccharide during fruit-softening. The amount of cell wall synthesis that took place in intact fruits probably reflected the remnants of some constitutive processes that were more active in earlier phases of fruit development. Differences in labelling patterns between intact fruit labelled *in vivo* with $^{14}CO_2$ and freshly excised discs labelled *in vitro* with ^{14}C-glucose, suggested that results obtained using fruit discs must be treated with caution.

EXPERIMENTAL

Plant material. Kiwifruit (*Actinidia deliciosa* [A. Chev.] C. F. Liang et A. R. Ferguson var. *deliciosa* cv. Hayward) of uniform size (110–130 g) were harvested 21 May 1992, from the Kumeu Research Orchard, Auckland, New Zealand, while still hard and unripe [% soluble solids concn (SSC) 10.8; firmness 88 N].

$^{14}CO_2$-labelling of intact fruit. Harvested fruits (30) were enclosed in a polyethylene bag and exposed to 37 MBq of $^{14}CO_2$ under Hg vapour lamp lighting (93 μmol m^{-2} sec^{-1}) for 2 hr at 20°. The $^{14}CO_2$ was generated as previously described [10]. Fruits were removed from the bag in a fume hood and kept in a fruit tray overnight at 20°. Labelling was repeated in an identical manner on the same fruit on each of the 2 subsequent days. Thus, labelling took a total of 3 days.

Sampling of intact fruit. After the final labelling period, fruit were sampled at 3 ripening stages, immediately after labelling (unripe fruit), 7 days after labelled fruit were exposed to ethylene (ripe fruit [5]) and 7 days after labelled fruit were held at 20° without an ethylene treatment when they were still relatively unripe (control). Triplicate samples were taken (one fruit per sample) and each fruit measured for SSC and firmness [11], peeled and the OP tissue cut into 2-cm^3 pieces (total *ca* 35 g). Samples were frozen in liquid N_2 and immersed in 100 ml MCW (20:5:1). Tissue suspensions were stored at 4° pending extraction.

To determine the total amount of radioactivity in the skin, OP, IP and core zones of the fruit, respective tissues were excised from individual fruit, homogenized to a uniform suspension in H_2O (200 ml) and the radioactivity in 1-ml aliquots determined by liquid scintillation counting.

Prepn and labelling of fruit discs. Whole fruit were surface sterilized (2.5% hypochlorite, 10 min) and rinsed well in sterile distilled H_2O in a laminar flow cabinet where all subsequent operations were done. Discs (0.7 cm × 4 mm) were cut from the OP and suspended with stirring in 10 ml 0.4 M mannitol for 3 min. Discs were washed twice more with fresh 0.4 M mannitol and blotted, and 25 discs were placed in a Petri dish on 0.7% agar containing 0.4 M mannitol. Dishes were incubated at 25° and 3 dish replicates (25 discs per dish) taken at time 0, 24 and 72 hr after disc excision from the fruit. Tissue was frozen in liquid N_2 and stored at −80° pending extraction, which was done as described for intact fruit. An Instron Model 4301 materials testing machine (Instron, Canton, MA, U.S.A.) was used to measure disc firmness by compression using a 13 mm probe which compressed the discs at a rate of 20 mm min^{-1}. Peak force was recorded for 8 fruit discs at each sampling time.

Two types of expt were done with discs. In one, discs were prepd from the $^{14}CO_2$-labelled intact fruit (immediately after the third labelling period) and samples taken as described above. In the second, discs were prepd from unlabelled intact fruit and 10 μl of 1 mM glucose soln containing 37 kBq ^{14}C-[U]-glucose applied to the surface of each disc. Samples were taken 24 and 72 hr after application of the ^{14}C-glucose.

Extraction and sepn of labelled products. Tissue–MCW suspensions were sepd into MCW-soluble compounds, H_2O-soluble polymers and CWMs according to ref. [12].

Fractionation of CWMs. CWMs were fractionated into CDTA-, Na_2CO_3-, KOH-soluble and residue frs as described in ref. [6]. Polysaccharides solubilized in each extractant, and the final residue were dialysed and freeze-dried.

Chemical analyses. Sugar composition of MCW-soluble frs was analysed by capillary GC of TMS derivatives of the sugars contained in the neutral fr. not retained on either SP-C-25 or QAE-A-25 ion-exchangers [13].

Composition of polymer frs (10 mg) was analysed following hydrolysis in 2 M TFA (1 ml, 121°, 1 hr). The hydrolysate was sepd into sugar, acidic and amino acid frs by ion-exchange chromatography using SP- and QAE-Sephadex [13]. Monosaccharide composition of the neutral fr. was determined by capillary GC of alditol acetates [6]. Radioactive components were located by autoradiography on TLC (MN-300 cellulose) plates and PC following chromatography using *n*-BuOH–HOAc–H_2O (12:3:5) and EtOAc pyridine–H_2O (8:2:1) in the same dimension. Radioactivity of sepd bands was assayed by liquid scintillation counting [14]. Monosaccharides were detected using *p*-anisidine [15] followed by heating at 110° for 10 min. Uronic acid was determined by the method of ref. [16].

The neutral fr. contained the galactosyl and arabinosyl residues, which occurred as side-chains attached to the acidic backbone of the pectic polysaccharides, as well as rhamnosyl residues from the backbone. In addition, it contained all the monosaccharides derived from the hemicellulosic polysaccharides, such as xyloglucan. The acidic fr. contained mostly monogalacturonic acid and oligomers of galacturonic acid derived from the pectic backbone.

TFA (2 M) hydrolysed the protein of the polymer frs to a mixt. of amino acids and peptides which were retained on SP-Sephadex and recovered with 0.25 M NH_4OH. This was confirmed by thin-layer electrophoresis of the amino acids and peptides at pH 2.0 using an HOAc–HCO_2H buffer [17]. Components were detected by autoradiography, as well as with ninhydrin reagent.

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