

# Polysaccharide Composition of Unlignified Cell Walls of Pineapple [*Ananas comosus* (L.) Merr.] Fruit<sup>1</sup>

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The polysaccharides of cell walls isolated from the fleshy, edible part of the fruit of the monocotyledon pineapple [*Ananas comosus* (L.) Merr.] (family Bromeliaceae) were analyzed chemically. These cell walls were derived mostly from parenchyma cells and were shown histochemically to be unlignified, but they contained ester-linked ferulic acid. The analyses indicated that the noncellulosic polysaccharide composition of the cell walls was intermediate between that of unlignified cell walls of species of the monocotyledon family Poaceae (grasses and cereals) and that of unlignified cell walls of dicotyledons. Glucuronoarabinoxylans were the major noncellulosic polysaccharides in the pineapple cell walls. Xyloglucans were also present, together with small amounts of pectic polysaccharides and glucomannans (or galactoglucomannans). The large amounts of glucuronoarabinoxylans and small amounts of pectic polysaccharides resemble the noncellulosic polysaccharide composition of the unlignified cell walls of the Poaceae. However, the absence of (1→3,1→4)- $\beta$ -glucans, the presence of relatively large amounts of xyloglucans, and the possible structure of the xyloglucans resemble the noncellulosic polysaccharide composition of the unlignified cell walls of dicotyledons.

Except for members of the economically important family Poaceae (grasses and cereals), the polysaccharide compositions of cell walls of monocotyledons have not been studied extensively. Although the Poaceae is a large family, containing approximately 8000 species, it constitutes only about 16% of all species of monocotyledons (Cronquist, 1988). The other monocotyledons include numerous economically important plants, many of which are tropical or subtropical. An example of these is the pineapple (*Ananas comosus*), a member of the family Bromeliaceae, which produces an economically important fruit.

Although the unlignified primary cell walls of the Poaceae and dicotyledons have similar physical properties that are important during growth, the noncellulosic polysaccharides of the cell walls of these two groups are quite different (Bacic et al., 1988; Carpita and Gibeau, 1993). Typically the unlignified cell walls of the Poaceae contain highly substituted heteroxylans (GAXs) as the major noncellulosic polysaccharides, together with variable amounts of (1→3,1→4)- $\beta$ -glucans. Pectic polysaccharides and xylo-

glucans are usually present in small amounts. In contrast, the unlignified cell walls of dicotyledons typically contain large amounts of pectic polysaccharides, which are a complex class of polysaccharides comprising a family of acidic polysaccharides (rhamnogalacturonans) and several neutral polysaccharides (arabinans, galactans, and arabinogalactans). Smaller amounts of xyloglucans are also present.

The differences between the polysaccharide compositions of the cell walls of the Poaceae and the polysaccharide compositions of the cell walls of dicotyledons are much fewer for lignified secondary cell walls than for unlignified primary cell walls. The lignified cell walls of both the Poaceae and dicotyledons contain heteroxylans as the major noncellulosic polysaccharides (Bacic et al., 1988). The heteroxylans of the lignified cell walls of the Poaceae are GAXs that have structures similar to those in the unlignified cell walls of the Poaceae, but they have a lower degree of substitution by glycosyl residues. The heteroxylans of the lignified cell walls of dicotyledons are 4-O-methylglucuronoxylans.

It is often assumed that the cell walls of the Poaceae are representative of all monocotyledons. However, analyses of the polysaccharides of the unlignified cell walls of bulbs of onion (*Allium cepa*) (Alliaceae) (Mankarios et al., 1980; Redgwell and Selvendran, 1986; Ohsumi and Hayashi, 1994) and of shoots of asparagus (*Asparagus officinalis*) (Asparagaceae) (Waldron and Selvendran, 1990) showed that these cell walls have noncellulosic polysaccharide compositions similar to those of dicotyledons.

The presence of ester-linked ferulic acid in the unlignified cell walls of monocotyledons appears to be a guide to the presence of GAXs in these walls. Ester-linked ferulic acid occurs in the unlignified cell walls of approximately half of the families of monocotyledons (Harris and Hartley, 1980). In the Poaceae, this ferulic acid is ester-linked to GAXs and it may also be linked to GAXs in the same way in the unlignified cell walls of other families of monocotyledons where it occurs. Thus, the monocotyledon species that have ester-linked ferulic acid in their unlignified cell walls may contain GAXs similar in structure to those in the unlignified cell walls of the Poaceae (Bacic et al., 1988). Evidence that supports this has recently been obtained from the neutral-monosaccharide compositions of unligni-

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Abbreviations: CDTA, *trans*-1,2-diamino-cyclo-hexane *N*'*N*'*N*'-tetraacetic acid; GAX, glucuronoarabinoxylan; Rha, rhamnose. Nomenclature: An *f* or *p* after the name of a sugar indicates furanose or pyranose, respectively (e.g. Glcp, glucopyranose).

fied cell walls of monocotyledons (P.J. Harris, R.J. McKenzie, M.F. Kendon, and M.R. Kelderman, unpublished data). The proportions of neutral monosaccharides in acid hydrolysates of unligified cell walls (neutral-monosaccharide compositions) of a range of monocotyledon species were determined. Those species that had ester-linked ferulic acid in their cell walls also had high proportions of Ara and Xyl in the acid hydrolysates. This is consistent with the presence of GAXs in these cell walls. An exception was a species of the palm family, *Arecaceae*. The other monocotyledons had unligified cell walls with neutral-monosaccharide compositions similar to those of the unligified cell walls of dicotyledons.

In this paper we report an investigation of the polysaccharides of the unligified cell walls of pineapple (*Ananas comosus*), a monocotyledon species that has ester-linked ferulic acid in these cell walls. We show that these cell walls contain GAXs and have an overall polysaccharide composition intermediate between that of unligified cell walls of dicotyledons and that of unligified cell walls of the Poaceae.

## MATERIALS AND METHODS

### Plant Material

Pineapple [*Ananas comosus* (L.) Merr. cv Smooth Cayenne] fruits from Queensland, Australia, were purchased from a store at a ripeness suitable for eating.

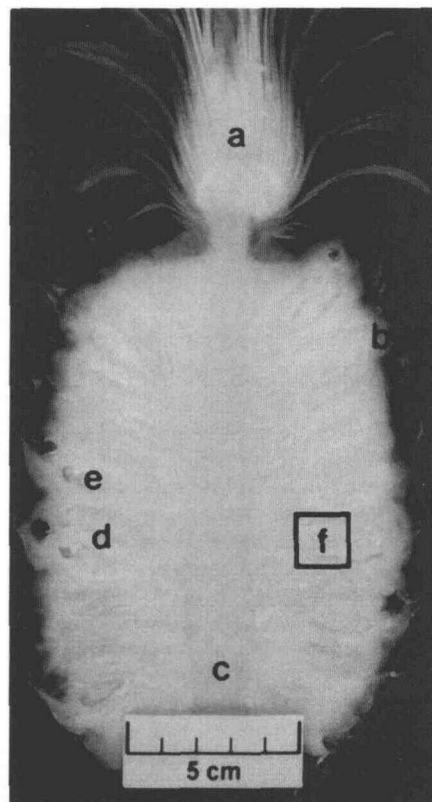
### Chemicals

A purified *endo*-(1→4)- $\beta$ -D-xylanase (M1) from *Trichoderma viride* (Gibson and McCleary, 1987) and kits for quantifying (1→3,1→4)- $\beta$ -D-glucans and Glc (McCleary and Codd, 1991) were obtained from Megazyme (Aust) (Warriewood, New South Wales, Australia). Oat spelt xylan, birchwood xylan, and wheat flour arabinoxylan were obtained from Serva (Heidelberg, Germany), Sigma, and Megazyme (Aust), respectively. All other reagents were of analytical grade.

### Microscopy

Bright-field and fluorescence microscopy was carried out using a Carl Zeiss research microscope (Oberkochen, Germany) fitted with a 50-W tungsten lamp, a mercury-vapor lamp (HBO 50), an incidence illuminator, and the appropriate filters.

Fresh sections (transverse and longitudinal) cut from the fruit (Figs. 1 and 2) and isolated cell walls were stained, using the methods described by Harris et al. (1994), in solutions of the bright-field stains ruthenium red, Alcian blue, and toluidine blue, the color reagents *p*-nitrobenzenediazonium tetrafluoroborate and phloroglucinol-HCl, and the aniline-blue fluorochrome. They were also stained with Sudan red 7B (0.1%, w/v) in a solution consisting of 50% (v/v) PEG, 45% (v/v) glycerol, and 5% (v/v) water (Brundrett et al., 1991). Control sections, mounted in the solvent for the stain, color reagent, or fluorochrome, were also examined.

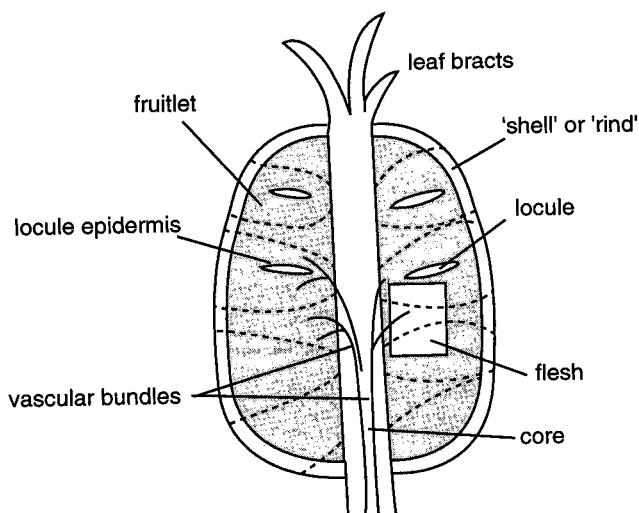


**Figure 1.** Photograph of a pineapple fruit cut longitudinally. a, Leaf bracts; b, shell or rind; c, core; d, fruitlet; e, locule; and f, flesh.

The autofluorescence of the cell walls in UV radiation was examined in sections mounted in water and in 0.1 M ammonium hydroxide (Harris and Hartley, 1976). The following fluorescence filters were used: G365 excitation filter, FT395 chromatic beam splitter, and LP420 barrier filter (for autofluorescence); and BP450–490 excitation filter, FT510 chromatic beam splitter, and LP520 barrier filter (for the aniline blue fluorochrome). Starch in sections and cell-wall preparations was detected using iodine in potassium iodide (0.2 g of iodine and 2 g of potassium iodide in 100 mL of water).

### Isolation of Cell Walls

Cell walls were isolated from the flesh of the fruit (flesh cell walls) and from the epidermis of the locule linings (Figs. 1 and 2). The lining of the locule was peeled away with forceps, and examination by bright-field microscopy showed that it consisted of the epidermis and underlying parenchyma cells. All procedures were carried out at 4°C. Tissues were homogenized in Hepes-KOH buffer (20 mM, pH 7.2) containing 10 mM 2-mercaptoethanol, using a specially constructed metal homogenizer in a Spex mixer/mill (model 8000; Spex, Edison, NJ) (Harris, 1983). The homogenate was centrifuged (2000g, 10 min) and the pellet washed with water three times by centrifugation, resuspended in water, and sonicated (ultrasonic processor W380; Heat Systems-Ultrasonics, Inc., Farmingdale, NY)



**Figure 2.** A schematic representation of a longitudinal section through a pineapple fruit showing the principal anatomical and morphological features (adapted from Dull, 1971).

for 1 min at full power, and centrifuged at 550g for 5 min. The cell walls were washed an additional four times by centrifugation and filtered onto nylon mesh (pore size 11  $\mu\text{m}$ ). The residue on the mesh was washed with water until the filtrate was clear, then dried by solvent exchange by successively washing with ethanol, methanol, and *n*-pentane, and stored under vacuum over silica gel.

#### Preparation of an Ethanol-Insoluble Residue from Pineapple Flesh

Cubes of flesh (sides 3 mm long, 15 g) were heated under reflux in 85% (v/v) aqueous ethanol (240 mL) for 20 min. After cooling the tube was centrifuged (2000g, 5 min), and the supernatant was removed, replaced by 80% (v/v) aqueous ethanol (240 mL), and heated under reflux for 20 min. Heating under reflux was done twice more. The residue was then washed by centrifugation (as above) with ethanol, methanol, and *n*-pentane and stored under vacuum over silica gel. The residue was then ground to pass a 0.5-mm screen using a Tecator Cyclone Sample Mill (Tecator AB, Hogenas, Sweden) and then further dried over  $\text{P}_2\text{O}_5$ .

#### Fractionation of Flesh Cell Walls

Flesh cell walls (50 mg) were incubated in 50 mM CDTA (adjusted to pH 6.5 with 1 M KOH, 5 mL) under Ar at 80°C for 2 h with frequent mixing. The suspension was centrifuged (2500g, 5 min) and the supernatant removed. The pellet was washed twice with 50 mM CDTA (pH 6.5), and the washings combined with the supernatant were dialyzed against water (20 h, 4°C) and freeze dried (CDTA fraction). The washed pellet was incubated in 1 M KOH containing 25 mM  $\text{NaBH}_4$  (10 mL) under Ar at 20°C for 2 h with frequent mixing. The suspension was centrifuged (as above) and the supernatant removed and adjusted to pH 5.0 with 18 M acetic acid. The pellet was washed twice with

1 M KOH containing 25 mM  $\text{NaBH}_4$  (10 mL), and the washings were adjusted to pH 5.0, combined with the supernatant, dialyzed (as above), and freeze-dried (1 M KOH fraction). The pellet was then extracted in 4 M KOH containing 25 mM  $\text{NaBH}_4$  (10 mL) in a similar way (4 M KOH fraction). The residue was resuspended in water (50 mL), adjusted to pH 5.0 with 18 M acetic acid, dialyzed, and freeze-dried ( $\alpha$ -cellulose fraction).

#### Neutral-Monosaccharide Composition of Cell Walls and Cell-Wall Fractions

The neutral-monosaccharide composition was determined by hydrolyzing the cell walls and cell-wall fractions in acid, converting the resultant monosaccharides to alditol acetates, and analyzing these by GC. Acid hydrolysis was done using two methods: by a two-stage  $\text{H}_2\text{SO}_4$  procedure and by TFA. The two-stage  $\text{H}_2\text{SO}_4$  procedure of Harris et al. (1988) was used except that the neutralized hydrolysate was filtered using a polytetrafluoroethylene disposable filter (diameter 13 mm, pore size 0.22  $\mu\text{m}$ ; Alltech, Deerfield, IL). Cell walls (2 mg) and cell-wall fractions (1 mg) were hydrolyzed using 2 M TFA (0.5 mL, 121°C, 1 h) in a sealed tube under Ar. After cooling, the hydrolysate was filtered as above and the filtrate evaporated to dryness in a stream of air. Water (0.1 mL) followed by 15 M ammonia (20  $\mu\text{L}$ ) was added to the residue, and the neutral monosaccharides were reduced and acetylated as described by Harris et al. (1988). The alditol acetates were separated and quantified on a BPX70 wall-coated, open tubular fused silica column (SGE Pty., Melbourne, Victoria, Australia) (25-m  $\times$  0.33-mm i.d., film thickness 0.25  $\mu\text{m}$ ) in a Hewlett-Packard 5890A gas chromatograph fitted with a flame-ionization detector and a dedicated cool on-column capillary inlet. Helium (zero grade; BOC Gases New Zealand Ltd., Penrose, Auckland, New Zealand) was used as the carrier gas at a column head pressure of 40 kPa. The initial oven temperature, 38°C, was maintained for 30 s following injection, then increased to 170°C at 50°C/min, then to 230°C at 2°C/min, and kept at 230°C for 5 min. The detector temperature was held at 250°C.

#### Uronic Acid Composition of Cell Walls and Cell-Wall Fractions

The total uronic acid content of the cell walls and cell-wall fractions was determined colorimetrically (as galacturonic acid) using the method of Blumenkrantz and Asboe-Hansen (1973) as modified by Rae et al. (1985). Galacturonic acid, glucuronic acid, and 4-*O*-methylglucuronic acid in TFA hydrolysates were separated and quantified by HPLC using a Dionex BioLC series 4000i HPLC (Dionex, Sunnyvale, CA). Cell walls and fractions were hydrolyzed using 2 M TFA as described above, but after evaporation to dryness more complete removal of the TFA was achieved by adding 2-propanol (0.6 mL) to the residue and again evaporating to dryness. The final residue was taken up in water (1.0 mL). Aliquots were injected (25- $\mu\text{L}$  loop) and run isocratically with 150 mM sodium acetate in 100 mM sodium hydroxide as the eluant, for 16 min at a

flow rate of 1 mL/min. Peak areas were corrected for the relative responses given by the detector by equal weights of galacturonic and glucuronic acids. Solutions containing reference 4-*O*-methylglucuronic acid were obtained by hydrolysis of 4-*O*-methylglucuronoxylan and the extracellular polysaccharide of *Rhizobium japonicum* strain 71a (Dudman, 1978). The polysaccharides (2 mg) were hydrolyzed as above using 2 M TFA, and the dry hydrolysates were dissolved in water (150  $\mu$ L) and 50- $\mu$ L aliquots subjected to descending paper chromatography on Whatman No. 1 paper with ethyl acetate:pyridine:water (8:2:1, v/v/v) as the solvent system (Fry, 1988). Material at the origin was eluted with water and run by HPLC. A major peak (4-*O*-methylglucuronic acid) with the same elution time (7.0 min) was obtained from both hydrolysates. It was assumed that on a weight basis 4-*O*-methylglucuronic acid gave the same detector response as glucuronic acid.

### Linkage Analysis of the Cell-Wall Polysaccharides

Methylation analysis was carried out using the method described by Harris et al. (1984). Partially methylated alditol acetates were identified by their mass spectra and GC retention times relative to *myo*-inositol hexaacetate. Combined GC-MS was done using a Hewlett-Packard 5890 gas chromatograph interfaced with a VG 70-SE mass spectrometer (VG Analytical, Wythenshawe, Manchester, UK) connected to a VG analytical 11-250J computer. The partially methylated alditol acetates were separated on the BPX70 column. Helium (zero grade, BOC Gases New Zealand Ltd.) was used as the carrier gas at a column head pressure of 95 kPa and flow rate of 30.0 cm/s. The injection port temperature was 220°C and samples (1.0  $\mu$ L) were injected under splitless conditions. The initial oven temperature (140°C) was increased to 230°C at 3°C/min and finally held at 230°C for 10 min. For MS, electron impact ionization at an ionization potential of 70 eV was used throughout. Compounds eluting from the column were detected in the mass spectrometer using the total ion current scanning from *m/e* 90 to 350 with a 1.6-s/decade scan rate and a 0.16-s interscan delay. The order of elution of the partially methylated alditol acetates was determined using standards (Bacic et al., 1984) and was the same as that reported by Lau and Bacic (1993) for a BPX70 column. The partially methylated alditol acetates were quantified using a flame-ionization detector after separation on the BPX70 column. The chromatography conditions were as described for the separation of the alditol acetates except that the oven temperature was increased from the initial temperature of 38 to 150°C at 50°C/min, then to 230°C at 3°C/min, and kept at 230°C for 10 min. The peak areas were corrected to mol% using the effective carbon responses calculated by Sweet et al. (1975). The partially methylated alditol acetates obtained from 2,4-linked and 3,4-linked xylopyranose co-eluted, as did those obtained from 2-linked and 4-linked xylopyranose. The relative amounts of the different partially methylated alditol acetates in the two peaks were determined by monitoring unique ions in the mass spectra.

### Partial Acid Hydrolysis of Cell Walls and the 1 M KOH Fraction

Flesh cell walls (10 mg) and the 1 M KOH fraction (10 mg) (fruit 2) were heated with 0.1 M TFA (1 mL) for 1 h at 100°C under Ar (Fry, 1988). The resulting suspension and solution were dialyzed against water (20 h, 4°C) and freeze-dried.

### Treatment of Cell Walls and the 1 M KOH Fraction with Endo-(1 $\rightarrow$ 4)- $\beta$ -Xylanase

Flesh cell walls (5 mg) and the 1 M KOH fraction (5 mg) (fruit 2), before and after partial acid hydrolysis, were treated with the (1 $\rightarrow$ 4)- $\beta$ -xylanase. The preparations were first incubated with sodium borohydride (20 g/L in 0.25 M NaOH) (1 mL/5 mg cell walls) at 40°C for 60 min after which 2 M acetic acid (1 mL) was added to destroy the sodium borohydride (Henry and Blakeney, 1988). Sodium acetate buffer (100 mM, pH 4.5, 2 mL) and the (1 $\rightarrow$ 4)- $\beta$ -xylanase (50  $\mu$ L, 10 units) were added, and the solution was incubated at 30°C for 10 min. The reducing sugars released were quantified (as Xyl) using the 4-hydroxybenzhydrazide assay (Blakeney and Mutton, 1980).

### Quantification of (1 $\rightarrow$ 3,1 $\rightarrow$ 4)- $\beta$ -Glucans in Cell Walls

This was done on the ethanol-insoluble residue of the flesh of fruit 2 (100 mg) by the method of McCleary and Codd (1991) using the (1 $\rightarrow$ 3,1 $\rightarrow$ 4)- $\beta$ -glucan and Glc assay kits from Megazyme. This method is a direct and specific enzymatic one that depends on the use of specific *Bacillus subtilis* (1 $\rightarrow$ 3,1 $\rightarrow$ 4)- $\beta$ -glucan hydrolase. Barley flour provided in the (1 $\rightarrow$ 3,1 $\rightarrow$ 4)- $\beta$ -glucan assay kit was used as a positive control.

## RESULTS

### Cell Types Present in the Fruit

Pineapple fruit is a collection of many individual fruitlets fused with one another and to the core (Figs. 1 and 2). The fruitlets are the fleshy, edible part of the fruit (flesh) and consist of ovary, bract, and sepal tissues, all of which are composed predominantly of parenchyma cells. On the external surface of the fruit, tissues of the fruitlets combine with floral remnants to form the "rind" or "shell." The core of the fruit consists mainly of vascular bundles with sclerenchyma fibers forming sheaths around the bundles (Okimoto, 1948). There are some vascular bundles in the flesh and, where possible, these were avoided during cell wall isolation because they contain lignified cell walls.

### Histochemistry of the Fruit Cell Walls

The staining properties of cell walls in sections of pineapple fruit are summarized in Table I. The cell walls of the sclerenchyma fibers, the xylem tracheary elements, and the epidermis of the shell gave a positive (red) coloration with phloroglucinol-HCl and in UV radiation showed a blue autofluorescence, which increased in intensity after treatment with ammonium hydroxide. These results indicate

**Table I.** Staining properties of pineapple fruit cell walls

–, No staining; +, weak staining; ++, moderate staining; +++, strong staining.

Stain or Color Reagent	Cell Type						Specificity
	Parenchyma of flesh	Sclerenchyma fibers	Xylem tracheary elements	Phloem	Epidermis of locule lining	Epidermis of shell	
Alcian blue	+	–	–	+	+	–	Polyanions (e.g. rhamnogalacturonans)
Ruthenium red	+	–	–	+	+	–	Polyanions (e.g. rhamnogalacturonans)
Toluidine blue	++ Pink-purple	+++ Blue-green	++ Blue-green	++ Pink-purple	++ Pink-purple	+++ Blue-green	Stains walls polychromatically Lignin and other phenolic components stain green or blue-green; polyanions (e.g. rhamnogalacturonans) pink or reddish purple
<i>p</i> -Nitrobenzene-diazonium tetrafluoroborate	++ Orange	+++ Orange-red	+++ Orange-red	++ Orange	++ Orange	+++ Orange-red	Phenolic components
Phloroglucinol-HCl	–	+++	+++	–	–	+++	Lignin
Aniline blue fluorochrome	–	–	–	+	–	–	(1 → 3)-β-D-Glucans (callose) Yellow

that the cell walls are lignified (Harris and Hartley, 1976). In addition, these cell walls gave a strong color reaction with *p*-nitrobenzenediazonium tetrafluoroborate and stained blue-green with toluidine blue, a result that is consistent with the cell walls being lignified. The cell walls did not stain with Alcian blue or ruthenium red.

In contrast, the cell walls of the parenchyma, phloem, and epidermis of the locule lining gave a negative color reaction with phloroglucinol-HCl, indicating that they are unlignified. The cell walls also stained pink-purple with toluidine blue and stained weakly with Alcian blue and ruthenium red. These cell walls fluoresced blue in UV radiation when mounted in water and green when mounted in ammonium hydroxide, indicating the presence of ester-linked ferulic acid (Harris and Hartley, 1976). The moderate color reaction of these cell walls with *p*-nitrobenzenediazonium tetrafluoroborate is also consistent with the presence of ferulic acid (Harris et al., 1982). Staining with Sudan red 7B revealed a cuticle on the shell epidermis but

stained no other structures. Starch granules were not detected in any of the cells or cell-wall preparations.

### Monosaccharide Composition of Cell Walls

The neutral-monosaccharide composition of the cell walls is shown in Table II. In sulfuric hydrolysates of cell walls from the flesh and epidermis of the locule lining, Glc was the most abundant neutral monosaccharide. The second most abundant neutral monosaccharide was Xyl, followed by Ara and Gal, with smaller proportions of Man, Rha, and Fuc. In TFA hydrolysates Xyl was the most abundant neutral monosaccharide, followed by Ara and Gal, whereas Glc accounted for less than 10% of the total, probably because cellulose is poorly hydrolyzed under these conditions (Mankarios et al., 1979). When calculated with Glc omitted the proportions of neutral monosaccharides in the sulfuric acid and TFA hydrolysates were similar (Table II).

**Table II.** Neutral-monosaccharide composition of cell walls from the flesh and epidermis of locule linings of pineapple fruits

Average of determinations on two hydrolysates. Figures in parentheses are calculated with Glc omitted.

Cell Wall	Acid Hydrolysis	Monosaccharide						
		Rha	Fuc	Ara	Xyl	Man	Gal	Glc
		<i>mol %</i>						
Flesh	H <sub>2</sub> SO <sub>4</sub>	1 (1)	1 (1)	15 (29)	27 (53)	3 (5)	5 (11)	50
(Fruit 1)	TFA	1 (1)	1 (1)	30 (31)	55 (56)	1 (1)	10 (10)	3
Epidermis <sup>a</sup>	H <sub>2</sub> SO <sub>4</sub>	tr <sup>b</sup> (1)	tr (1)	14 (25)	36 (63)	2 (4)	4 (7)	44
(Fruit 1)	TFA	1 (1)	1 (1)	25 (25)	65 (67)	1 (1)	6 (6)	2
Flesh	H <sub>2</sub> SO <sub>4</sub>	1 (1)	1 (1)	14 (25)	26 (48)	2 (4)	12 (22)	45
(Fruit 2)	TFA	2 (2)	1 (1)	25 (26)	43 (46)	2 (3)	20 (22)	7
Flesh	H <sub>2</sub> SO <sub>4</sub>	tr (1)	1 (1)	13 (24)	27 (53)	2 (5)	8 (16)	49
(Fruit 3)	TFA	2 (2)	1 (1)	25 (27)	47 (50)	3 (3)	16 (17)	6

<sup>a</sup> Epidermis of locule lining.

<sup>b</sup> tr, <0.5%.

Acid hydrolysates of the cell walls of the epidermis of the locule lining contained relatively more Xyl, and smaller proportions of the other neutral monosaccharides, than the hydrolysates of the flesh cell walls of the same fruit. Acid hydrolysates of cell walls of fruit 2 contained higher proportions of Gal than the other two fruits.

The flesh cell walls contained only 6 to 8% by weight of uronic acids determined colorimetrically (Table III). Galacturonic and glucuronic acids accounted for most of the uronic acids detected by HPLC of TFA hydrolysates of these cell walls (Table III). There was a slightly higher proportion of galacturonic than glucuronic acid. 4-O-Methylglucuronic acid accounted for only approximately 2% (w/w) of the uronic acids detected by HPLC.

### Monosaccharide Composition of Cell-Wall Fractions

The proportions of the cell-wall fractions obtained and the percentages of the neutral monosaccharides in acid hydrolysates of these fractions are shown in Table IV. The 1 M KOH and  $\alpha$ -cellulose fractions were the largest and represented 35 and 44%, respectively, of the total weight of the fractions obtained from the cell walls of fruit 1. The CDTA and 4 M KOH fractions represented only 11 and 10%, respectively, of the total weight of the fractions obtained. The fractions were hydrolyzed with TFA, except for the  $\alpha$ -cellulose fraction for which sulfuric acid was also used. The CDTA fraction contained high proportions of Xyl, Ara, and Gal and lower proportions of Rha, Glc, and Man. The proportion of Gal in all fractions from fruit 2 was higher than in fractions obtained in the same way from fruit 1. Hydrolysates of the 1 M KOH fraction contained mainly Xyl and Ara with smaller amounts of Gal, Glc, and Rha. The neutral-monosaccharide composition of hydrolysates of the 4 M KOH fraction was quite different from that

of hydrolysates of the CDTA and 1 M KOH fractions. It contained high proportions of Glc and Xyl with smaller proportions of Ara, Man, and Gal. Glc was the predominant neutral monosaccharide in  $H_2SO_4$  hydrolysates of the  $\alpha$ -cellulose fraction but not in TFA hydrolysates of this fraction. This is consistent with the  $\alpha$ -cellulose fraction containing a high proportion of cellulose, which is poorly hydrolyzed by 2 M TFA.

The uronic acid content (percentage by weight determined colorimetrically) of the fractions is shown in Table III. The CDTA fraction had the highest content (25%) and the 4 M KOH fraction had the lowest content (2%). Galacturonic and glucuronic acids accounted for most of the uronic acids detected by HPLC in hydrolysates of all of the fractions. Hydrolysates of the CDTA fraction contained mainly galacturonic acid, but hydrolysates of the 1 and 4 M KOH fractions contained a much higher proportion of glucuronic than galacturonic acid. Hydrolysates of the  $\alpha$ -cellulose fraction also contained more glucuronic acid than galacturonic acid (Table III). Small proportions of 4-O-methylglucuronic acid were found in hydrolysates of the 1 and 4 M KOH fractions (3% in both), hydrolysates of the CDTA fraction contained only trace amounts, and hydrolysates of the  $\alpha$ -cellulose fraction contained none. Galacturonic acid is characteristic of acidic pectic polysaccharides (rhamnogalacturonans), whereas glucuronic acid is characteristic of heteroxylans. The results are consistent with the proportion of rhamnogalacturonans to heteroxylans being much higher in the CDTA fraction than in the other fractions.

### Linkage Analysis of the Cell-Wall Polysaccharides

The positions of the glycosidic linkages between the monosaccharide residues in the polysaccharides in the unfractionated flesh cell walls of fruit 1 and fractions obtained from these are shown in Table V. The positions of particular glycosidic linkages are often characteristic of particular cell-wall polysaccharides (Bacic et al., 1988). The predominant glycosyl residues in the CDTA and 1 M KOH fractions were 4-linked Xylp; 3,4-linked Xylp; and terminal Araf, which are characteristic of GAXs. These residues were also in the 4 M KOH fraction, but the predominant residues in this fraction were 4-linked Glcp and 4,6-linked Glcp, which are characteristic of xyloglucans. In the  $\alpha$ -cellulose fraction the predominant glycosyl residue was 4-linked Glcp, which is characteristic of fractions containing predominantly cellulose.

The CDTA fraction also contained smaller proportions of residues characteristic of pectic polysaccharides. These residues were 5-linked Araf (arabinans), 4-linked Galp (galactans), and 2-linked and 2,4-linked Rhap (rhamnogalacturonans). The 4 M KOH fraction also contained smaller proportions of 4-linked Manp, which is characteristic of glucomannans.

The amounts of different polysaccharides present can be estimated as the summed mol% of their individual glycosyl residues using linkage structures characteristic of individual polysaccharides (Bacic et al., 1988). However, this sum does not include uronic acid residues. The unfractionated

**Table III.** Uronic acid content of cell walls and cell-wall fractions from pineapple flesh

Figures in parentheses are the calculated content (% w/w) of each uronic acid in the cell walls or cell-wall fractions. These were calculated from the total uronic acid content (colorimetric) of the cell wall or cell-wall fraction and the percentage of each uronic acid in hydrolysates of the cell wall or cell-wall fraction. GalA, Galacturonic acid; GlcA, glucuronic acid; 4-O-Me GlcA, 4-O-methylglucuronic acid.

Cell Wall or Cell-Wall Fraction	Total Uronic Acid Content <sup>a</sup>	Uronic Acid <sup>b</sup>		
		GalA	GlcA	4-O-Me GlcA
	% w/w			
Fruit 1	8	49 (4)	49 (4)	2 (tr)
Fruit 2	6	54 (4)	44 (3)	2 (tr)
Fruit 3	8	54 (5)	44 (4)	2 (tr)
CDTA fraction	25	89 (22)	11 (3)	tr (tr)
1 M KOH fraction	11	22 (2)	75 (8)	3 (tr)
4 M KOH fraction	2	29 (1)	68 (1)	3 (tr)
$\alpha$ -Cellulose fraction	8	46 (4)	55 (4)	0 (0)

<sup>a</sup> Determined colorimetrically (as galacturonic acid) (average of determinations on two hydrolysates). <sup>b</sup> Percentages (w/w) of the uronic acids GalA, GlcA, and 4-O-Me GlcA in the hydrolysates, detected and quantified by HPLC. tr, <0.5%.

**Table IV.** Neutral-monosaccharide composition of cell-wall fractions from pineapple flesh

Fraction (Fruit) (Yield <sup>a</sup> [%, w/w])	Acid Hydrolysis	Neutral Monosaccharide <sup>b</sup>						
		Rha <sup>c</sup>	Fuc	Ara	Xyl	Man	Gal	Glc
					<i>mol %</i>			
CDTA fraction (fruit 1) (11)	TFA	7	1	30	34	5	18	6
CDTA fraction (fruit 2) (9)		8	tr <sup>d</sup>	21	24	2	40	5
1 M KOH fraction (fruit 1) (35)	TFA	1	1	31	53	1	7	7
1 M KOH fraction (fruit 2) (30)		1	1	28	47	tr	15	8
4 M KOH fraction (fruit 1) (10)	TFA	1	2	11	36	7	7	37
4 M KOH fraction (fruit 2) (14)		1	2	11	34	6	13	33
$\alpha$ -Cellulose fraction (fruit 1) (44)	TFA	2	tr	27	35	2	10	25
$\alpha$ -Cellulose fraction (fruit 2) (47)		2	1	20	27	2	22	28
$\alpha$ -Cellulose fraction (fruit 1)	H <sub>2</sub> SO <sub>4</sub>	0	0	7	10	2	3	78
$\alpha$ -Cellulose fraction (fruit 2)		0	0	7	9	1	8	75

<sup>a</sup> Average yield from two (fruit 1) or three (fruit 2) fractionations. <sup>b</sup> Average of determinations on two hydrolysates. <sup>c</sup> Rha, Rhamnose. <sup>d</sup> tr, <0.5%.

**Table V.** Glycosyl linkage composition of pineapple flesh cell walls and cell-wall fractions

Glycosyl Derivative	Determined Position of O-Methyl Group <sup>a</sup>	Deduced Glycosidic Linkage	Flesh Cell Walls	Cell-Wall Fraction <sup>b</sup>			
				1	2	3	4
				<i>mol %</i>			
Xylopyranosyl	2,3,4	Terminal	4	6	5	7	3
	2,3	4	7	26	19	7	5
	2,4	3	tr <sup>c</sup>	1	1	–	tr
	3,4	2	2	3	4	5	2
	2	3,4	11	17	26	9	4
Arabinofuranosyl	3	2,4	2	3	4	2	tr
	2,3,5	Terminal	8	14	18	4	3
	2,3	5 or 4 (p)	tr	2	tr	tr	tr
	2,5	3	1	tr	2	1	tr
Glucopyranosyl	3,5	2	tr	2	2	tr	tr
	2,3,4,6	Terminal	3	tr	–	2	6
	2,3,6	4	44	3	5	28	67
	2,4,6	3	tr	–	tr	–	tr
	2,3,4	6	–	–	–	–	tr
	2,3	4,6	5	3	5	19	3
	2,6	3,4	tr	tr	–	1	tr
	3,6	2,4	tr	tr	–	tr	tr
	2	3,4,6	tr	–	–	tr	tr
Galactopyranosyl	6	2,3,4	–	–	–	–	tr
	2,3,4,6	Terminal	1	3	2	2	tr
	2,3,6	4	2	11	2	–	–
	2,4,6	3	tr	3	1	tr	tr
	3,4,6	2	tr	–	tr	tr	–
	2,3,4	6	tr	–	tr	tr	–
Rhamnopyranosyl	3,6	2,4	–	tr	–	–	–
	3,4	2	1	2	3	1	tr
	3	2,4	tr	1	–	–	tr
Fucopyranosyl	2,3,4	Terminal	2	tr	tr	2	–
Mannopyranosyl	2,3,6	4	3	tr	tr	9	2
	2,6	3,4	–	–	–	tr	–
	3,6	2,4	–	–	–	tr	–
	2,3	4,6	tr	–	–	tr	–

<sup>a</sup> 2,3,4-*O*-Methyl xylitol, 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methyl xylitol, etc. <sup>b</sup> Fractions 1, 2, 3, and 4 are the CDTA, 1 M KOH, 4 M KOH, and  $\alpha$ -cellulose fractions, respectively. <sup>c</sup> tr, Trace (<0.5 mol%). Derivatives in trace amounts total approximately 4% in flesh cell walls, 1% in the CDTA fraction, 1% in the 1 M KOH fraction, 1% in the 4 M KOH fraction, and 5% in the  $\alpha$ -cellulose fraction.

cell walls thus contained approximately 28% GAXs calculated from the sum of 4-, 3,4-, and 2,4-linked Xylp and terminal, 2-, and 3-linked Araf. Xyloglucans accounted for approximately 16%, calculated from the sum of the 4,6-linked Glcp, terminal, and 2-linked Xylp, terminal Galp, 2-linked Galp, terminal Fucp, and 4-Glcp (equal to one-third of the 4,6-Glcp). The proportions of 4-Glcp were based on the structure of xyloglucans from cell walls of dicotyledons and onions (Hayashi, 1989; Ohsumi and Hayashi, 1994). The proportion of pectic polysaccharides was calculated as approximately 6% from the sum of 5-linked Araf, 3-, 4-, and 6-linked Galp, and 2- and 2,4-linked Rhap. The proportion of glucomannans was approximately 5%, from the sum of 4-linked Manp and 4-linked Glcp, which is equal to half the 4-linked Manp. This is based on a ratio of 1:2 for Glc:Man in glucomannans of angiosperm cell walls (Bacic et al., 1988). The remaining 4-linked Glcp (40%) was assumed to be cellulose.

#### Partial Acid Hydrolysis of Cell Walls and the 1 M KOH Fraction

Arabinofuranosyl residues of GAXs are known to be removed preferentially by mild acid hydrolysis, for example by using 0.1 M TFA for 1 h at 100°C (Fry, 1988). This treatment reduced substantially the proportions of Ara in 2 M TFA hydrolysates of flesh cell walls and of the 1 M KOH fraction (fruit 2). In hydrolysates of untreated flesh cell walls and of the 1 M KOH fraction, the percentages of Ara were 25 and 28%, respectively (Tables II and IV). After treatment with 0.1 M TFA for 1 h at 100°C, the monosaccharide compositions of the flesh cell walls and of the 1 M KOH fraction (in parentheses) were: Rha, 4% (6%); Ara, 5% (2%); Xyl, 54% (43%); Man, 5% (4%); Gal, 13% (12%); Glc, 20% (34%). This result is consistent with the Ara being in the furanose form.

#### Treatment of Cell Walls and the 1 M KOH Fraction with *Endo*-(1→4)- $\beta$ -Xylanase

Reducing sugars were released by the xylanase from the flesh cell walls and the 1 M KOH fraction, indicating that the flesh cell walls and the 1 M KOH fraction contained (1→4)- $\beta$ -D-xylans. The amounts of reducing sugars released ( $\mu$ g of Xyl released from 5 mg in 10 min at 30°C by 10 units of xylanase) were as follows (values obtained using flesh cell walls and the 1 M KOH fraction after treatment with 0.1 M TFA are in parentheses): flesh cell walls, 73 (116); 1 M KOH fraction, 121 (132). This indicates that removal of arabinofuranosyl residues by partial acid hydrolysis increases the extent of hydrolysis. However, the amounts were still low compared to the amounts released from the positive controls: oat spelt xylan, 420; birchwood xylan, 906; and wheat flour arabinoxylan, 1871.

#### Quantification of (1→3,1→4)- $\beta$ -Glucans in Cell Walls

No (1→3,1→4)- $\beta$ -glucans were detected in the ethanol-insoluble residue of the flesh. The assay was done on an ethanol-insoluble residue because some of the (1→3,1→4)-

$\beta$ -glucans in the cell walls of some species of Poaceae are water soluble.

### DISCUSSION

Our results indicate that the noncellulosic polysaccharide composition of the unignified cell walls of pineapple fruits is intermediate between that of the unignified cell walls of species of the monocotyledon family Poaceae and that of the unignified cell walls of dicotyledons. The results are consistent with GAXs, similar to those of the Poaceae, being the major noncellulosic polysaccharides of the unignified cell walls of pineapple fruits. Furthermore, like the cell walls of the Poaceae, the pineapple cell walls contain only small amounts of pectic polysaccharides. However, in contrast to the unignified cell walls of the Poaceae, we found no (1→3,1→4)- $\beta$ -glucans in the cell walls of pineapple. The xyloglucan content of the pineapple cell walls is also more similar to the xyloglucan content of the unignified cell walls of dicotyledons than that of the unignified cell walls of the Poaceae. In addition, the possible structures of the xyloglucans in pineapple cell walls are more similar to those in the unignified cell walls of dicotyledons than those in the unignified cell walls of a number of species of the Poaceae. Small amounts of glucomannans (or galactoglucomannans) are also present in the pineapple cell walls.

Except for the  $\alpha$ -cellulose fraction, the largest fraction obtained from the pineapple flesh cell walls was that extracted with 1 M KOH. Our results are consistent with this fraction containing GAXs with structures similar to those GAXs that occur in the unignified cell walls of species of the Poaceae. The GAXs from the unignified cell walls of the Poaceae have a linear backbone of (1→4)- $\beta$ -xylopyranosyl residues substituted by mono- and oligosaccharide side chains. The main substituents are single  $\alpha$ -L-arabinofuranosyl and  $\alpha$ -D-glucuronosyl (or its 4-O-methyl derivative) residues linked to the backbone xylopyranosyl residues at C(O)3 and C(O)2, respectively (Bacic et al., 1988; Carpita and Gibeau, 1993). In the unignified cell walls of both pineapple and maize (maize coleoptiles and leaves) more substituents were linked to the xylopyranosyl residues at C(O)3 than at C(O)2. Maize cell walls contain GAXs with different amounts of substitution, the highly substituted GAXs being most abundant in dividing and elongating cells. The ratio of branched Xyl (2,4- plus 3,4-xylosyl) to unbranched Xyl (4-xylosyl) for GAXs in maize coleoptiles varied from 0.9 to 6.2 (Carpita, 1984). This compares with a ratio of 1.6 for the GAXs extracted with 1 M KOH from pineapple flesh cell walls. In the monosaccharide compositions of the pineapple cell walls, the higher proportion of Xyl to Ara in the epidermal cell walls of the locule lining compared to that in the cell walls of the flesh may be due to differences in the degree of substitution of the GAXs in different cell types. Gordon et al. (1985) also found that the epidermal cell walls of the leaves of perennial and Italian ryegrass had a higher proportion of Xyl to Ara than did the mesophyll cell walls. In view of the high proportion of GAXs in the unignified cell walls of pineapple, which have a similar structure to the GAXs of the Poaceae, it is likely



that the ester-linked ferulic acid we detected histochemically in pineapple cell walls is linked to these polysaccharides in the same way as in the Poaceae. GAXs have also been isolated from lignified cell walls of pineapple leaf fiber (Bhaduri et al., 1983). As with GAXs from lignified cell walls of species of the Poaceae, they were much less substituted (branched to unbranched Xyl ratio of 0.2) than for GAXs in unligified pineapple cell walls in the present study.

Our linkage-analysis results are also consistent with the presence of xyloglucans in the unligified cell walls of pineapple fruits as indicated by the presence of 4,6-linked Glc residues. As with the cell walls of other plants, these xyloglucans were extracted mainly with strong alkali (4 M KOH). In the xyloglucans of dicotyledons some of the Xyl residue side chains have Gal or Gal and Fuc residues attached to them. The Fuc is terminal and is attached at the C(O)2 of Gal, which is in turn attached to Xyl at C(O)2. It has recently been found that the xyloglucans in the unligified cell walls of bulbs of onion, garlic (*Allium sativum*), and their hybrid have structures similar to those of dicotyledon xyloglucans (Ohsumi and Hayashi, 1994). However, xyloglucans from the unligified cell walls of the Poaceae contain less Xyl and much less Gal than xyloglucans from dicotyledons (Hayashi, 1989). Xyloglucans from the unligified cell walls of a number of species of the Poaceae were found to contain no terminal Fuc (Hayashi, 1989), but fucosylated xyloglucans have recently been identified in the walls of suspension culture cells of the grass tall fescue (*Festuca arundinacea*) (McDougall and Fry, 1994). The presence of terminal Fuc residues in the 4 M KOH fraction of the pineapple cell walls indicates that the xyloglucans in these cell walls may contain terminal Fuc residues and thus may be more similar in structure to the xyloglucans of dicotyledons than to the xyloglucans from species of the Poaceae other than *F. arundinacea*. The presence of terminal and 2-linked Gal in the 4 M KOH fraction is also consistent with the presence in this fraction of xyloglucans similar in structure to the xyloglucans of dicotyledons. Furthermore, the calculated xyloglucan content of pineapple cell walls of 16% is more similar to the xyloglucan content of the unligified cell walls of dicotyledons than those of the Poaceae. For example, Fry (1988) indicated that xyloglucans typically make up 20% of the unligified cell walls of dicotyledons and 1 to 5% of the unligified cell walls of the Poaceae.

The unligified cell walls of pineapple fruits contain only small amounts of pectic polysaccharides. These pectic polysaccharides were found mostly in the fraction extracted with CDTA, and the monosaccharide and linkage analyses are consistent with the presence of rhamnogalacturonans and neutral pectic polysaccharides rich in 4-linked Gal residues, either galactans or type I arabinogalactans rich in Gal residues. The presence of trace amounts of 2,4-linked Gal residues in the CDTA fraction is consistent with the presence of small amounts of the acidic pectic polysaccharide rhamnogalacturonan II (Bacic et al., 1988).

Jarvis et al. (1988) determined the pectic galacturonan content of unligified cell walls from a range of monocoty-

ledons, including pineapple (leaves) and two species of the family Poaceae, oats (*Avena sativa*) (coleoptile and first leaf) and bamboo (*Dendrocalamus giganteus*) (leaf lamina). The pectic galacturonan was isolated by partial hydrolysis and characterized by electrophoresis and degradation by purified polygalacturonase. The pectic galacturonan contents of the pineapple, oat, and bamboo cell walls were 55, 46, and 6 mg/g, respectively. These values contrast with the much higher ones of 257 and 157 mg/g that were obtained for the two dicotyledons, fodder rape (*Brassica napus*) (stem) and pea (*Pisum sativum*) (epicotyl), respectively, which were included for comparison.

Unlike on the structures of the pectic polysaccharides in the unligified cell walls of dicotyledons, little work has been done on the structures of the pectic polysaccharides in the unligified cell walls of the Poaceae. Carpita (1989) examined the small amounts of pectic polysaccharides in the cell walls of maize (*Zea mays*) coleoptiles and the cell walls of a liquid suspension culture of proso millet (*Panicum miliaceum*). The linkage analysis was also consistent with the presence of rhamnogalacturonans and neutral pectic polysaccharides. However, the rhamnosyl residues were more highly branched than in pineapple or dicotyledons (Bacic et al., 1988): with both grass cell walls there were 4 times more 2,4-rhamnosyl residues than 2-rhamnosyl residues. The presence of considerable amounts of 3-, 6-, and 3,6-linked Gal in the maize and proso millet pectic fraction (ammonium oxalate soluble fraction) indicated the presence in these grass cell walls of type II arabinogalactans (arabinogalactan proteins). However, the absence of 3,6-linked Gal in the pineapple cell walls suggests that arabinogalactan proteins are not present in these cell walls. The 3-linked Gal in the CDTA fraction may be in side chains attached to rhamnogalacturonans (Bacic et al., 1988).

The weak staining by Alcian blue and ruthenium red of the unligified cell walls in pineapple fruits is consistent with the presence of only small amounts of acidic pectic polysaccharides, such as rhamnogalacturonans. However, the moderate pink-purple staining of these cell walls with toluidine blue suggests that this stain may be more sensitive to the presence of small amounts of polyanions than are Alcian blue and ruthenium red.

Because fruit softening during ripening is believed to result from the enzymic modification of cell walls, changes in the cell-wall composition of dicotyledonous fruits during ripening have been studied extensively. However, similar work has not been carried out with monocotyledonous fruits. In the ripening of dicotyledonous fruits, much attention was initially focused on the depolymerization of acidic pectic polysaccharides (rhamnogalacturonans) by polygalacturonase. However, experiments with transgenic plants have shown that, although polygalacturonase is important for the degradation of acidic pectic polysaccharides, it is not the primary determinant of softening in tomato fruit (Gray et al., 1992). More recently, attention has shifted to the loss of Gal from the neutral pectic galactans of fruit cell walls during ripening, which has been reported from a variety of dicotyledonous fruits (e.g. Redgwell et al.,

1992). Our results indicate that it is possible that Gal is also lost from the monocotyledonous pineapple cell walls during ripening. Although the objective of the present study was not to determine the changes in the composition of pineapple cell walls during ripening, and care was taken to obtain three fruits at the same stage of ripeness, the flesh cell walls of one of the pineapple fruits examined (fruit 2) contained a higher proportion of Gal than the other two and may have been at an earlier stage of ripening than the other two. With dicotyledonous fruits attention has also recently been focused on changes in the cell-wall xyloglucans and the possible enzymes involved (Redgwell and Fry, 1993). It would be interesting to know whether the xyloglucans and/or the GAXs of pineapple fruit cell walls are modified during ripening and result in changes in fruit texture.

The presence in the pineapple flesh cell walls of small amounts of 4-linked Man (which was concentrated after fractionation mainly in the 4 M KOH fraction) is consistent with the presence in the cell walls of glucomannans or possibly galactoglucomannans. A galactoglucomannan has been isolated from the cell walls of suspension cultures of the dicotyledon tobacco (*Nicotiana tabacum*) (Eda et al., 1985). The presence of small amounts of Man in hydrolysates of unligified cell walls (or fractions from these cell walls) of the Poaceae has also been reported, for example, in the cell walls of oat (*A. sativa*) coleoptiles (Wada and Ray, 1978), but the structures of the polysaccharides have not been investigated.

No (1→3,1→4)- $\beta$ -glucans were detected in the pineapple flesh cell walls using a direct and specific enzymatic method. The traces of 3-linked Glc that were detected in the cell walls probably derived from the (1→3)- $\beta$ -glucans (callose) that were detected histochemically in the phloem sieve plates using the aniline blue fluorochrome. The absence of (1→3,1→4)- $\beta$ -glucans in the flesh cell walls of the pineapple fruit is consistent with the results of Stinard and Nevins (1980), who examined the cell walls of a number of monocotyledons from the family Poaceae and from species of 10 other families, including *Billbergia nutans*, a member of the Bromeliaceae, for the presence of (1→3,1→4)- $\beta$ -glucans. They used cell walls obtained from nonendospermic tissue (elongating subapical tissue) and found the glucans only in cell walls of the Poaceae. From this they concluded that these glucans may be restricted to the cell walls of the Poaceae. However, in the Poaceae the amounts of (1→3,1→4)- $\beta$ -glucans in unligified cell walls vary considerably. For example, large amounts of (1→3,1→4)- $\beta$ -glucans occur in the cell walls of the starchy endosperm cells (e.g. 75% in barley), but only small amounts (<3%) occur in the cell walls of ryegrass mesophyll cells (Bacic et al., 1988). Furthermore, the amounts of (1→3,1→4)- $\beta$ -glucans in cell walls vary with stage of cell development. For example, the amount of (1→3,1→4)- $\beta$ -glucans in the cell walls of maize coleoptiles is approximately 1% before elongation, but at the end of elongation it is approximately 14% (Luttenegeger and Nevins, 1985).

In conclusion, our results show that the unligified flesh cell walls of pineapple fruits have a noncellulosic polysac-

charide composition intermediate between that of the unligified cell walls of the Poaceae and that of the unligified cell walls of dicotyledons. This is the first study not based on the Poaceae of the polysaccharides of unligified, monocotyledon cell walls that contain ester-linked ferulic acid.

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