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POST-HARVEST CHANGES IN *MANGIFERA INDICA* MESOCARP CELL WALLS AND CYTOPLASMIC POLYSACCHARIDES

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Abstract—During ripening, mango mesocarp cell walls undergo degradation with the net loss of arabinose, galactose and galacturonic acid. Hot water fractions of the cell walls from unripe fruits were rich in galactose and arabinose and contained only 7% galacturonic acid in comparison with those from unripe fruits which contained 90% uronic acid: little change occurred in the alkali-soluble (hemicellulose) fraction during ripening. The ripening-associated changes in the cold water-soluble cytoplasmic polysaccharides in the mesocarp were also examined. As the mesocarp softened these increased in amount and bound uronic acid increased three-fold. Gel-filtration and ion-exchange chromatography were used to examine these cytoplasmic polysaccharides. Their average M_r decreased on ripening and most of the fractions were complex with respect to monosaccharide composition. However, polysaccharides which are essentially an arabinoxylan and a galacturonan appeared to be present in the unripe and ripe tissue, respectively.

INTRODUCTION

Over the last 15 years there has been renewed interest in the structural polysaccharides of higher plants partly as a result of improved procedures for investigating what is perhaps the most complex group of macromolecules in living organisms. However, as a consequence of this complexity there is still no clear understanding of the detailed architecture of even the simplest, primary wall [1–3]. Needless to say, the biochemistry of these materials is also limited although there is a rich literature on the topic [4] and recent special interest connected with claims that carbohydrates may possess regulatory activities in plants [5].

The cell walls of fruits have received considerable attention [6, 7] as changes occurring during maturation and ripening are commercially important in connection with marketing and storage. Little is known about the biochemistry of mango fruits [8, 9], a major crop in the Far East and parts of Africa which is exported worldwide.

Roe and Bruemmer [10] have examined the changes occurring in high-M_r carbohydrates in mango fruits during ripening. Their results show that total alcohol-insoluble solids (cell wall plus cytoplasmic components) decreases significantly during fruit softening and that approximately 40% of this weight loss is due to a decrease in combined uronic acid, presumed to be present as galacturonan. Further, they equate softening with an increase in polygalacturonase and cellulase activities during ripening although both of these reported activities were low.

Apart from this work, there is little information on the structural polysaccharides of mango mesocarp tissue. The present study provides more detailed information concerning changes in the cell wall and cytoplasmic polysaccharides which occur when the mature green fruits ripen.

RESULTS AND DISCUSSION

Cell wall polysaccharides

Preparations of cell walls from the mesocarp of mangoes (cultivar 'Ngowe') at two stages of ripening were obtained by homogenisation of the tissues, centrifugation and washing with sodium phosphate buffer (pH 6.9) followed by water and organic solvents, all at 4°. The yields of cell walls from the mango tissues based on fresh weight were 9.2 and 3.3% for unripe and ripe fruits, respectively, and the total uronic acid contents of the walls were 25.0 and 19.1%. The walls of ripe fruits contained 21.1% protein in contrast to 7.3% for unripe fruits.

The combined monosaccharides released from the cell walls by hydrolysis with 2M TFA are shown in Table 1. A significantly higher yield of monosaccharides was obtained from the unripe material than from the more mature fruits. This presumably relates to the neutral polysaccharide/galacturonan ratio which is higher in the unripe wall material than the ripe, coupled with the greater acid lability of the neutral polymers compared to the galacturonans. There is a marked reduction in the arabinose, galactose and galacturonic acid contents of the mesocarp cell walls during the ripening process indicating loss of the pectin complex. Some of the glucose from the cell wall preparations is likely to be derived from starch which has been reported to constitute up to 13% of the mature fruit tissue and then undergo post-harvest hydrolysis [11].

Table 1. Determination of monosaccharides in acid hydrolysates of cell wall preparations from unripe and ripe mango mesocarp tissues

Monosaccharide	Monosaccharide (mg) released from 10 mg (dry wt) cell wall	
	Unripe	Ripe
Rha	0.01	0.03
Fuc	0.20	0.09
Ara	0.87	0.28
Xyl	0.43	0.29
Gal	1.14	0.18
Glu	1.12	0.94
Galacturonic acid	0.38	0.16
Total	4.15	1.96

Table 2. Yield of cell wall fractions from unripe and ripe mango mesocarp tissues

Fraction	Cell wall fraction (g) obtained from 100 g fr. wt of pulp	
	Unripe	Ripe
Hot H_2O-soluble	4.42 ± 0.31	0.36 ± 0.03
4 M NaOH-soluble	2.02 ± 0.07	0.79 ± 0.03
Residue	2.75 ± 0.23	2.14 ± 0.17

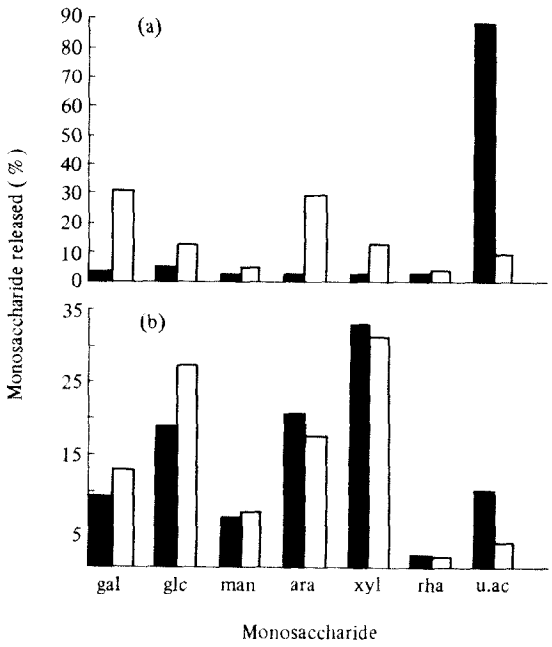

Fig. 1. Monosaccharide compositions of (a) hot-water-soluble; (b) alkali-soluble fractions of cell walls prepared from unripe (empty bars) and ripe (filled bars) of mango mesocarp tissues.

The polysaccharides in the cell wall preparations from unripe and ripe tissues were subjected to a simple fractionation by sequential extraction with H_2O (100°) and 4 M NaOH (25°). Ripening resulted in a reduction in the amount of extractable hot water- and alkali-soluble polysaccharides and the insoluble 'cellulosic' residue (Table 2). Analysis of the monosaccharide components of these fractions by TLC after hydrolysis with 2 M TFA showed a significant difference in the galacturonic acid content of the hot water (pectin) fractions (Fig. 1a): the ripe tissue fraction contained approximately 90% galacturonic acid whereas the unripe material possessed only 7% and was particularly rich in galactose and arabinose. Fig. 1b shows the changes in the composition of the alkali-soluble (hemicellulose with some pectin) cell wall fractions. High levels of xylose were detected in very similar proportions in the fractions from both ripe and unripe fruits. The other monosaccharide constituents (apart from glucose, which decreased on ripening and galacturonic acid which increased) were also present in similar proportions, in the alkaline fractions from both physiological stages. Hemicellulose materials would appear to undergo little change during the ripening process in comparison with the water-soluble pectins.

Mesocarp water-soluble carbohydrates

The changes in the mesocarp water-soluble carbohydrates which accompanied the breakdown of the cell walls were also examined. Mosocarp preparations were obtained by macerating the tissues with cold (4°) acetate buffer (pH 5.0) and removing the cell wall debris by centrifugation. The dry weights of solids in the resulting supernatants were *ca* 2 and 32% of the total dry weight of solids in the pulp macerates of the unripe and ripe fruits, respectively. Furthermore, the solids in the supernatants were composed largely of glucose, fructose and sucrose (as judged by TLC); 84% in the unripe fruit fraction and 91% in the ripe.

The soluble polysaccharides were examined on Bio-gel P-150 columns (Fig. 2). Those from the unripe fruit (containing 16.7% uronic acid) yielded one main carbohydrate-containing peak (P1: $M_r > 150\,000$) when the column fractions were analysed for total carbohydrate (Fig. 2a). This was obviously heterogenous as a different profile was obtained when the column fractions were assayed for uronic acids. A shoulder (P2; average M_r 45 000) was also apparent. In contrast the polysaccharides from the ripe mango preparation (containing 53.2% uronic acid) eluted as a single peak (P3) with average M_r 45 000; again, with indications of heterogeneity (Fig. 2b).

Fractions P1 and P2 from unripe tissues differed markedly in their combined monosaccharide compositions: P1 was made up of seven different monosaccharides, the major sugar being galacturonic acid (36%) (see Table 3) whilst P2 consisted essentially of arabinose (38%) and xylose (46%). The column fractions from the ripe mango preparations represented by peak P3 were divided into three aliquots, P3a (elution vol. 24–28 ml), P3b (30–34 ml) and P3c (36–40 ml) in order of decreasing M_r. Monosaccharide analysis revealed that the highest M_r subfraction (P3a) was composed almost entirely of galacturonan. In contrast to P3a, the P3b and P3c subfractions contained large amounts of neutral monosaccharides which were present in very similar proportions in the two

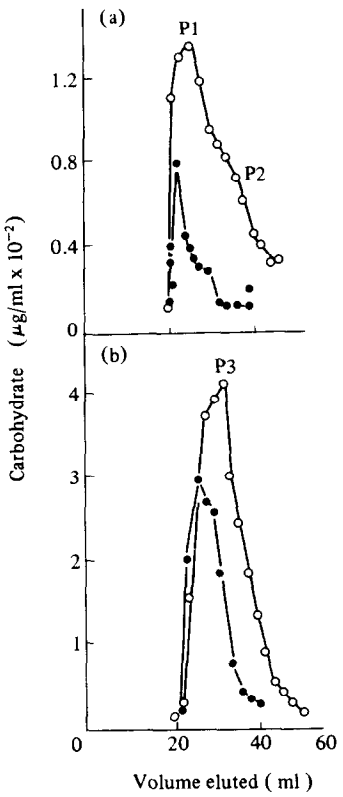

Fig. 2. Bio-Gel P-150 gel-filtration of soluble polysaccharides of (a), unripe and (b), ripe mesocarp tissue of mango. —○—○—, total carbohydrate; —●—●—, uronic acid.

fractions: the proportions of combined uronic acid decreased with the decreasing M_r of the subfractions.

The dialysed buffer extracts of unripe and ripe mango mesocarp tissues were also examined on DEAE Sephadex ion-exchange columns. Approximately 20% of the water-soluble polysaccharides from the unripe tissues and 50% from the ripe tissues were eluted unretarded from the ion-exchanger with 50 mM sodium acetate buffer, pH 5.0. These 'neutral' fractions (not shown in Fig. 3) were pooled in each case and freeze-dried prior to monosaccharide analysis. The bound 'acidic' polysaccharides were eluted from the columns with a gradient of NaCl. Fractions from the unripe tissues (Fig. 3a) consisted of a single, major component (U1) eluting at 0.07 M NaCl together with several minor and more acidic components eluting over a NaCl concentration range of 0.25 to 0.52 M. The acidic polysaccharides from ripe mesocarp also contained a major acidic component (R1) eluting at 0.07 M NaCl (Fig. 3b).

The monosaccharide composition of the 'neutral' and major acidic polysaccharide fractions are shown in Table 4. The 'neutral' fractions from unripe and ripe fruits were quite similar. The most striking observation was the marked difference between the composition of the major acidic peaks (U1) and (R1). The latter was composed largely (78%) of uronic acid whilst the former contained much less acidic sugar (13%) together with relatively large amounts of galactose, arabinose and xylose.

The results of this study show that mango cell walls undergo a net degradation during ripening and that like many other fruits, such as the tomato, combined monosaccharides of the pectin complex are lost [4, 12–15]. The hot water-soluble fraction of the unripe mango walls would appear to lose arabinose and galactose leaving a water-soluble galacturonan-rich material in the ripe mesocarp.

Accompanying these changes is an increase in the cold water-soluble polysaccharide content of the cells. In the mature, unripe fruits the polysaccharide mixture is complex with a high M_r fraction which is rich in uronic acid and neutral sugars together with a lower M_r fraction which is largely composed of arabinose and xylose: arabinoxylans have been isolated from a number of plant sources [2]. As the mesocarp ripens there is a three-fold increase in the total combined uronic acid in the polymers and a decrease in the average molecular weight of these polysaccharides. A fraction composed largely of galacturonan can also be resolved by both gel-filtration and ion-exchange chromatography from the ripe tissues.

Recent studies by Gross [16] have shown that in ripening 'Rutger' tomatoes there is an increase in ethanol-

Table 3. Monosaccharide compositions of pooled polysaccharide fractions from unripe and ripe mango mesocarp tissues separated on Bio-Gel P-150 columns (see Fig. 2)

	Monosaccharide (%)*				
	Unripe [see Fig. 2(a)]		Ripe [see Fig. 2(b)]		
Monosaccharide	P1 (22–28 ml)	P2 (30–40 ml)	P3a (24–28 ml)	P3b (30–34 ml)	P3c (36–40 ml)
Gal	13.2	0.0	1.6	5.6	9.3
Glu	10.2	0.0	0.4	7.6	12.9
Man	11.4	0.0	0.0	2.2	4.9
Ara	9.1	38.2	3.1	26.8	33.9
Xyl	18.1	45.5	1.7	11.3	19.2
Rha	2.4	4.9	0.0	0.5	3.3
Uronic acids	35.6	11.4	93.2	46.0	16.5

*Based on total monosaccharides released by acid hydrolysis.

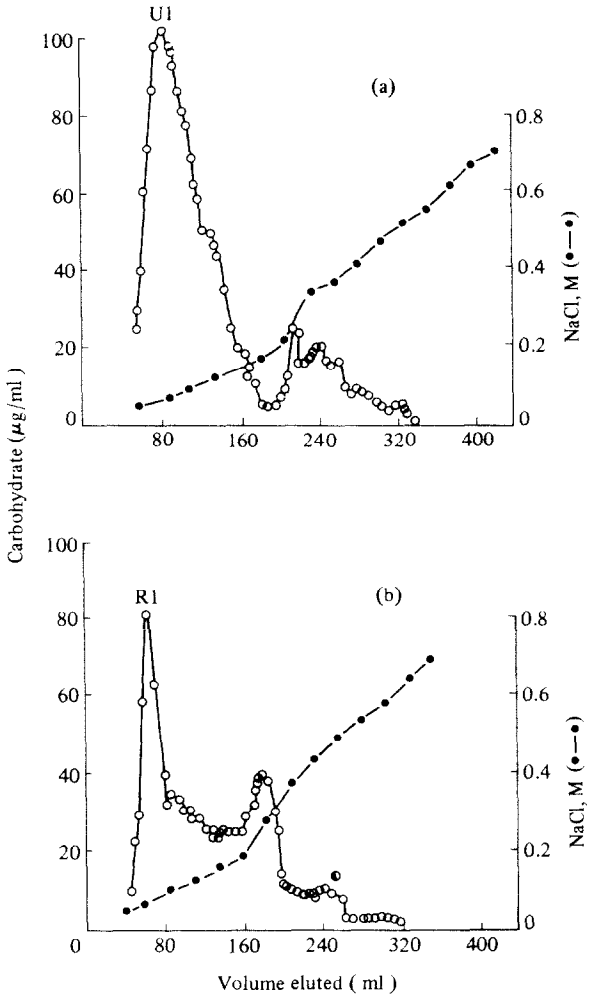

Fig. 3. DEAE-Sephadex A 25 chromatography of soluble polysaccharides present in the mesocarp tissues of (a) unripe and (b) ripe mangoes. The profiles show the elution of column-bound polysaccharides only.

insoluble polysaccharides (EIP) in water extracts of the fruits, as occurs in mangoes. Furthermore, the galacturonic acid and rhamnose contents (and to a much smaller extent arabinose and galactose) of the total EIP from tomatoes increased on ripening. Gross suggests that the removal of galacturonan-rich material from the tomato cell wall involves endopolygalacturonase activity, as has been assumed in the case of other fruits [12]. However, we have been unable to detect endopolygalacturonase in mango mesocarp (unpublished results) and there have been claims that it is absent from the tissues of some other fruits such as cranberry, grape and melon [17]. Furthermore, there is the question of whether fruit cell sap polysaccharides arise wholly from cell wall degradation or whether they, at least in part, represent material destined for incorporation into the wall which has been synthesized in the golgi membrane system. Undoubtedly cell wall turnover in the true sense (i.e. where there is continual synthesis and degradation) does occur in some plant tissues [4] and in fruits it is probable that during maturation there is turnover but as ripening proceeds synthesis decreases and degradation increases. The water-soluble polysaccharides of the cell may, therefore, reflect different metabolic processes at different physiological stages.

The observations on polysaccharide changes during mango ripening serve as a basis for studies in progress in this laboratory on enzymes involved in the fruit softening process.

EXPERIMENTAL

Materials. General laboratory chemicals were of analytical grade. Monosaccharide standards for TLC analysis were from Koch-Light, Colnbrook, Bucks., plastic-backed silica-gel TLC plates from Schleicher and Schüll, Dassel, F.R.G., DEAE-Sephadex and Dextran markers from Pharmacia (S.B.) Ltd., London and Bio-gel from Bio-Rad Laboratories, Watford, Herts. Mangoes, cultivar 'Ngowe', were obtained from Geest Associates Ltd., Burnham, Bucks. Mature, unripe mangoes were hard with dark green skins and 12–15 cm in length. The underlying mesocarp tissue at this stage was white in colour. Fruits which were allowed to ripen at room temperature, possessed bright

Table 4. Monosaccharide compositions of pooled polysaccharide fractions from unripe and ripe mango mesocarp tissues separated on DEAE-Sephadex A 25 columns (see Fig. 3)

	Monosaccharide (%)*			
	Unripe		Ripe	
Monosaccharide	'Neutral' fraction	Acidic fraction [U1; see Fig. 3(a)]	'Neutral' fraction	Acidic fraction [R1; see Fig. 3(b)]
Gal	21.0	24.2	16.5	4.1
Glu	12.3	9.1	12.0	2.8
Man	5.4	5.7	7.5	1.1
Ara	22.9	23.5	34.5	4.8
Xyl	27.3	22.1	23.3	8.9
Rha	2.7	2.7	1.5	1.0
Uronic acids	8.4	12.7	4.7	77.5

*Based on total monosaccharides released by acid hydrolysis.

yellow skins with soft and dark yellow mesocarps. Fruits were stored as necessary at −20°.

Analyses. N_2 analyses of cell wall preparations were carried out by Elemental Micro-Analysis Limited, Devon and the approximate protein contents obtained by multiplying % N by 6.25; average values were calculated from 3 estimates for each cell wall sample. Total carbohydrate in the column fractions was measured by the PhOH–H_2SO_4 method of Dubois [18] with some modification in the assay vols. To the aq. carbohydrate soln (1 ml), PhOH (25 μl; 80% w/v in H_2O) was added followed by conc. H_2SO_4 (2.5 ml). The A_{480} was noted after cooling the mixture. A standard curve was prepared using glucose. Hydrolysis of cell wall and other polysaccharide fractions was achieved by adding 2 M trifluoroacetic acid (TFA; 1 ml) to the polysaccharide (*ca* 0.2 mg) in a screwcap glass bottle and heating for 6 hr at 100°. Acid was removed by drying over KOH *in vacuo*. The galacturonic acid and neutral sugar content of cell wall hydrolysates was measured by conversion to alditol acetate derivatives followed by GC [19]. The neutral sugars present in the TFA hydrolysates of cell wall fractions and water-soluble cytoplasmic polysaccharides were determined by quantitative TLC [20]. The uronic acid in these hydrolysates was determined by the method of ref. [21].

Preparative methods. Cell wall material used for protein and monosaccharide analyses (Table 1) was prepared by a method based on that of ref. [22]. Mango mesocarp was thinly sliced longitudinally, macerated in 20 mM Pi buffer, pH 6.9 at 4° and centrifuged at 40 000 *g* for 1 hr. The pellet was suspended and further washed with buffer followed by water then stirred at room temp with Me_2CO for 1 hr and filtered on sintered glass. The product was finally washed with $CHCl_3$–MeOH (1:1) and dried over P_2O_5 under vacuum.

Cell wall material used for polysaccharide fractionation (Table 2) was prepared by taking equal amounts of mesocarp tissue (20 g) from 5 unripe or ripe mangoes and homogenising in 50 mM NaOAc–AcOH buffer (100 ml), pH 5.0, at 4°. Following sonication (4 × 30 sec with 2 min intervals) the suspension was filtered through 5 layers of muslin and the residue generously washed with cold H_2O. The residue was then resuspended in the same buffer, sonicated , washed and filtered. This protocol was repeated until starch granules were not detectable microscopically in the prepn after I_2 staining. The wall material was finally washed with $CHCl_3$–MeOH and dried as described above. Water-soluble cell wall fractions were prepared by suspending the cell walls in water (10 mg/ml) followed by heating in a boiling water bath for 6 hr and filtering on sintered glass. The residue was washed with boiling water and filtrates pooled and freeze dried. The alkali-soluble cell wall fractions were prepared from the hot-water residues by incubation with 4 M NaOH (10 mg residue/ml) at 25° for 4 hr under N_2. The suspensions were filtered through sintered glass and the residues washed with NaOH. The final 'cellulosic' residues were washed with 80% aq. EtOH and freeze-dried. To the combined alkaline filtrates and washings was added 80% aq. EtOH (3 vols). After leaving overnight at −20° the ppts were separated by filtration, washed with 80% EtOH and freeze-dried. The water-soluble carbohydrate preparations from mango mesocarps were prepared by homogenising the tissues (100 g; 20 g taken separately from 5 unripe or ripe mangoes) in 50 mM NaOAc–AcOH buffer (1 mg/g), pH 5.0 at 4°. The suspensions were centrifuged at 10 000 *g* for 1 hr and the soluble carbohydrate contents of the supernatants determined by the PhOH–H_2SO_4 method [18]. The solns were analysed for mono- and oligo-saccharides [20]. Dialysed samples (1 ml) of the above solns were gel-filtered using a Bio-Gel P-150 column (76 × 0.8 cm) equilibrated with 50 mM NaOAc–AcOH buffer, pH 5.0, and eluted with the same buffer (0.1 ml/min). The fractions (2 ml/fraction) were assayed for total carbohydrate [18], and uronic acid [21]. The gel column was calibrated using the following polysaccharide standards; Dextran T10 (M_r 10 000), Dextran T40 (40 000), Dextran T70 (70 000) and Blue Dextran 2000 (for determining the void vol.).

Dialysed samples (4 ml) of soluble carbohydrates were also applied to a DEAE-sephadex A-25 column (11 × 2.5 cm), equilibrated with 50 mM NaOAc buffer, pH 5.0. This buffer was also used for elution (4 ml/fraction). Unretarded carbohydrates ('neutral' fractions) eluting in the first 50 fractions were pooled and freeze-dried. The column-bound carbohydrates were eluted with a NaCl gradient, obtained by using 200 ml of equilibrating buffer and 200 ml 0.8 M NaCl soln in this buffer. Fractions were assayed for carbohydrate [18]. Carbohydrate-containing peaks were pooled, dialysed and freeze-dried for further analysis.

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