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Compositional changes in cell wall polymers during mango fruit ripening

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Softening of mango fruit has been investigated by analysis of ripening related changes in the composition of the fruit cell walls. There is an apparent overall loss of galactosyl and deoxyhexosyl residues during ripening, the latter indicating degradation of the pectin component of the wall. The loss of galactose appears to be restricted to the chelator soluble fraction of the wall pectin, whilst loss of deoxyhexose seems to be more evenly distributed amongst the pectin. The chelator soluble pectin fraction is progressively depolymerised and becomes more polydisperse during ripening. These changes are similar to those occurring in other fruit and are related to the action of wall hydrolases during ripening.

INTRODUCTION

The textural changes which occur during fruit ripening are thought to be related to alterations in cell wall structure (Huber, 1983; Tucker & Grierson, 1987). These changes are often most apparent in the pectic components of the wall and usually involve an increase in pectin solubility, a decrease in pectin molecular weight and a loss of pectic natural sugars such as arabinose and galactose from one or more of the pectic fractions (Seymour *et al.*, 1987, 1990; Gross & Sams, 1984). The exact biochemical mechanisms which are responsible for these events are not clear, but it is likely that some of these wall modifications are brought about by pectolytic enzymes such as polygalacturonase (PG), pectinesterase (PE) or galactosidase.

Cell wall changes have been studied in several fruit. Perhaps the most extensively studied is the tomato fruit. In this fruit, pectin is solubilised during ripening but this would appear to be independent of either PG or PE activity (Tucker, 1992). The soluble pectin is depolymerised during ripening and this aspect of wall metabolism has been closely linked to the level of PG activity (Smith *et al.*, 1990). There is a loss of neutral sugar, predominantly galactose, during ripening and this appears to come from both the soluble and insoluble pectin fractions of the wall (Seymour *et al.*, 1990).

Very little is known about the structure of the cell walls of tropical fruit such as mango. Roe and Bruemner (1981) examined gross changes in the cell walls of 'Keitt' mangoes. They solubilised material from mesocarp alcohol insoluble residues with water, ammonium oxalate and dilute alkali. Their data indicated that softening in mangoes was accompanied by a decline in the amounts of both water and alkaline soluble pectin. In contrast, oxalate soluble pectin increased during ripening. The decline in alkaline soluble pectin was most closely correlated with the loss of firmness of the mango fruit and was also closely correlated with the increase in PG activity observed as the fruit ripened. However, the levels of PG activity detectable in ripe mango fruit were about 300-fold lower than those found in ripe tomato fruit. Brinson *et al.* (1988) analysed total cell wall sugar content in 'Ngowe' mango fruit and showed a net loss of arabinosyl, galactosyl and galacturonosyl residues during ripening. This study, however, used water based methods of cell wall preparation which may have resulted in a loss or degradation of soluble polymers during wall extraction (Seymour *et al.*, 1987). In a similar study, but taking measures to eliminate enzymic degradation of wall polymers during the extraction, Tucker and Seymour (1991) demonstrated a loss of galactosyl residues during ripening of 'Sensation' mango fruit. More recently Mitcham and McDonald (1992) analysed total cell wall changes in 'Keitt' and 'Tommy Atkins' mango fruit. In both cases there was a ripening associated increase in soluble pectin and a decline in arabinosyl, rhamnosyl and galactosyl residues.

In the present study, some of the structural features of pectic polymers from 'Tommy Atkins', a commer-

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cially important mango variety are examined. This work is intended to form the basis for identifying the factors responsible for changes in the pectic components of mango fruit during ripening.

MATERIALS AND METHODS

Plant material

Hard green preclimacteric mango fruit (*Mangifera indica* L.) cultivar Tommy Atkins were obtained from a commercial source in the UK. Five preclimacteric fruit were peeled, and the mesocarp tissue was frozen in liquid nitrogen and stored at −20°C. The remaining fruit were ripened at 20°C, under high humidity, following exposure to ethylene gas at 10 μl/l for the first 24 h. Fruit were taken at various times following initiation of ripening, pulp firmness was measured using a penetrometer (Medlicott, 1985) and the mesocarp tissue frozen and stored as described above.

Preparation of cell wall material

Acetone insoluble solids (AIS) were prepared by powdering the frozen mango mesocarp tissue in an electric coffee grinder and then homogenising in acetone as described by Seymour *et al.* (1987). The AIS were then treated with phenol : acetic acid : water to inactivate endogenous enzymes as described by Seymour *et al.* (1987).

Fractionation of the AIS

The AIS was fractionated into CDTA (1,2-diaminocyclohexane *N,N,N,N*-tetra acetic acid), Na_2CO_3 and KOH soluble material by procedures similar to those described by Jarvis *et al.* (1981), Jarvis (1982), Gross (1984) and Redgewell and Selvendran (1986). AIS (400 mg) were incubated, while stirring, in 80 ml of 50 mM sodium acetate, 50 mM CDTA, pH 6·5 for 6 h at room temperature. The suspension was then filtered through GF/A paper and the filtrate collected. The residue was extracted in 80 ml of 50 mM Na_2CO_3 for 20 h at 1°C and then for a further 2 h at 20°C. The suspension was again filtered and the residue further extracted with 4 KOH containing 20 mM $NaBH_4$ for 2 h at 20°C under nitrogen. All filtrates were collected, neutralised as appropriate, extensively dialysed against water and stored frozen at −20°C.

Carbohydrate analysis

Uronic acids were determined using the colorimetric method of Blumenkrantz and Ashboe-Hansen (1973). The neutral sugar content of AIS or freeze dried fractions was determined using a modified Seaman hydrolysis (Selvandran *et al.*, 1979). The sugars released were converted to their alditol acetates as described by Englyst and Cummings (1984). These derivatives were then analysed by GC using an FID detector and a Supelco SP-2330 fused silica glass capillary column (30 cm × 0·75 mm) operating at 200–240°C at 4°C/min during each analysis.

Starch determinations were made on the AIS and all related fractions. Starch was estimated using a Starch analysis kit (Boehringer Mannheim) by following the manufacturer's instructions. AIS (12 mg) or related fractions (up to 10 mg) were used and starch was solubilised prior to analysis, using DMSO in 8 M HCl as recommended by the manufacturers.

Pectin molecular size determination

Molecular size distributions were obtained by gel filtration chromatography on Sephacryl S-500. CDTA fractions were concentrated by rotary evaporation to about 1 mg/ml. Concentrated sample (2 ml) was then applied to a S-500 column (1·6 cm × 75 cm) and eluted with 0·1 M sodium acetate, 20 mM EDTA pH 6·5. Fractions were assayed for uronic acid as described previously.

The weight-average molecular weights of the chelator soluble pectins from mangoes at days 0 and 6 were determined using analytical ultracentrifugation. Sedimentation equilibrium experiments were carried out in 30 mm pathlength cells using a Beckman Model E analytical ultracentrifuge equipped with Rayleigh interference optics. Rotor speed was 9948 r.p.m. The pectins were dissolved in a buffer comprising sodium acetate (50 mM) and CDTA (40 mM) (pH = 4·5).

The (apparent) molecular weights were calculated from the solute distribution at sedimentation equilibrium using the method described in Creeth and Harding (1982). Several concentrations of solution were used in each case and the apparent molecular weight values extrapolated to infinite dilution to obtain the weight-average molecular weight.

RESULTS AND DISCUSSION

Preclimacteric mangoes, cultivar Tommy Atkins, were initiated to ripen with ethylene (day 0) and the course of ripening followed over 6 days by measurement of ethylene evolution, CO_2 production and changes in mesocarp pH and soluble solids. The fruit exhibited a classical climacteric pattern for both ethylene production and respiration. These both peaked at 2 days from initiation and then declined. The fruit changed colour and underwent increases in both pH and soluble solids (data not shown). These changes are as expected for mangoes undergoing normal ripening. Changes in pulp texture were also measured and are illustrated in Fig. 1. Unripe preclimacteric fruit (day 0) had a firm texture which declined rapidly over the first 4 days of ripening.

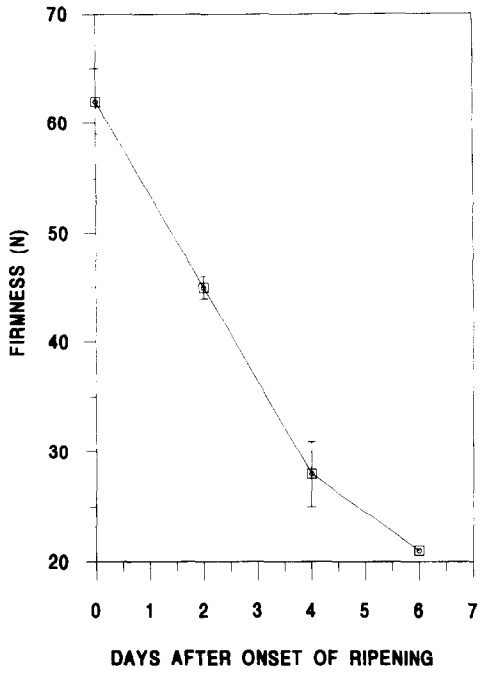

Fig. 1. Changes in Tommy Atkins fruit texture during ripening at 20°C.

At day 6 the fruit were post climacteric and fully ripe as judged by both colour development and texture.

Acetone insoluble solids, freed from any detectable endogenous cell wall degrading activity, were prepared from fruit mesocarp at various stages of ripening. The AIS consisted primarily of cell wall material and starch. In Tommy Atkins mangoes, starch accounted for about 11% of the dry weight of the AIS from unripe fruit, declining to a point at day 6 where no starch was detectable.

Changes in overall sugar composition of Tommy Atkins fruit cell walls during ripening are shown in Table 1. The values in this table have been corrected for the contribution made by starch and free glucose. These data indicate that there are significant changes in the levels of several cell wall associated sugars during ripening, particularly decreases in the levels of glucosyl and galactosyl residues. Loss of glucosyl residues during ripening may reflect our inability to estimate with complete accuracy all the glucose present as starch and may, therefore, represent starch degradation. The changes in galactosyl residues imply degradation of the neutral pectic components of mango cell walls. Loss of galactosyl residues has been reported in many types of fruit during ripening (Gross, 1984; Gross & Sams, 1984).

Table 1. Changes in overall sugar composition of Tommy Atkins fruit cell walls during ripening (SE $n = 3$)

Sugar residue (mg/g fresh wt)	Days after onset of ripening			
	0	2	4	6
Deoxyhexose	0·7 ± 0·19	0·5 ± 0·04	0·6 ± 0·17	0·3 ± 0·04
Arabinose	1·6 ± 0·19	2·0 ± 0·08	1·4 ± 0·04	1·5 ± 0·06
Xylose	1·0 ± 0·09	1·0 ± 0·02	0·9 ± 0·03	0·7 ± 0·04
Mannose	0·4 ± 0·14	0·4 ± 0·01	0·3 ± 0·01	0·3 ± 0·02
Galactose	1·6 ± 0·25	1·9 ± 0·02	1·1 ± 0·06	0·8 ± 0·04
Glucose	10·5 ± 0·55	14·6 ± 0·11	7·5 ± 0·38	5·8 ± 0·32
Uronic acid	13·7 ± 1·70	17·0 ± 1·75	16·9 ± 0·59	12·9 ± 0·25

The AIS from Tommy Atkins mangoes were sequentially extracted with CDTA, Na_2CO_3 and KOH to yield chelator soluble pectin rich, carbonate soluble pectin rich and hemicellulose rich fractions, respectively. The residue remaining after these extractions is essentially α-cellulose. The sugar compositions of these fractions are shown in Tables 2 and 3. The CDTA soluble fraction showed an approximately 50% decrease in galactosyl content during ripening. There was little or no loss of galactose from any of the other fractions. Thus it is likely that the changes in galactosyl residues observed in the total AIS are due to changes occurring mainly in the chelator soluble fraction. Deoxyhexose in comparison seems to be lost from both the chelator and carbonate soluble pectin rich fractions.

Analysis of the yields of material solubilised by CDTA and Na_2CO_3 at different stages of ripening

Table 2. Composition of CDTA and Na_2CO_3 soluble material from Tommy Atkins fruit cell walls at various days after the onset of ripening (SE $n = 3$)

Fraction	Days after the onset of ripening	Deoxy-hexose	Arabinose	Xylose	Sugar (mol%) Mannose	Galactose	Glucose[a]	Uronic acid
CDTA	0	24 ± 2·8	11 ± 2·0	1 ± 0·1	t	8 ± 1·5	3 ± 0·4	53 ± 5·0
	2	9 ± 1·0	24 ± 3·6	1 ± 0·1	t	7 ± 2·0	3 ± 0·3	56 ± 3·5
	4	9 ± 1·2	26 ± 2·9	1 ± 0·1	t	6 ± 0·1	2 ± 0·1	57 ± 1·4
	6	8 ± 1·8	24 ± 1·8	1 ± 0·0	t	4 ± 0·2	2 ± 0·1	65 ± 3·0
Na_2CO_3	0	12 ± 1·6	6 ± 0·6	1 ± 0·7	1 ± 0·1	5 ± 1·0	3 ± 0·7	74 ± 2·5
	2	7 ± 0·7	10 ± 1·3	1 ± 0·1	t	7 ± 1·8	1 ± 0·2	74 ± 3·4
	4	4 ± 0·8	10 ± 3·4	2 ± 0·3	1 ± 0·4	7 ± 2·2	2 ± 0·5	74 ± 6·3
	6	4 ± 1·0	7 ± 0·7	1 ± 0·1	t	5 ± 1·0	1 ± 0·1	82 ± 2·6

[a] All glucose values have been corrected for contributions made by starch and free glucose.
t = trace.

Table 3. Composition of KOH soluble and residue material from Tommy Atkins fruit cell walls at various days after the onset of ripening (SE $n = 3$)

Fraction	Days after the onset of ripening	Deoxy-hexose	Arabinose	Xylose	Sugar (mol%) Mannose	Galactose	Glucose[a]	Uronic acid
KOH	0	12 ± 0·2	5 ± 0·2	23 ± 2·6	4 ± 0·5	7 ± 1·3	23 ± 2·3	27 ± 8·3
	2	12 ± 1·8	5 ± 0·2	25 ± 1·2	5 ± 0·4	9 ± 1·0	28 ± 1·6	16 ± 4·7
	4	11 ± 1·3	3 ± 0·4	31 ± 1·0	6 ± 0·3	9 ± 1·2	27 ± 2·1	14 ± 4·1
	6	16 ± 1·2	2 ± 0·4	31 ± 0·5	6 ± 0·5	9 ± 0·2	24 ± 2·1	13 ± 4·6
Residue	0	6 ± 2·3	4 ± 0·6	5 ± 0·6	5 ± 4	3 ± 0·1	72 ± 5·4	5 ± 1·8
	2	3 ± 1·0	4 ± 0·1	6 ± 0·6	2 ± 0·1	3 ± 0·1	74 ± 3·7	5 ± 1·8
	4	4 ± 1·7	2 ± 0·5	8 ± 1·8	1 ± 0·3	2 ± 0·1	74 ± 2·6	11 ± 2·2
	6	4 ± 1·8	1 ± 0·3	9 ± 0·7	1 ± 0·1	2 ± 0·2	72 ± 2·9	10 ± 0·7

[a]All glucose values have been corrected for contributions made by starch and free glucose.
t = trace.

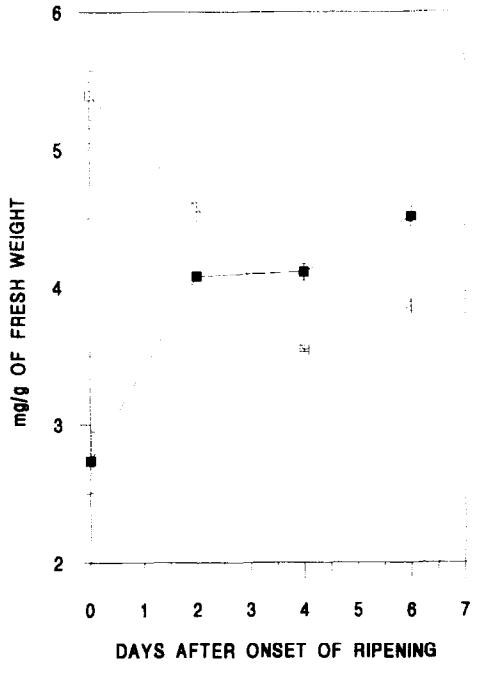

Fig. 2. Weight of CDTA (■) and Na_2CO_3 (□) soluble cell wall material from Tommy Atkins fruit cell walls during ripening.

revealed that an increase in the CDTA fraction was accompanied by a corresponding decrease in the Na_2CO_3 fraction (Fig. 2) and the combined yields of the two fractions remained approximately constant. These observations suggest that the increase in the amount of material extracted with CDTA during ripening may result from the altered solubility of polymers associated with the carbonate fraction in unripe fruit.

The CDTA soluble polyuronides were further analysed for molecular weight distribution by gel filtration on Sephacryl S-500 (Fig. 3). The results show that a progressive depolymerisation is occurring in this fraction during ripening. This results in both a decrease in the average molecular weight and an increase in the polydispersity of the polymers. The absolute molecular weights of the chelator soluble fraction from day 0 and day 6 fruit were determined, by sedimentation equilibrium ultracentrifugation, to be 200,000 + 10,000 and 78,000 + 4,000, respectively.

This study has used only one mango cultivar, Tommy Atkins. It is possible that changes in the cell walls of other mango cultivars may be different. Very few comparative studies of ripening changes in major mango cultivars have been reported (Lizada, 1991). It would appear, however, that mango cell walls undergo changes during ripening which are similar to those occurring in a wide range of fruit (Huber, 1983; Tucker & Grierson, 1987; Brady, 1987). These changes can be summarised as a loss of galactose, increased chelator solubility of polyuronides and a progressive depolymerisation of these soluble polymers. However, the significance of these changes with respect to the structural properties of the cell wall and textural changes in fruit is unknown.

The enzymic mechanisms acting in mango may, therefore, also be similar to those in other fruit. Precise biochemical mechanisms for these cell wall changes are again unknown. Depolymerisation of pectin could result from the action of PG and perhaps pectinesterase (PE), while loss of galactose may be the result of galactanase action. All three enzyme activities have been found in mango fruit (Roe and Bruemner, 1981; Lazan *et al.*, 1986; Muda & Tucker, personal communication). However, compared with many fruit, PG and PE activity in mango is comparatively low, and in some cases, PG is undetectable (Brinson *et al.*, 1988). In tomato fruit the inhibition of PG activity, to levels approaching those found in mango, resulted in a gross inhibition of pectin depolymerisation (Smith *et al.*, 1990). Both mangoes and tomato (Gross, 1984) show a loss of galactose during ripening. Evidence from tomato implicates a β-1-4 galactanase activity in this change (Pressey, 1983; Pressey & Himmelsbach, 1984; Seymour *et al.*, 1990). Very high levels of β-galactosidase activity are apparent in mango fruit, and this activity increases dramatically during ripening (Muda and Tucker, unpublished). This observation, coupled with the

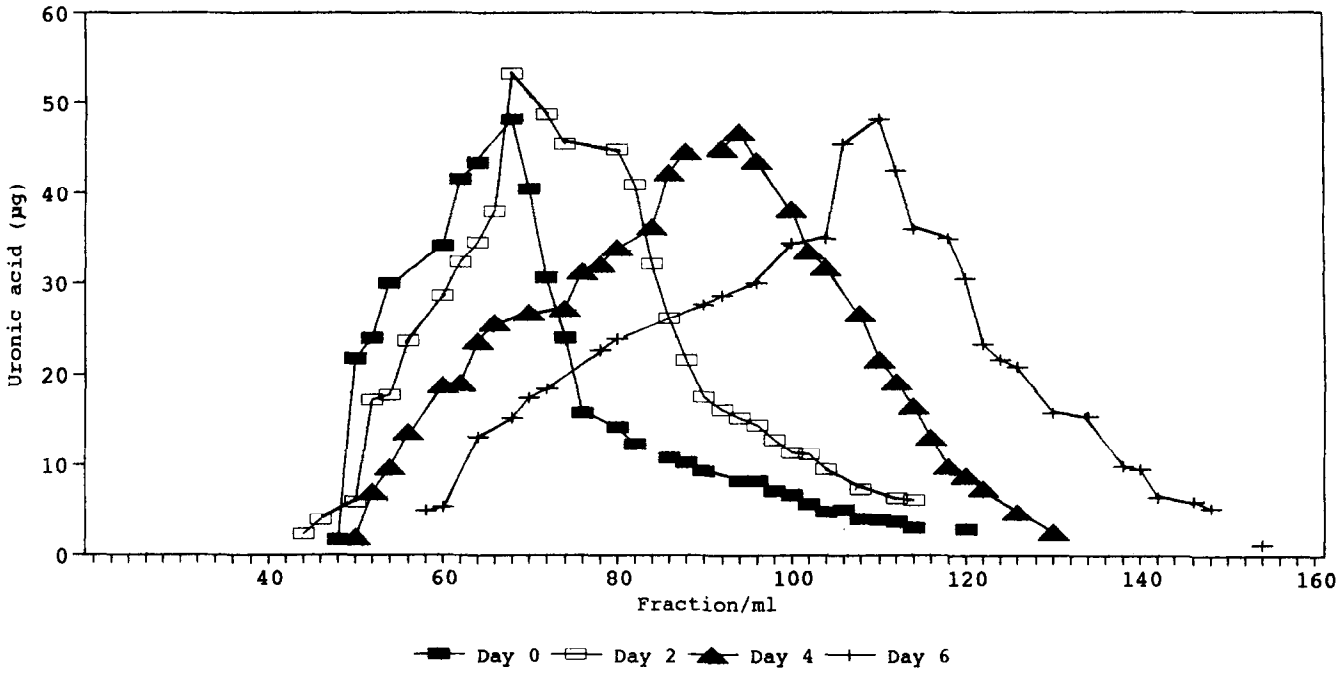

Fig. 3. Sephacryl S-500 profiles of polyuronides in the CDTA soluble material from Tommy Atkins cell walls at various days after the onset of ripening.

marked loss of galactose during ripening may imply a key role for this enzyme in texture change and this possibility is currently being investigated.

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