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Cell Wall Changes in Nectarines (*Prunus persica*)[1]

Solubilization and Depolymerization of Pectic and Neutral Polymers during Ripening and in Mealy Fruit

Debra M. Dawson*, Laurence D. Melton, and Christopher B. Watkins

University of Otago, P. O. Box 56, Dunedin, New Zealand (D.M.D., L.D.M.); and Horticulture and Food Research Institute of New Zealand, Private Bag 92 169, Auckland, New Zealand (D.M.D., C.B.W.)

ABSTRACT

Nectarine fruit (*Prunus persica* L. Batsch var nectarina [Ait] maxim) cultivar Fantasia were either ripened immediately after harvest at 20°C or stored for 5 weeks at 2°C prior to ripening. Fruit ripened after 5 weeks of storage did not soften to the same extent as normally ripened fruit, they lacked juice, and had a dry, mealy texture. Pectic and hemicellulosic polysaccharides were solubilized from the mesocarp of the fruit using phenol:acetic acid:water (PAW) treatment to yield PAW-soluble material and cell wall material (CWM). The carbohydrate composition and relative molecular weight (M_r) of polysaccharide fractions released from the CWM by sequential treatment with cyclohexane-*trans*-1,2-diamine tetra-acetate, 0.05 M Na_2CO_3, 6 M guanidinium thiocyanate, and 4 M KOH were determined. Normal ripening of nectarines resulted in solubilization of pectic polymers of high M_r from CWM during the first 2 d at ripening temperatures. Concurrently, galactan side chains were removed from pectic polymers. Solubilized pectic polymers were depolymerized to lower M_r species during the latter stages of ripening. Upon removal from cool storage, fruit had undergone some pectic polymer solubilization, and after ripening, pectins were not depolymerized and were of high M_r. Side chains did not appear to be removed from insoluble pectic polymers and branched pectins accumulated in the CWM. The molecular weight profiles obtained by gel filtration of the hemicellulosic fractions from normally ripening and mealy fruit were similar. The results suggest that mealiness results as a consequence of altered pectic polymer breakdown, including that associated with neutral side chains.

Mealiness or woolliness is a physiological disorder of nectarines and peaches (*Prunus persica*) when they fail to ripen normally after prolonged periods of cool storage, resulting in a lack of juice and a dry, mealy texture. Mealiness has been attributed to impaired solubilization of pectic substances with accumulation of insoluble low methoxyl pectin of high mol wt (2). Attempts to understand mealiness have focused on activities of PE[2] and PG, but results have been conflicting. Ben-Arie and Sonego (3) suggested that the accumulation of the high mol wt pectin was due to an increase in PE activity and inhibition of PG activity in cool stored fruit. Buescher and Furmanski (6) detected activity of PE and PG in mealy fruit, although at lower levels than in normal fruit, and proposed that mealy fruit had impaired capacity to provide adequate levels of both enzymes to ripen the fruit. In contrast, Von Mollendorff and de Villiers (21) reported increased PG activity during the ripening period in fruit that developed mealy characteristics, although activity was low during storage. No role for PE in the development of mealiness was found.

Another approach to the problem of mealiness development is to examine cell wall pectic polymer changes that have the commonality of accumulation of high M_r-insoluble pectins. Such information may be expected to give a greater understanding of the exact processes affected by prolonged periods of low temperature storage and the subsequent development of mealiness. Unfractionated cell wall extracts do not allow discrimination between pectic and hemicellulosic polymers. We have studied the sequence of cell wall changes in pectic polysaccharides during normal ripening and in fruit at removal from storage and after development of mealiness. Previous studies have reported changes in the cell walls throughout development (10) and in the pectic fractions of the wall at harvest and for ripe and mealy fruit (11). Therefore, hemicellulosic fractions have also been analyzed as these fractions have increasingly been found to undergo changes during ripening (12, 15, 20, 23). Significant amounts of pectin may be associated with hemicellulosic polymers (16).

MATERIALS AND METHODS

Plant Material

Nectarines (*Prunus persica* L. Batsch var nectarina [Ait] maxim) cv Fantasia were harvested in Central Otago in February 1990. A harvest sample (H) was taken and the remaining fruit were either ripened at 20°C with sampling after 2, 4, and 6 d (D2, D4, D6, respectively) or stored at 2°C for 5 weeks. Fruit stored for 5 weeks were sampled at removal from storage (W5) and after an additional 6 d at 20°C (M). Each fruit was measured for flesh firmness (kgf) using an Effegi penetrometer (FT 011) fitted with a 7-mm diameter

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[2] Abbreviations: PE, pectinmethylesterase; PG, polygalacturonase; CDTA, cyclohexane-*trans*-1,2-diamine tetra-acetate; GTC, guanidinium thiocyanate; PAW, phenol:acetic acid:water (2:1:1, w/v/v); CWM, cell wall material; DE, degree of esterification.

plunger, after the removal of a 1-mm thick disc of skin from the shoulder of the fruit. Extractable juice was measured using a modification of the cellular integrity test (14). A 1-cm diameter core from the fruit mesocarp was inserted into a 2.5-mL syringe barrel with a glass wool plug and centrifuged for 5 min at 5000*g*, after which the extractable juice was weighed. The density of the juice was determined and the extractable juice expressed as mL. At each sampling stage, 20 to 25 fruit were assessed, but only the five most "average" fruit for flesh firmness and extractable juice content were used for cell wall analysis.

Preparation of CWM

CWM was extracted from single samples composed of five fruit at progressive stages of ripeness (H, D2, D4, and D6), at removal from storage (W5), and from three mealy (M) samples. The samples were frozen in liquid nitrogen and stored at −80°C prior to grinding to a fine powder in a Waring grinder precooled with liquid nitrogen. The powder (200 g) was then homogenized in a Waring blender with 250 mL of PAW. The slurry was centrifuged at 5000*g* for 20 min at 4°C and the pellet was further washed with 250 mL of PAW followed by two washes with 200 mL of cold distilled water. All supernatants were combined, filtered through GF/A glass fiber filter, and lyophilized after exhaustive dialysis (M_r cut-off 14,000) against cold distilled water at 4°C (PAW-soluble material). The pellet was dialyzed as above and lyophilized to give CWM. Yields represent the dialyzed/lyophilized material recovered from an initial 200 g of frozen powdered mesocarp.

Fractionation of CWM

Pectic polymers were extracted using a modified method of Selvendran et al. (18). CWM (750 mg) was suspended in 75 mL of 0.05 M acetate buffer (pH 6.5) containing 0.05 M CDTA and stirred for 16 h at 23°C. The suspension was centrifuged (5000*g* for 20 min at 4°C) and the pellet was washed with 75 mL of cold distilled water. The combined supernatants were dialyzed exhaustively against distilled water and lyophilized to give the CDTA-soluble fraction.

To the pellet 75 mL of 0.05 $NaCO_3$ containing 0.02 M $NaBH_4$ was added and the suspension incubated with constant stirring for 16 h at 23°C. The suspension was centrifuged (5000*g* for 20 min at 4°C) and the pellet washed twice with 75 mL of cold distilled water. All supernatants were combined, dialyzed exhaustively against distilled water, and lyophilized to give the Na_2CO_3-soluble fraction. Neutral polymers were extracted from the cell wall as previously described (16) to yield GTC- and KOH-soluble fractions, and a KOH-insoluble fraction. Yields represent the dialyzed/lyophilized material recovered from an initial 750 mg of CWM.

Gel Filtration

The size distribution of pectic and neutral polymers from PAW-soluble and CWM was determined by gel filtration chromatography on a Bio-Gel A-50m column (1.6 × 65 cm). The column was equilibrated and eluted with 50 mM acetate buffer (pH 6.0) containing 0.125 M NaCl. Lyophilized material was rehydrated (5 mg/mL) in elution buffer with constant stirring for 8 h at 23°C. Rehydrated material was centrifuged (5000*g* for 10 min at 4°C) and 1 mL of the supernatant applied to the column. Fractions (1.7 mL) were collected and assayed for uronic acids and neutral sugars.

General Methods

Pectic polysaccharides were quantified as anhydrogalacturonic acid using the *m*-hydroxydiphenyl method (5). Reagents were made up as described by Kintner and Van Buren (13). The anthrone method of Dische (7) was used to quantify neutral sugars. Glucose was used as the standard. Protein was estimated using the micro Kjeldahl method (N × 6.25).

The DE of the CWM was determined using a modification of the method of Wood and Siddiqui (22). Sodium arsenite at 0.7 M was used instead of 0.5 M, and after addition of the dye, the sample was held for 1 h at room temperature.

Preparation of Derivatives for GLC

The monosaccharide composition of all materials was determined by capillary GLC. Samples (10 mg) were hydrolyzed in 1.5 mL of 2 M TFA at 121°C for 1 h. Samples were dried under nitrogen at 50°C, washed in 0.75 mL of 1 M NH_4OH, and dried under nitrogen at 50°C.

The hydrolyzed material was reduced to its alditols using the method of Fry (8) and acetylated using the method of Albersheim et al. (1). Chromatography was carried out on a Hewlett-Packard 589A GLC fitted with a SP-2380 fused silica capillary column (30 m × 0.25 mm i.d.) (Supelco, Inc.). The column was maintained at 100°C for 2 min and increased to a final temperature of 230°C at a rate of 25°C/min. *Myo*-inositol, added after TFA hydrolysis of wall materials, was used as an internal standard at 3 mg/mL.

RESULTS

After harvest, fruit ripened normally, softening to less than 1.5 kgf and reaching a high extractable juice content (Table I). On removal from storage at 2°C, fruit (W5) had softened slightly and had a higher extractable juice content than fruit at harvest (Table I). Fruit that ripened for 6 d at 20°C after storage softened but failed to ripen normally, developing mealy characteristics, i.e. the fruit had a low extractable juice content and a dry mealy texture.

Extraction of CWM

Ripening resulted in increased solubilization of CWM, reflected by a decreased ratio of CWM:PAW-soluble material (Table I). The ratio in W5 fruit was similar to that of H fruit, and that of mealy fruit was between H and D2 (Table I).

PAW removed greater than 75% of protein from CWM (Table II). Amino acid analysis revealed no differences in composition of the protein between ripening and/or storage treatments (data not shown). The DE of the pectin in the CWM decreased as the fruit ripened (Table II). Both the W5 and M fruit had a DE similar to that observed in normally ripened fruit (Table II).

Composition of the CWM and PAW-Soluble Materials

A 64% loss of uronic acid from the CWM at harvest occurred during normal ripening (Table II). Arabinose was the major neutral sugar residue present in the CWM. Ripening was associated with loss of arabinose and galactose and an increase in xylose was present in the CWM. No consistent trends were observed for rhamnose, glucose (Table II), fucose, or mannose (data not shown).

The PAW-soluble fraction represents polymers that would be found in an aqueous homogenate of the tissue. PAW-soluble uronic acid increased markedly as ripening progressed. PAW-soluble arabinose increased during ripening in parallel with the observed decrease in arabinose residues in the CWM. Glucose levels decreased (Table II). No consistent trends were observed for rhamnose, xylose, galactose (Table II), fucose, or mannose (data not shown) during ripening.

The uronic acid content of CWM of W5 fruit was lower than that of H and decreased further during subsequent ripening (Table II). Storage did not appear to alter the neutral sugar composition of the CWM, and M fruit appeared similar to D6 fruit (Table II). PAW-soluble materials from W5 and M fruit were similar and appeared similar to the PAW-soluble materials from fruit at H.

Table II. *Neutral Sugar Composition, Uronic Acid and Protein Content, and DE of the CWM and PAW-Soluble Material from Nectarines during Storage and Ripening Treatments*

Fraction	Composition[a]					Uronic Acid	Protein	DE
	Rha	Ara	Xyl	Gal	Glu			
	mol%						%	
CWM								
H	3.0	51.9	14.6	23.3	4.0	36.0	2.8	79.5
D2	3.3	47.8	17.6	20.3	6.9	28.5	5.0	79.1
D4	4.0	46.3	22.9	15.3	5.7		6.0	
D6	3.6	45.6	23.4	15.9	5.6	23.0	5.1	67.5
W5	2.6	53.4	20.1	15.1	4.9	29.3	3.4	68.8
M	4.6	45.1	22.4	16.5	6.6	32.3	6.7	69.8
PAW-Soluble								
H	1.1	20.8	4.4	15.7	54.7	7.0	25.0	
D2	2.5	39.1	4.8	17.5	33.0	28.0	19.3	
D4	1.8	56.7	4.8	14.8	18.4	21.0	17.2	
D6	2.9	65.7	6.0	13.6	7.8	20.8	18.0	
W5	0.9	29.2	5.3	16.9	44.8	12.5	17.6	
M	1.4	24.9	7.1	17.1	45.9	20.8	29.6	

[a] Anhydro-values after TFA hydrolysis. Values are the mean of duplicate analyses and for any value the error is less than 10%.

Composition of the Fractionated CWM

CDTA-soluble yields did not change appreciably during normal ripening, whereas the Na_2CO_3-soluble yield decreased. The hemicellulosic materials, GTC- and KOH-soluble, were less than 15% of the wall and varied only slightly in the amount of material solubilized from the CWM during ripening.

Fractionation of the CWM from stored tissue either with or without ripening showed increased yields of the two pectic fractions with CDTA-soluble material present at levels greater than at any stage of normal ripening and the Na_2CO_3-soluble fraction present at H levels.

As ripening progressed, the uronide content of both pectic fractions decreased (Table III). Arabinose was the major neutral sugar in the pectic fractions and increased throughout ripening. Galactose decreased during ripening in both the CDTA- and Na_2CO_3-soluble fractions. Rhamnose was present in variable amounts in the CDTA-soluble fraction, and in the Na_2CO_3-soluble fraction, levels decreased as the fruit ripened. Xylose in the CDTA-soluble fraction decreased between H and D2 and then between D4 and D6. However, xylose levels in the Na_2CO_3-soluble fraction increased as ripening progressed. The GTC- and KOH-soluble fractions contained about 10% uronic acid. During ripening, the arabinose, galactose, and rhamnose levels decreased, whereas the xylose and glucose levels increased.

W5 fruit had a similar CWM composition to fruit during the early stages of ripening (H to D2), whereas M fruit appeared similar to ripe fruit (Table III). However, fractionation of the CWM revealed differences between M and D6, especially in the pectic fractions. The CDTA-soluble fraction from M fruit contained uronic acid at D6 levels, yet the neutral sugar composition appeared similar to fruit between H and D2. The Na_2CO_3-soluble fraction from M fruit contained uronic acid levels between H and D2; rhamnose was present at levels greater than at any stage of ripening.

The hemicellulosic fractions, GTC- and KOH-soluble, contained uronic acid at levels comparable with ripe fruit (Table III). Xylose and glucose were the major residues present in these fractions. The composition of W5 fruit was similar to

Table I. *Flesh Firmness, Extractable Juice Content, and Yields of CWM and PAW-Soluble Materials Extracted from Nectarines during Storage and Ripening Treatments*

	Firmness	Extractable Juice	CWM	PAW-Soluble	Material Recovered	Ratio of CWM:PAW-Soluble Material
	kgf	*mL*		*g*		
H	4.80 ± 0.27	0.20 ± 0.02	2.69	1.04	3.73	1:0.4
D2	1.40 ± 0.09	0.49 ± 0.02	1.73	1.63	3.36	1:0.9
D4	0.65 ± 0.03	0.72 ± 0.02	1.17	1.88	3.05	1:1.6
D6	0.60 ± 0.03	0.75 ± 0.03	1.53	1.64	3.17	1:1.1
W5	3.44 ± 0.27	0.28 ± 0.03	2.89	1.13	4.02	1:0.4
M	1.02 ± 0.09	0.30 ± 0.01	2.19	1.38	3.57	1:0.6

that of partially ripened fruit (D2–D4), and that of M fruit was similar to D6 fruit.

Gel Filtration Chromatography

Uronic Acid-Rich Polymers

The M_r distribution of the uronic acid and neutral polymers from fractionated CWM and PAW-soluble material was determined by gel filtration in normal ripening and in W5 and M fruit. PAW-soluble material from fruit at harvest contained low amounts of uronic acid that were heterogeneous in M_r (Fig. 1). However, at D2 of ripening, considerable amounts of high and intermediate M_r polymers were present that at D6 had changed to predominantly intermediate and low M_r (Fig. 1). PAW-soluble material from W5 and M fruit contained similar amounts of uronic acid-rich polymer as D6 fruit, although unlike ripe fruit, the polymers were predominantly high and intermediate M_r (Fig. 1).

At H, the uronic acid-rich polymers in the CDTA-soluble fraction were predominantly of high M_r (Fig. 2). After D2 of ripening, high M_r species eluting at V_0 had decreased and by

Table III. *Neutral Sugar Composition, Uronic Acid Content, and Yield of Fractions from the CWM of Nectarines during Storage and Ripening Treatments*

Fraction	Composition[a]					Uronic Acid	Yield
	Rha	Ara	Xyl	Gal	Glu		
	mol%					%	
CDTA-Soluble							
H	4.2	58.5	12.5	18.9	3.5	45.0	24.0
D2	7.2	69.1	5.1	12.5	4.7	42.0	18.0
D4	3.6	73.3	5.0	10.4	3.7	ND[b]	18.6
D6	5.7	74.3	0.8	8.9	5.9	24.0	21.2
W5	8.3	62.7	4.6	12.0	6.4	24.4	29.7
M	7.3	59.1	11.5	14.9	3.9	29.3	30.6
Na_2CO_3-Soluble							
H	5.8	69.5	1.9	20.9	1.4	47.0	27.1
D2	5.0	79.5	1.8	12.1	1.1	31.0	24.5
D4	4.4	78.7	2.9	11.2	2.0	33.0	17.6
D6	3.3	79.0	3.0	11.5	2.4	32.5	17.3
W5	4.4	81.1	2.4	10.1	1.3	39.5	26.2
M	8.5	71.9	5.5	10.3	2.2	40.7	26.8
GTC-Soluble							
H	2.5	38.9	17.8	22.4	13.1	9.0	6.9
D2	2.4	34.7	22.6	18.7	14.4	5.0	9.2
D4	1.8	21.5	26.1	14.0	27.3	ND	7.3
D6	1.3	24.6	29.3	12.2	23.0	10.0	7.7
W5	2.0	34.1	19.7	13.9	21.6	12.5	6.6
M	1.1	13.6	32.6	15.1	27.5	9.0	4.5
KOH-Soluble							
H	0.8	27.0	28.0	17.9	19.8	7.0	3.3
D2	0.9	19.0	29.8	14.5	27.9	3.5	4.7
D4	0.3	8.2	41.6	11.9	29.8	ND	4.5
D6	0.5	8.5	46.7	12.0	25.8	ND	6.3
W5	0.4	12.7	38.2	16.6	21.8	4.7	10.1
M	0.4	8.2	32.7	13.5	36.0	4.1	5.2

[a] Anhydro-values after TFA hydrolysis. Values are the mean of duplicate analyses and for any value the error is less than 10%. [b] ND, Not determined.

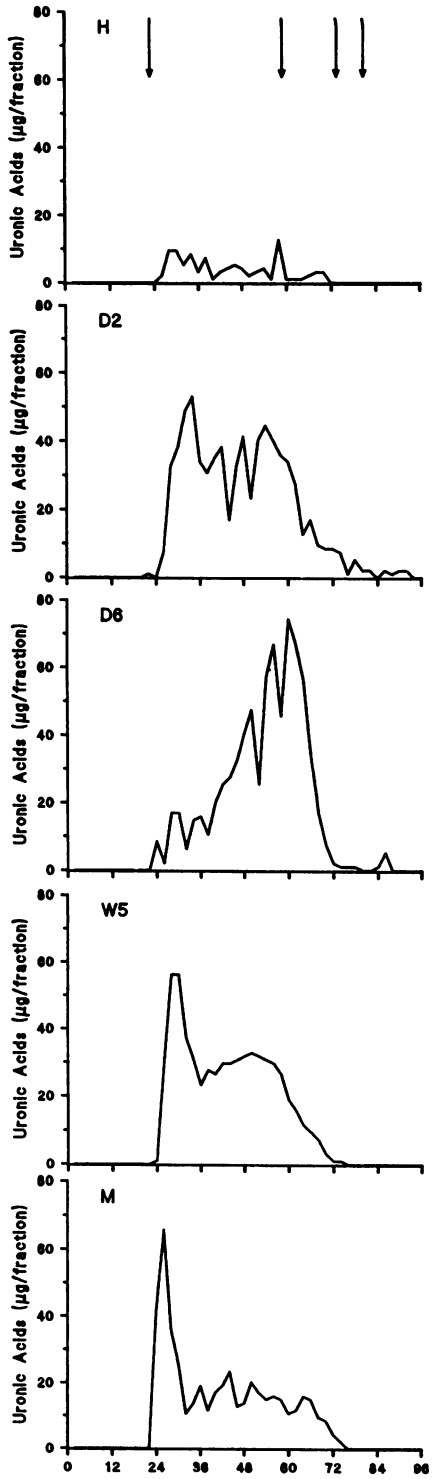

Figure 1. Gel filtration profiles of PAW-soluble materials from nectarine tissue during ripening, at removal from storage, and in mealy fruit on Biol-Gel A-50m. Column fractions (1.7 mL) were assayed for uronic acid using the *m*-hydroxydiphenyl method (5). The arrows represent, from left to right, the void volume and the dextran mol wt markers Dextran $M_r = 2 \times 10^6$, Dextran $M_r = 5 \times 10^5$, and Dextran $M_r = 4 \times 10^4$. H, Harvest; D2, 2 d at 20°C; D6, 6 d at 20°C; W5, removal from storage after 5 weeks at 2°C; M, mealy fruit.

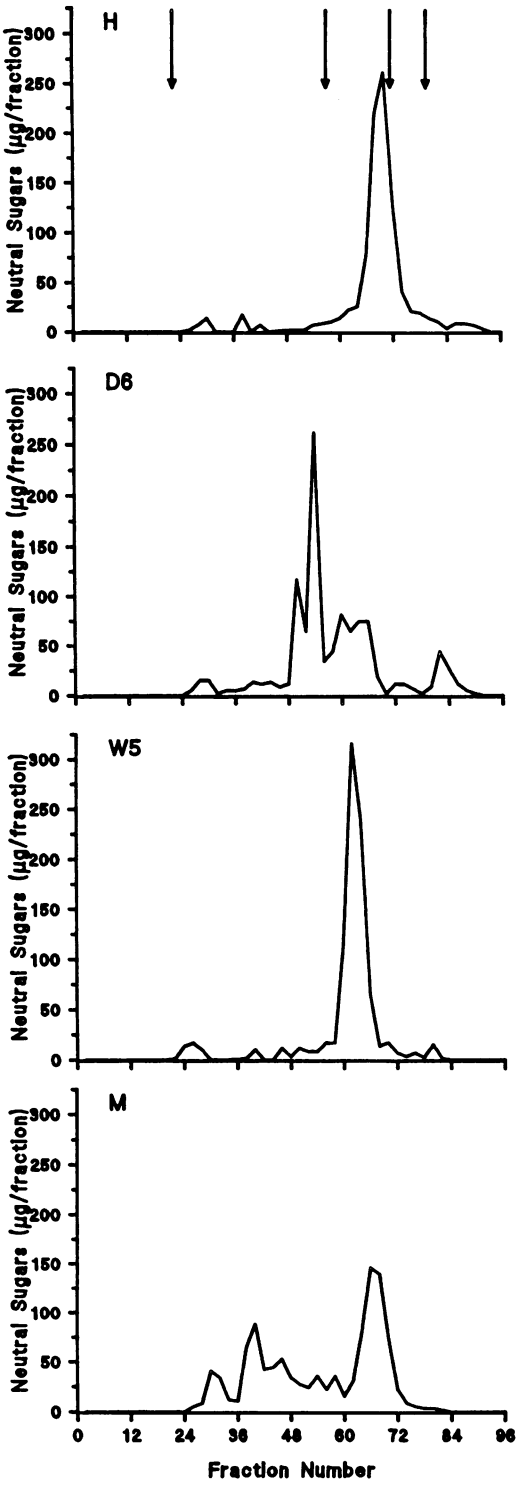

Figure 2. Gel filtration profiles of CDTA-soluble fraction from nectarine CWM during ripening, at removal from storage, and in mealy fruit on Bio-Gel A-50m. Column fractions (1.7 mL) were assayed for uronic acid content using the *m*-hydroxydiphenyl method (5). The arrows represent, from left to right, the void volume and the dextran mol wt markers Dextran $M_r = 2 \times 10^6$, Dextran $M_r = 5 \times 10^5$, and Dextran $M_r = 4 \times 10^4$. H, Harvest; D2, 2 d at 20°C; D6, 6 d at 20°C; W5, removal from storage after 5 weeks at 2°C; M, mealy fruit.

D6 of ripening had almost disappeared (Fig. 2). The CDTA-soluble fraction from W5 and M fruit contained large amounts of high M_r polymers eluting at V_0, similar to fruit after D2 of ripening (Fig. 2).

The M_r of the uronic acid-rich polymers present in the Na_2CO_3-, GTC-, and KOH-soluble fractions did not change appreciably during ripening. In the respective fractions, W5 and M fruit exhibited the same M_r profiles as D6 fruit (data not shown). However, the polymer distribution in the Na_2CO_3-soluble fraction remained unchanged during ripening, with a single intermediate M_r peak, but the amount of material progressively decreased (data not shown).

Neutral Sugar-Rich Polymers

Large amounts of neutral sugars were present in the PAW-soluble material (Fig. 3). At H, the polymers were predominantly low M_r species eluting at 120 mL. During normal ripening, this peak diminished and a higher M_r peak eluting at 100 mL, not present at H, became the major neutral species. W5 and M fruit contained similar low M_r species to those observed at H. However, M fruit also contained a high M_r peak eluting at 70 mL, which was not present at any stage of normal ripening (Fig. 3).

The M_r of neutral sugar-rich polymers in the CDTA-, Na_2CO_3-, GTC-, and KOH-soluble fractions did not change appreciably during ripening and/or storage treatments (data not shown).

DISCUSSION

Normal Ripening

Nectarine fruit held at 20°C after harvest ripened normally. Loss of flesh firmness and the increase in extractable juice content underwent the greatest change during the initial 2 d at 20°C (Table I), and materials extracted from the tissue at progressive stages of ripening revealed that the most dramatic changes in cell wall composition also occurred during this time. Although the total protein content, as a percentage of the sample, remained relatively constant throughout ripening, that associated with the CWM increased at the onset of ripening. This coincided with a decrease in the cell wall esterification, suggesting a change in ion-exchange properties of the wall rather than in vivo apoplastic protein content.

Cell wall analysis revealed that pectins, as indicated by uronic acid content, were solubilized from the wall during ripening (Table II). Concomitantly, soluble uronic acid polymers of high and intermediate M_r increased (Fig. 1). As ripening progressed, further uronic acid polymers were solubilized from the wall and these solubilized polymers were then depolymerized to lower M_r species. It appears that solubilization of pectins precedes their depolymerization and, therefore, depolymerization is not a necessary first step in the degradation of pectin, results also found in kiwifruit (18). The CDTA- and Na_2CO_3-soluble fractions both showed large losses of uronic acid during ripening, consistent with solubilization of pectins from the wall (Table III). Uronic acid-rich polymer from the Na_2CO_3-soluble fraction decreased dramatically at the onset of ripening. The rhamnose content of this fraction implied that the pectins were not highly

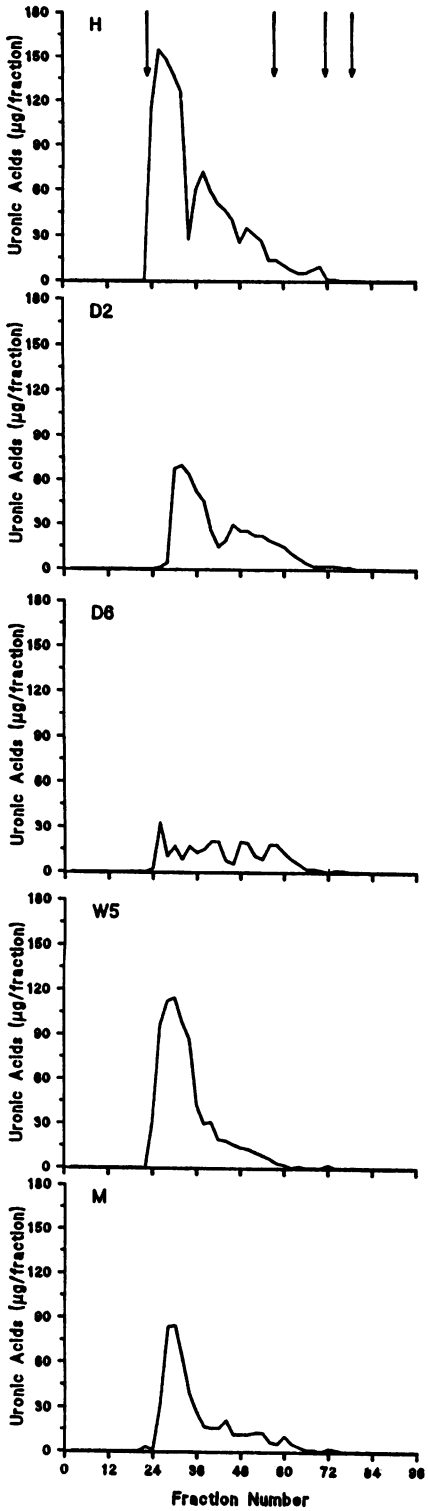

Figure 3. Gel filtration profiles of PAW-soluble materials from nectarine tissue during ripening, at removal from storage, and in mealy fruit on Bio-Gel A-50m. Column fractions (1.7 mL) were assayed for neutral sugars using the anthrone method (7). The arrows represent, from left to right, the void volume and the dextran mol wt markers Dextran $M_r = 2 \times 10^6$, Dextran $M_r = 5 \times 10^5$, and Dextran $M_r = 4 \times 10^4$. H, Harvest; D6, 6 d at 20°C; W5, removal from storage after 5 weeks at 2°C; M, mealy fruit.

branched. However, in the CDTA-soluble fraction, the uronic acid content did not show an immediate decrease at the onset of ripening and thus may indicate that material solubilized in vivo from the Na_2CO_3-soluble fraction then becomes ionically bound in the wall. It is not possible to categorically determine whether the PAW-soluble material is solubilized directly from the Na_2CO_3-soluble fraction or via the CDTA-soluble fraction.

Hobbs et al. (11) fractionated CWM from nectarines into two pectic fractions and an insoluble residue. They described a decrease in arabinose in all fractions, the greatest being in the insoluble residue, suggesting that arabinose may be hemicellulosic in origin. In the present study, CWM was fractionated into pectic- and hemicellulosic-rich fractions. The results show no loss of arabinose from the CDTA- and Na_2CO_3-soluble fractions during ripening. However, both hemicellulosic fractions showed large losses of arabinose. It is noteworthy that both the GTC- and KOH-soluble fractions contained significant amounts of pectin, which appeared to be highly branched due to the high rhamnose-to-uronic acid content. Therefore, it appears that the arabinose loss reported here is from highly branched pectins present in the cell wall in close association with hemicelluloses, which are deeply embedded in the wall matrix.

Loss of galactose was observed from all CWM fractions, both pectic and hemicellulosic, during ripening, yet no concomitant increase in soluble galactose was seen. Galactose may be present as polymeric galactans and/or arabinogalactans attached to the pectic backbone. No loss of arabinose was observed in the CDTA- and Na_2CO_3-soluble fractions, suggesting that galactan side chains were removed and that this was an early event in the ripening sequence. Removal of galactan side chains, by galactosidase activity or cell wall turnover, may allow loosening of the structure of the wall and improved solubilization of pectins. Whether galactan side chain removal is a prerequisite for pectic solubilization is unclear. Galactose in the hemicellulosic fractions is most probably of pectic origin. A net decrease in cell wall galactose (9, 17) and increases in soluble galactose during ripening of tomato and kiwifruit have been reported (17). Although no increase in soluble polymeric galactose was found in our study, it is possible that galactose polymers solubilized from the CWM are depolymerized, the products of this being oligomers of sufficiently low M_r to enable them to pass through the dialysis membrane and be lost (17). However, whether the loss of galactose is due to increased solubilization or metabolism is unclear.

The large amount of glucose present in the PAW-soluble material at harvest may be due to a soluble glucan, as this material gave a negative KI/I_2 starch test. Low amounts of glucose were associated with the CWM. Fractionation of the wall showed the glucose to be present in the hemicellulosic fractions and insoluble residue. The xylose-to-glucose ratio in the GTC- and KOH-soluble fractions suggests that glucose is present as xyloglucan, and that this is the major hemicellulose found in nectarine tissue. The xylose content of the PAW-soluble material increased slightly during ripening, whereas in the CDTA-soluble fraction there is a concomitant decrease in xylose. This effect has also been observed by Hobbs et al. (11).

The apparent increase in M_r observed in the PAW-soluble material (Fig. 3) can be explained by changes in polymeric neutral sugars during ripening. At harvest, 55 mol% of residues were glucose and 21 mol% arabinose, whereas in ripe fruit glucose residues make up only 8 mol% and arabinose 66 mol%. The predominantly arabinose-rich polymer solubilized from the CWM during ripening is of higher M_r than soluble material present at harvest. No changes in the M_r were detected in other fractions for neutral sugars during ripening.

The data presented suggest that during the initial stages of ripening solubilization of high M_r uronic acid-rich polymers from the cell wall occurred and, concurrently, galactan side chains were removed from pectic polymers retained in the CWM. These changes coincided with the greatest loss of flesh firmness and increase in extractable juice content. After 2 d of ripening, solubilized uronic acid-rich polymers were depolymerized to lower M_r species. Solubilization of the polymers continued after D2 but at a slower rate than during the initial 2 d. Arabinose was lost from pectins and an increase in soluble neutral polymers of intermediate M_r was observed. During the latter stages of ripening (D4–D6), only minor compositional changes were observed.

Mealy Fruit Ripening

Extraction and fractionation of CWM from fruit at removal from storage and mealy fruit revealed differences in uronic acid and neutral sugar composition, and uronic acid depolymerization relative to harvest and normal ripening. Cell wall analysis showed that solubilization of pectins from the CWM had occurred during cool storage, as indicated by increased uronic acid in the PAW-soluble material. During subsequent ripening, further pectin solubilization was not as extensive as during normal ripening, consistent with previous findings (4). Gel filtration showed the solubilized uronic acid-rich polymers to be of high M_r, and unlike normal ripening, these polymers were not depolymerized to lower M_r species. Inhibition of PG activity in fruit at low temperatures has been suggested (3, 4) and therefore solubilization of pectins by PG would not occur. The extent of pectic solubilization in our study suggests that inhibition is not complete in vivo at low temperatures or that enzymic processes are not involved in the solubilization observed. Subsequent failure to show a substantial decrease in the M_r of pectic fractions does indicate that PG was largely inhibited. Activity of PE was not affected by low temperatures, as shown by the similar DE in fruit at removal from storage and in ripe fruit.

Fractionation of the CWM from mealy fruit also resulted in increased yields of pectic fractions, relative to normal ripening (Table III), consistent with previous findings (3, 4, 6). Loss of uronic acid-rich polymer from the Na_2CO_3-soluble fraction during ripening after storage may involve conversion to the CDTA-extractable form, which may, therefore, remain ionically bound in the wall. This may explain the increased CDTA-soluble fraction yield in mealy fruit. Subsequent extraction of uronic acid from the CDTA fraction would therefore account for the uronic acid from the CWM that appears in the PAW-soluble material.

The CDTA and Na_2CO_3 fractions both contained high rhamnose levels, relative to normal ripening (Table III). Rhamnose present along the pectic backbone is a major site of side chain attachment. Therefore, the increased amounts of rhamnose in these pectic fractions suggest that branched pectins remain in the CWM and are not solubilized during ripening in mealy fruit. This is supported by the neutral sugar analysis for the CDTA and Na_2CO_3 fractions, both of which are similar to fruit at harvest. Therefore, it appears that without removal of neutral side chains, branched pectins are not solubilized from the CWM and that removal of side chains is an essential part of pectic solubilization. The factor limiting solubilization of pectic polymers from mealy fruit may be removal of neutral sugar side chains.

The neutral sugar composition of hemicellulosic fractions from W5 and M fruit is generally similar in composition to that found in normally ripened fruit, with W5 fruit being comparable to partially ripe fruit (D2–D4) and M fruit being comparable to fully ripe fruit (D6). However, there are some exceptions when M fruit are compared with normally ripened fruit. The xylose and glucose levels in the KOH-soluble fraction are different for M and D6 fruit. Whether a xyloglucan or xylan or glucan is involved in mealiness will require further investigation.

It is concluded that mealiness predominantly affects the pectic component of the cell wall. Solubilization of uronic acid-rich polymers in mealy fruit is altered relative to that in normally ripened fruit. Solubilized polymers are of high M_r and are not depolymerized to lower M_r species. During cool storage, pectins in the cell wall are deesterified, suggesting continued activity of PE at low temperature. Branched pectins accumulate in the CWM and galactan side chains, which are removed from the pectic backbone during the initial stages of normal ripening, remain attached to the backbone in mealy fruit.

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