Analysis of Cell Wall Components in Juice of 'Flavortop' Nectarines during Normal Ripening and Woolliness Development

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ABSTRACT. Harvested nectarine fruit [*Prunus persica* (L.) Batsch 'Flavortop'] were held for 5 days at 20 °C, or stored at 0 °C either immediately (control), or after 2 days at 20 °C (delayed-cooling). Observations were conducted after removal from storage for 1, 3, or 5 weeks and a shelf life of 5 additional days at 20 °C. After 5 weeks storage, 87% of control fruit developed woolliness (mealiness in texture accompanied by dry tasting fruit as a result of reduced juice content), while only 7% of delayed-cooling fruit showed signs of woolliness. Firmness of fruit in the delayed-cooling treatment was less at the beginning of ripening than control fruit, but after shelf life in both treatments, fruit reached the same final softness. Expressible juice was lower in woolly fruit (46%) than in healthy fruit (65%). Along with woolliness, viscosity of the resuspended alcohol insoluble residue (cell wall material) of expressed juice increased, implying accumulation of large molecular-weight polymers. The high performance liquid chromatography profile confirmed there were more large pectin polymers (2000 to 76 Ku) in the cell wall components of juice from woolly fruit and a lower arabinose content in these polymers reflected greater side chain removal from pectins in the juice of woolly fruit. Accumulation of larger sized pectin polymers along with high viscosity correlated with lower polygalacturonase activity in woolly fruit. Degradation of soluble pectin released into the juice of woolly fruit may have been impeded by repressed polygalacturonase activity.

Nectarines (*Prunus persica*) are extremely perishable during postharvest handling procedures and cold storage has been beneficial due to its ability to delay ripening and senescence. However, cold storage brings with it a drawback of chilling injury for most nectarine cultivars if they are held longer than 2 to 3 weeks (Lill et al., 1989). The injured fruit have a good appearance when removed from storage, but do not ripen normally. The main symptom of chilling injury is a woolliness or mealiness in texture whereby the fruit taste dry, and without juice. This disorder is not apparent externally and the fruit are often marketed at this stage leading to decreased consumer acceptance.

During normal ripening, insoluble-pectin in the cell wall is converted into soluble pectin by the action of cell wall hydrolases. Formation of water-soluble pectin parallels the increase in polygalacturonase (PG) activity in normally ripening peaches, and the molecular weight of pectin decreases progressively (Pressey et al., 1971; Pressey and Avants, 1973). During woolliness development in the chill-injured fruit, however, changes in the activity of cell wall hydrolases reduces solubilization of pectin. Repressed PG activity during and following storage impedes solubilization of insoluble-pectin from the cell wall (Artes et al., 1996; Ben-Arie and Sonogo, 1980; Buescher and Furmanski, 1978; Von Mollendorff and De Villiers, 1988). In woolly fruit, the total amount of insoluble pectin can increase (Ben-Arie and Lavee, 1971). Reduced levels of water soluble pectins and enhanced levels of insoluble pectins in peaches [*Prunus persica* (L.) Batsch] have been associated with woolliness (Buescher and Furmanski, 1978).

Viscosity of soluble pectins is influenced by the concentration, relative molecular weight, and degree of esterification of the pectins (Doesburg, 1965). In unripe peaches the molecular weight of most

of the water-soluble pectin is very high (>10,000,000), and at this stage of ripening PG activity is hardly detectable (Pressey et al., 1971). As ripening proceeds, the molecular weight of solubilized pectin decreases concomitantly with increased PG activity (Bartley and Knee, 1982; Pressey et al., 1971; Pressey and Avants, 1973). VonMollendorff and De Villiers (1988) showed that viscosity of soluble pectin in woolly fruit increases during ripening and suggested it is possibly the result of an increase of relative molecular weight rather than an increased concentration of the soluble pectins. However, it has not been determined what causes this increase in viscosity.

Pectin consists principally of galacturonan interspersed with rhamnose to which side chains, composed of arabinan and galactan, are attached. Fruit ripening is accompanied by removal of side chains. There is a significant decrease in the amounts of arabinose and galactose in the side-chains of pectin from ripe tomato (*Lycopersicon esculentum* Mill.) (Carrington et al., 1993; Gross and Wallner, 1979; Seymour et al., 1990). In normal ripening persimmon (*Diospyros kaki* L.), cell wall pectic polysaccharides are solubilized and arabinose and galactose are lost from the cell wall (Grant et al., 1992). However, in woolly nectarines the side chains do not appear to be removed from insoluble pectic polymers and branched pectins remain high in the cell wall (Dawson et al., 1992).

In this study we investigated the components and characteristics of the cell wall material released into expressed juice of woolly and healthy nectarine fruit during ripening. Our hypothesis was that changes in juice viscosity which accompany fruit softening are due to changes in the amount and type of cell wall material released into the juice, and that differences in viscosity between healthy and woolly fruit will be reflected by differences in the juice cell wall components. Further, it was of interest to see if the decrease in PG activity which is found during ripening after extended storage would be reflected in the amount or composition of cell wall components in the juice.

Materials and Methods

Nectarines (*Prunus persica* 'Flavortop') were harvested at com-

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mercial maturity. Fruit were divided into three lots. One lot was ripened for 5 d at 20 °C, the second placed on the same day in cold storage at 0 °C (control), and the third held for 2 d at 20 °C and then stored at 0 °C (delayed cooling). Fruit were removed from cold storage after 1, 3, or 5 weeks and subsequently held at 20 °C for 5 d shelf life. On the day of removal and on day 1, 3, or 5 of shelf life, three replications of 10 fruit were sampled from each treatment and woolliness was estimated by visual observation and tasting the fruit. Woolly fruit show tissue separation not seen in normal softening and taste dry without the normal fruit juiciness in the mouth. Firmness was measured on two pared sides of each fruit using a penetrometer fitted with a 11-mm-diameter plunger. Expressible juice was determined by removing a tissue plug from each fruit with a cork borer, passing it through a 5 mL syringe into an Eppendorf tube, centrifuging 10 min at 10,000 g_n and separately weighing the juice and solids (Lill and Van Der Mespel, 1988). A wedge-shaped slice (≈ 5 g) was removed from each fruit in the replication and the pooled sample was passed through an electric juicer (Moulinex, model 753, Lyon, France). Thirty milliliters of the juice was boiled in a final concentration of 80% ethanol for 20 min. This was filtered and the residue collected as alcohol insoluble residue (AIR). The AIR was resuspended in water with stirring for 2 h and then centrifuged (12,000 g_n for 10 min). An aliquot of the supernatant was collected for measuring relative viscosity. The AIR was then lyophilized (dry weight) and stored at -20 °C before determination of uronic acid (UA) and neutral sugar (NS) content. Relative viscosity was measured in a Cannon-Fenske viscosimeter (Munich, Germany) at 30 °C and calculated according to Von Mollendorff and De Villiers (1988). Uronic acid and neutral sugar were determined by the m-hydroxydiphenol method (Blumenkranz and Asboe-Hansen, 1973) and anthrone method (Dische, 1962) using galacturonic acid and glucose as standards. Another slice from each fruit was pooled, frozen, and held at -20 °C for later analysis of PG activity.

AIR (150 mg) was resuspended in 0.2 mol·L⁻¹ imidazole (pH 6.8), passed through QAE Sephadex anionic exchange column (1.8×9 cm) and eluted by increasing concentrations of imidazole buffer (pH = 6.8) to separate pectin from hemicellulose according to Lurie et al. (1994). Both fractions were dialysed and lyophilized. The pectin fraction was redissolved into 1 mL imidazole buffer (pH = 6.8). An aliquot of redissolved pectin

polymers (50 μ L) was resolved on high performance liquid chromatography (HPLC) Bio-Sil SEC-400-5 column (Bio-Rad, Hercules, Calif.) to determine size. Imidazole buffer (0.2 mol·L⁻¹, pH = 6.8) was used as mobile phase at a flow rate 0.3 mL·min⁻¹. Dextrons (2000 Ku, 488 Ku, 76 Ku, and 39 Ku) were used as markers. The fractions were analyzed for UA content and peaks pooled for individual neutral sugar determination by gas chromatography

Fig. 1. (A) Fruit firmness, (B) percent expressible juice, (C) relative viscosity, and (D) dry weight of resolubilized juice AIR at removal and during shelf life. Legend in A applies to all figures. Asterisks indicate significant differences between means on day of assay at $P = 0.05$ by paired t test.

Fig. 2. (A) Uronic acid and (B) neutral sugar in AIR from 30 mL juice at removal from storage and after shelf life. Legend in B applies to both figures. Vertical lines represent SD.

(GC) (Albersheim et al., 1967; Blakeney et al., 1983). Samples were injected into a DB-225 capillary column (Supelco, Bellefonte, Pa.) in a gas chromatograph (GC) equipped with a flame ionization detector run isothermally at 210 °C, using He as a carrier gas. Peaks were quantified against an inositol internal standard.

Exo-PG and endo-PG activities were determined by using frozen fruit flesh according to the methods of Ben-Arie and Sonogo (1980) and Gross (1982). Protein content was determined by the procedures of Bradford (1976) using Bio-Rad reagent (Bio-Rad, Munich, Germany). Exo-PG activity was expressed as μg galacturonic acid released per mg protein in 1 h, while endo-PG was the viscosity change per mg protein in 1 h.

Each measurement was repeated at least three times and standard deviation of means calculated. In some cases (see Fig. 1) significant differences at $P = 0.05$ between the two treatments at each point of measurement were determined by a paired t test.

Results

During ripening of nonstored fruit and after 1 or 3 weeks of storage, fruit from both control and delayed cooling treatments were healthy without any symptoms of woolliness. At removal after 5 weeks storage all fruit appeared healthy, but by day 5 of shelf life woolliness was observed in 87% of the control fruit while only 7% of the fruit from the delayed-cooling treatment were woolly.

Although fruit from the control treatment had greater firmness than those from the delayed-cool-

ing treatment at removal from storage, there was no difference after 5 d shelf life (Fig. 1A). As time in storage increased, the difference in firmness at removal between the two treatments also increased.

Expressible juice increased as fruit softened (Fig. 1B). During ripening after 1 and 3 weeks storage, expressible juice in both control and delayed-cooling fruit increased in parallel, with delayed-cooling fruit having more juice than control fruit. After 5 weeks storage, however, expressible juice in control fruit declined from day 1 to day 3 of ripening but on day 5 returned to the level seen at removal from storage. After 5 weeks storage, expressible juice from delayed-storage fruit remained level through 3 d of ripening, then increased to an amount comparable to fruit from the other storage periods.

Viscosity of the resolubilized AIR increased and then decreased during ripening after 1 and 3 weeks storage when fruit from the control and delayed-cooling treatment were healthy (Fig. 1C). The differences were that viscosity of delayed-cooling fruit increased earlier than control fruit during the first days of ripening, and reached a lower level of viscosity than the control fruit on the last day of shelf life. However, after 5 weeks of storage the viscosity of the control fruit did not decrease during ripening, but continuously increased and reached its highest point on the fifth day when woolliness was apparent in these fruit. In contrast, viscosity values for delayed-cooling fruit showed an increase and decrease during ripening, as occurred after shorter periods of storage.

Fig. 3. (A) Exo-PG and (B) endo-PG activity in fruit flesh at removal from storage and after shelf life. Legend in A applies to both figures. Vertical lines represent SD.

Table 1. Fractionation of juice alcohol insoluble residue (AIR) polymers by QAE column and their components.^z The two treatments were sampled after 5 weeks storage and 5 d shelf life.

Treatment	Hemicellulose (mg/150 mg AIR) ^y			Pectin (mg/150 mg AIR)		
	UA	NS	UA/NS	UA	NS	UA/NS
Control	5.87	64.3	0.09	10.6	2.61	4.07
Delayed cooling	3.95	80.6	0.05	9.0	1.59	5.67

^zUA = uronic acid, NS = neutral sugar.

^yDry weight basis.

More cell wall material was present in the juice as the fruit ripened, as expressed by increased dry weight of AIR (Fig. 1D). However, this was similar in control and delayed-storage fruit, even after 5 weeks storage when control fruit was woolly and delayed storage fruit ripened normally.

During ripening after harvest and storage, total UA and NS in the AIR (Fig. 2) increased in the juice from control and delayed-cooling treatments with slightly higher UA and lower NS in the delayed-cooling treatment than control up to 3 weeks storage. However, at the last day of ripening after 5 weeks storage there was a slightly higher amount of UA and lower NS content in woolly fruit (control) than healthy fruit (delayed-cooling).

Both endo- and exo-PG activities increased with ripening (Fig. 3A and B), regardless of storage treatment, reaching peak activity after 3 weeks storage. However, as storage was extended to 3 and 5 weeks the rise in PG activity during ripening was less in control fruit than in delayed-storage fruit.

Fractionation by ion exchange chromatography of AIR from juice of woolly and healthy fruit into hemicellulose and pectin showed that in woolly fruit the juice contained more pectin polymers than healthy fruit (Table 1). In contrast, there was less hemicellulose in the juice of woolly fruit than in healthy fruit. Moreover, composition of the polymers was noticeably different in the woolly and healthy fruit (see below).

UA HPLC profiles of the pectin fraction from the ion exchange chromatography showed there was a greater content of large molecular weight pectins (2000 to 76 Ku) in the AIR of the juice from woolly fruit than from healthy fruit (Fig. 4A). However, hemicellulose in juice from both woolly and healthy fruit had the same molecular size (<39 Ku) (Fig. 4B).

The pectin fraction of the AIR from the fruit juice was collected into three fractions (2000 to 500 Ku, 500 to 50 Ku, and below 20 Ku) following HPLC fractionation and individual NS were analyzed (Fig. 5). In the large molecular and mid-sized polymers the major NS were arabinose, glucose, and galactose. In the small molecular weight fraction (below 20 Ku), the major neutral sugar was glucose, followed by mannose. Among the large and mid-sized molecules the percentage of arabinose, the major sugar, was the same in stored healthy fruit as in nonstored fruit, but was lower in woolly fruit. As a result, the percentage of glucose in woolly fruit was higher than in healthy fruit. There were also higher galactose and lower glucose levels in fruit ripened after harvest than after storage, whether the latter ones were healthy or woolly.

Discussion

Delaying storage by holding the fruit at 20 °C for 2 d before 0 °C storage prevented fruit from developing woolliness after 5 weeks at 0 °C, while fruit which were stored immediately developed woolliness. By this means normal cell wall autolysis in healthy fruit from delayed cooling treatment could be compared synchronously with abnormal autolysis occurring in woolly fruit.

VISCOSITY AND EXPRESSIBLE JUICE VERSUS WOOLLINESS. Viscosity decreased when expressible juice increased during ripening after 1 and 3 weeks storage. At the end of the 5 d shelf life there was a higher level of expressible juice and lower viscosity in fruit from delayed-storage than in control fruit. Furthermore, during ripening after 5 weeks storage expressible juice in control fruit remained low while viscosity reached its highest level in these fruit. In fruit from the delayed-cooling treatment expressible juice still showed a trend of increasing and viscosity increased and then decreased. Concomitant with the viscosity increase in the control fruit were visual symptoms of woolliness. Von Mollendorff et al. (1989, 1993) also found that viscosity of soluble pectins attains a maximum value during development of woolliness and the extractable juice decreases to a

Fig. 4. (A) Uronic acid (UA) content of pectin and (B) neutral sugar (NS) content of hemicellulose HPLC profiles in juice from woolly or healthy fruit, sampled after 5 weeks storage and 5 d of shelf life after QAE column separation. Legend in A applies to both figures. Standards are dextrans of known molecular weight.

Fig. 5. Neutral sugar components from HPLC fractions depicted in Fig. 4. (A) Large (2000 to 500 Ku), (B) mid-sized (500 to 50 Ku), and (C) small (<20 Ku) peaks. Legend in A applies to all figures. R = rhamnose, F = fucose, A = arabinose, X = xylose, M = mannose, Gal = galactose and Glu = glucose.

minimum value when woolliness is apparent in the fruit. Therefore, the viscosity of the AIR from the juice can be correlated both with woolliness, and could be used as a measurement of woolliness occurrence.

VISCOSITY AND WEIGHT OF AIR. Although during ripening after 1 and 3 weeks of storage the viscosity of the AIR increased initially and then decreased, the amount of AIR (dry weight basis) in the juice showed a continual increase. This dis-synchrony between viscosity and the amount of AIR indicates its composition is undergoing radical changes during ripening. Otherwise, after 1 week ripening, for example, when the amount of AIR obtained from 30 mL of juice increased 4-fold, a similar increase in viscosity would be expected. However, viscosity at the end of shelf life was almost the same as at removal, suggesting this large increase in AIR was in components that do not contribute to increasing viscosity. By contrast, between 3 and 5 d of shelf life after 3 weeks of storage there is a slight decrease in the dry weight of the AIR concomitant with the decrease in viscosity. This may reflect further metabolism of the cell wall polymers in the juice to components, some of which are too small to be precipitated by 80% ethanol. After 5 weeks storage this was not seen, instead AIR in the juice from fruit of both treatments increased steeply during shelf life.

VISCOSITY AND PECTINS. As fruit ripened the UA content of the juice of both control and delayed-cooling fruit increased, indicating increased solubilization of pectin from the cell wall. However, juice of control fruit had slightly less UA and slightly higher viscosity than delayed-cooling treated fruit at the end of ripening after 1 and 3 weeks storage. Control fruit accumulated more UA than delayed-cooling fruit in the juice at the end of ripening after 5 week storage

(when woolliness occurred). At this time they showed much higher viscosity than delayed-cooling fruit. This higher UA juice content in woolly fruit was due to large pectin molecules, verified by HPLC.

HPLC fractionation indicated that pectin polymers in woolly fruit underwent less degradation, leading to accumulation of larger pectins in the juice than in juice from the delayed cooling treatment (healthy fruit). Possibly, this is why the AIR from woolly fruit had a higher viscosity, compared with healthy fruit, despite equivalent levels of dry matter. Dawson et al. (1992) found that solubilized pectic polymers depolymerize to low molecular weight species during the latter stages of normal ripening but not during abnormal ripening.

PG ACTIVITY, VISCOSITY, AND PECTINS. The changes in pectin size in the AIR of the juice were associated with PG activity changes. PG activity increase was accompanied by a decrease in viscosity towards the end of shelf life after 1 and 3 weeks storage in both control and delayed-cooling treatments. By day 3 and 5 of ripening after 1 and 3 weeks storage, when PG had reached a higher level in delayed-cooling treatment than in control fruit, viscosity decreased to a lower level in delayed-cooling treatment than in the control. During ripening after 5 weeks storage, PG activity in the control fruit was lower than in previous weeks, and viscosity continued to increase. PG activities in delayed cooling fruit were as high after 5 weeks of storage as after 1 week storage and viscosity was also similar to ripening after 1 week.

PG activities in the fruit pulp increased during ripening in parallel with more UA being present in the AIR of juice. PG is considered to have an important role in converting insoluble pectin to water-soluble pectin during ripening (Carrington et al., 1993; Pressey et al., 1971). During normal ripening, PG appearance parallels solubilization of pectin, while during woolliness development, repressed PG activity is found (Artes et al., 1996; Ben-Arie and Sonego, 1980; Buescher and Furmanski, 1978). This lower activity was considered to impede solubilization of insoluble pectin leading to woolliness (Ben-Arie and Sonego, 1980). Our results support these findings. After 5 weeks storage, PG activity in control fruit was not only consistently lower than in delayed-cooling fruit but was also lower than during previous ripening after 1 and 3 weeks storage, whereas PG activity in the delayed cooling treatment still maintained a level similar to that measured after ripening without storage. Lower PG activity could contribute to less pectin degradation in woolly juice and the presence of more high molecular weight polymers than in juice of healthy fruit.

Sugar components

PECTINS. From measurements of NS, we found that in the AIR of woolly fruit juice (control) there was less arabinose than in nonwoolly (healthy) fruit juice, particularly among the large and mid-sized pectin polymers. Arabinose is considered the main component of pectin side chains in the cell wall (Chapman and Horvat, 1990; McCann and Roberts, 1991). During ripening, pectic polymer depolymerization is accompanied by side chain loss (Dawson et al., 1992). Our results indicate that soluble pectin substances in woolly fruit contain less arabinose, presumably as side chains, than soluble pectin in healthy fruit. It also implies that either more neutral sugar side chains remain attached to the insoluble pectins which had not been hydrolyzed into the juice in woolly fruit, or that the side chains had been detached and degraded into alcohol-soluble saccharides. In healthy and normally ripened fruit, more side chains were present on the pectins in the AIR of the juice. This result was consistent with that of normal ripening after controlled atmosphere storage (Lurie et al., 1994).

HEMICELLULOSE. On the last day of ripening after 5 weeks storage, most NS in the AIR of juice was in the hemicellulose fraction, not attached to pectin (Table 1). Their molecular weight was small (Fig. 4B) and the major components were glucose and mannose (Fig. 5C). Higher NS in healthy fruit implies that more hemicellulose could be cleaved and released into the juice during normal ripening, perhaps due to normal hemicellulose and cellulose hydrolases. β -1,4-Glucanase is involved in the hydrolysis of hemicellulose (Keegstra et al., 1973; Maclachlan and Brady, 1992, 1994; O'Donoghue and Huber, 1992; O'Donoghue et al., 1997). Its activity increases with the ripening of many fruit (Pressey, 1977) and xyloglucan molecular weight decreases during ripening in a number of fruit (Cutillas-Iturralde et al., 1994; O'Donoghue et al., 1997). Cellulolytic enzymes could degrade both cellulose and the β -1,4-glucan backbone of xyloglucan, a hemicellulosic polysaccharide prominent in walls of dicotyledons (Keegstra et al., 1973). If their activities are repressed under chilling injury, a lower hemicellulose content in the juice could result.

In conclusion, it appears the type of polymers released into juice of healthy or woolly fruit are qualitatively, though not very quantitatively, different and this affects AIR viscosity. During normal ripening, viscosity increases as fruit softening begins and cell wall material is increasingly released into the juice, but at the end of ripening although a great deal of cell wall material is present in the juice, the viscosity is low. This is apparently due to smaller molecular weight polymers prevailing in the ripe, healthy fruit juice. The higher molecular weight pectins in the juice of woolly fruit do not allow this later decrease in viscosity at the end of softening, and may be due to low PG activity impeding pectin depolymerization. The treatment of delayed storage prevents the decrease in PG activity found after extended storage, and is a practical way to allow longer storage of nectarines without a decrease in quality after shelf life.

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