# CHANGES IN CELL WALL NEUTRAL SUGAR COMPOSITION AND ETHYLENE EVOLUTION AS POTENTIAL INDICATORS OF WOOLLINESS IN COLD-STORED NECTARINE FRUIT

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## ABSTRACT

*Changes in cell wall composition during ripening of nectarine fruit (*Prunus persica *var* nectarina, *Ait. Max, cv. "Caldesi 2000") after harvest or after cold storage were investigated. Cell wall materials were prepared from nectarine fruits after harvest, after 2, 4 and 6 weeks of cold storage (0C) and after additional ripening at room temperature (20C) for 1 and 5 days, respectively. The materials were analyzed for neutral sugars and cellulose content. Incidence of chilling injury (CI) symptoms, expressed as woolliness and gaseous emissions of $CO_2$ and ethylene evolution, were also monitored. A significant decrease in arabinose and galactose contents as the woolliness symptoms became more intense was observed. After 6 weeks of cold storage and an additional 5 days at room temperature, arabinose and galactose contents decreased up to 63 and 34%, respectively. In addition, as the cold storage period increased, a linear decrease of total neutral sugars (*$r = 0.9832$, $P < 0.01$*) and arabinose content (*$r = 0.9903$, $P < 0.01$*) after 5 days of ripening at room temperature was recorded. Furthermore, reduced ethylene production after 6-week cold storage was accompanied by intense woolliness symptoms. Inversely, respiration rate and cellulose content did not show any consistent trend during nectarine ripening after harvest or after removal from cold storage, and therefore cannot be used as indicators of CI symptoms.*

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## INTRODUCTION

Fruit ripening of climacteric fruit is a complex, genetically programmed process that culminates in dramatic changes in color, texture, flavor and aroma of fruit flesh (Alexander and Grierson 2002), accompanied by a transient burst of ethylene production and respiration rate. This process has been attributed mainly to modifications of cell wall architecture (Fry 2004). However, nectarine fruit often develop chilling injury (CI) symptoms after extended storage periods at low temperatures, leading to abnormal ripening (Fernández-Trujillo *et al.* 1998; Zhou *et al.* 2000; Manganaris *et al.* 2005). The injured fruit have a good appearance when removed from storage but does not ripen normally, causing woolliness (mealiness) of the flesh that leads to a dry, mealy texture (Zhou *et al.* 2000).

During ripening, cell wall architecture and the polymers, of which it is composed, are progressively modified. Extensive research has been devoted to elucidate the mechanisms responsible for the textural changes that occur during postharvest ripening of fruit (Brummell *et al.* 2004; Fry 2004). Flesh woolliness has been mainly attributed to the imbalance between polygalacturonase and pectin esterase (Zhou *et al.* 2000). In addition, notable differences between the physicochemical properties of cell wall pectin of normally ripened and chilling-injured fruit have been well documented (Zhou *et al.* 2000; Manganaris *et al.* 2005). However, a direct relationship between fruit ripening and noncellulosic neutral sugar composition has not been defined clearly.

Neutral sugar side chains are covalently attached to the backbone of cell wall (Schmelter *et al.* 2002) and their composition, as well as their content during ripening, varies between different species, even within the same botanical group, like stone fruit (Redgwell *et al.* 1997a). It was demonstrated by an *in vitro* study that the removal of the main neutral sugars from pectin side chains can affect cell wall physicochemical properties through the increase of its solubility and extensive downshifts of its molecular mass, supporting the hypothesis that net loss of neutral sugars decreases the ability of pectin molecules to aggregate together (De Veau *et al.* 1993). Therefore, neutral sugar composition possesses a critical role in cell wall structure, because removal of neutral side chains has been regarded as an essential part of pectin solubilization (Dawson *et al.* 1992).

Besides neutral sugars, a substantial part of cell wall matrix consists of rigid, inextensible cellulose microfibrils held together by interpenetrating coextensive networks of matrix glycans, pectins and structural glycoproteins (Carpita and McCann 2000), and relatively few data regarding its content during nectarine ripening exist (Lurie *et al.* 1994).

In plants with fleshy fruit, a major focus has been devoted to the dissection of physicochemical and biochemical regulatory cascades controlling rip-

ening (White 2002). Based on objective criteria, the present work aimed to examine the changes in cell wall neutral sugars and cellulose composition, respiration rate and ethylene production during ripening upon removal from cold storage as potential biochemical indices of flesh woolliness.

## MATERIALS AND METHODS

### Plant Material

Nectarine fruits were harvested at the preclimacteric stage and divided into eight groups of 30 fruits. One group was analyzed after 1 day at 20C while another was allowed to ripen for 5 days (shelf life) at 20C. The other groups were stored (0C, 95% relative humidity) for 2, 4 and 6 weeks and were sampled 1 day after removal from cold storage and after an additional 5 days at 20C.

### Woolliness Symptoms

Expressible juice was used as an index of woolliness and was expressed as the percentage of free juice in total tissue according to Lill and Van Der Mespel (1988), with slight modifications in speed and time of centrifugation ($13{,}000 \times g$, 10 min).

### Ethylene and Respiration Determination

Six replications of three fruits were enclosed in 3-L airtight jars for 1 h at 20C. For headspace sampling, syringes were inserted through rubber septa affixed on the lid. Ethylene measurements were performed by injecting 1 mL of headspace gas sample into a gas chromatograph (model 3300, Varian Analytical Instruments Inc., Palo Alto, CA), equipped with a stainless steel column filled with Porapak Q (length 100 cm, diameter 0.32 cm) at 50C and a flame-ionization detector at 120C. The carrier gas was $N_2$ at a flow rate of 20 mL/min. Respiration rate was calculated by the $CO_2$ production in the gas phase of the jars, measured automatically by an infrared gas analyzer (Combo 280, David Bishop Instruments, UK) connected to a computer. Ethylene production and respiration rate were recorded 1, 3, 5 and 7 days after harvest or after removal from cold storage.

### Extraction of Cell Wall Material (CWM)

A wedge-shaped slice of mesocarp tissue from each fruit was removed, pooled, frozen in liquid nitrogen, stored at −80C and subsequently used for cell wall extraction according to Campbell *et al.* (1990).

### Determination of Neutral Sugars and Cellulose

Samples of the CWM fractions were analyzed to determine the component carbohydrates of the cell wall. Noncellulosic neutral sugars were derivitized to alditol acetates by hydrolysis in 2 N trifluroacetic acid (TFA), reduction and acetylation (Blakeney *et al.* 1983). The monosaccharide composition was determined by capillary gas chromatography (GC) of the alditol acetates on a Dani chromatograph 1000 (Dani Instruments SpA, Cologno Monzese, Milan, Italy) fitted with a 30-m fused silica capillary column (DB-225, J&W Scientific, Folsom, CA). The chromatograph oven was held at 210C and hydrogen was used as the carrier gas. Quantitation was based on integration of the peaks from the flame ionization detector with a Shimadzu C-R3A (Shimadzu, Kyoto, Japan) chromatography data system. Six analyses were run for each treatment. Each GC analysis consisted of triplicate injections of each sample, and data were averaged over the three injections. Neutral sugar contents were expressed as their relative concentrations in cell wall in $\mu$g/mg.

The cellulosic residue obtained after TFA hydrolysis was rinsed with three lots of methanol and then air-dried. The dried material was hydrolyzed in 67% (v/v) of $H_2SO_4$ for 1 h at 20C and quantified by anthrone colorimetric assay (Dische 1962).

### Statistical Analysis

All treatments were run with at least six replicates. Data were treated for multiple comparisons by analysis of variance (ANOVA), followed by the Duncan's multiple range test with the significance level set at $P < 0.05$. Data in percentages were subjected to arcsine transformation prior to statistical analysis. ANOVA was performed using the statistical software SPSS (SPSS Inc., Chicago, IL). Correlation coefficients ($r$) were also calculated.

## RESULTS AND DISCUSSION

### Woolliness Symptoms

Nectarine fruit ripened normally after harvest or 2-week cold storage, as indicated by the increased expressible juice content. However, expressible juice content decreased after 4 and 6 weeks of cold storage, indicating the substantial severity of woolliness symptoms as cold storage period increased (Table 1).

### Ethylene Evolution and Respiration Rate

Ethylene climacteric peak appeared on the third day after removal from cold storage. Differences in the ethylene production were recorded (Fig. 1).

TABLE 1.
EXPRESSIBLE JUICE (%) OF NECTARINE FRUIT
(CV. "CALDESI 2000")

| Treatment | Expressible juice (%) |
|---|---|
| 0 week + 1 days | 74.3b* |
| 0 week + 5 days | 83.4a |
| 2 weeks + 1 days | 78.4ab |
| 2 weeks + 5 days | 71.10b |
| 4 weeks + 1 days | 63.4c |
| 4 weeks + 5 days | 60.8c |
| 6 weeks + 1 days | 54.8d |
| 6 weeks + 5 days | 45.8e |

Treatments were done after 0, 2, 4 and 6 weeks of cold storage and their maintenance at room temperature (20C) for 1 and 5 days, respectively.

* Values within a column followed by the same letter are not significantly different to each other ($P < 0.05$).

The 4 weeks that followed the 2-week cold-storage period showed the highest climacteric peak, whereas the sixth week's cold storage climacteric peak was lower. It appears that the capacity to synthesize ethylene or to make new receptors in nectarine fruits subjected to cold storage for 6 weeks was suppressed, and the normal feedback regulation of ethylene was blocked. A direct relationship between low climacteric peak of nectarine fruits after prolonged cold storage and incidence of severe woolliness symptoms can be postulated. Our results are in agreement with those reported by Dong *et al.* (2001), postulated the same relationship for another nectarine cultivar, subjected to extended cold storage. Inversely, upon removal from shorter cold storage periods (2 and 4 weeks) the capability of ethylene autocatalysis resumed and the fruit held at 20C were able to make new receptors to compensate for the binding sites. Fernández-Trujillo *et al.* (1998) reported that ethylene production in peach fruit increased after their transfer from cold storage to ripening temperatures as this period increased, whereas loss of ethylene production capacity due to prolonged storage, possibly influenced by CI, has also been indicated (Valero *et al.* 1997).

Overall, ethylene possesses an important role in integrating developmental signals and responses to abiotic stresses (Alexander and Grierson 2002) like cold storage and is a critical component of many diverse developmental processes, including normal and abnormal fruit ripening.

Respiration rate did not show any consistent trend after harvest or removal from cold storage (Fig. 1b), coinciding with data reported for another nectarine cultivar (Dong *et al.* 2001). Therefore, respiration rate cannot be used as an indicator of CI symptoms in prolonged cold storage of nectarine fruit.

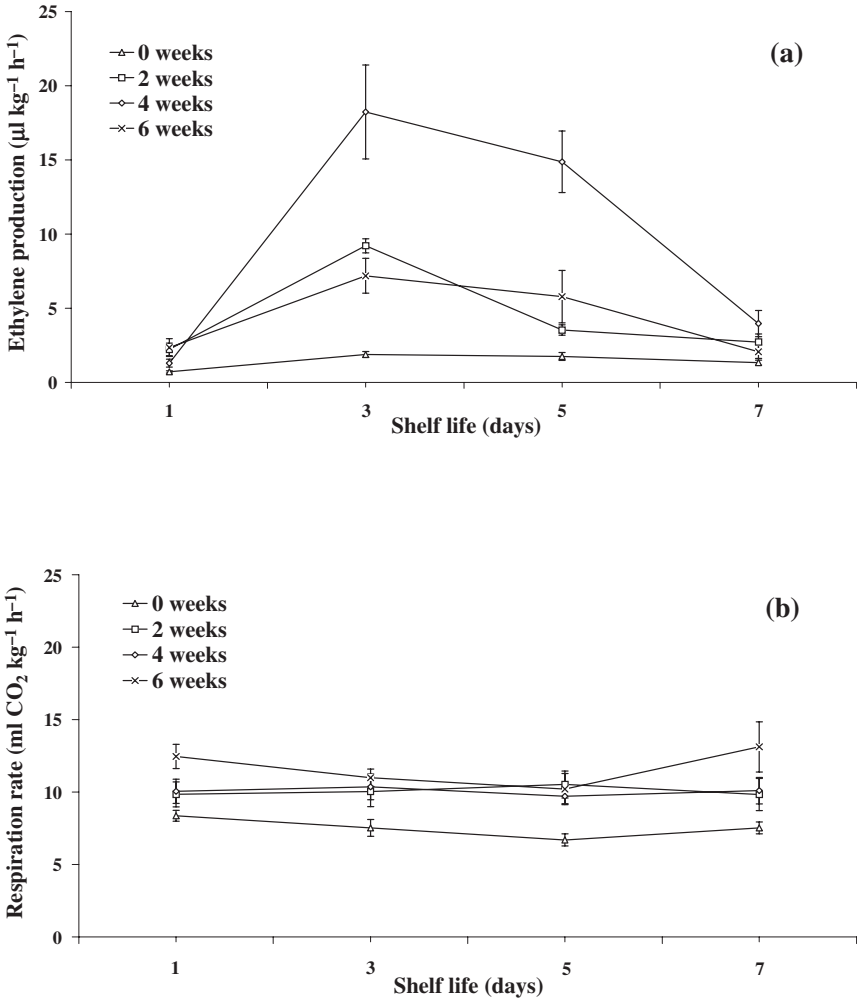


FIG. 1. ETHYLENE PRODUCTION ($\mu$L kg$^{-1}$ h$^{-1}$) (a) AND RESPIRATION RATE (mL $CO_2$ kg$^{-1}$ h$^{-1}$) (b) OF NECTARINE FRUIT (CV. "CALDESI 2000") DURING RIPENING AFTER 0, 2, 4 and 6 WEEKS COLD STORAGE
The lines represent the standard deviation of the mean (±SD, $n = 6$).

## Neutral Sugar Content

Neutral sugar composition changed substantially along with the cold storage time. Arabinose (Ara) consisted the predominant noncellulosic neutral sugar (Table 2), with which other nectarine cultivars have also been associated

TABLE 2.
MONOSACCHARIDE COMPOSITION OF NONCELLULOSIC NEUTRAL SUGARS OF CWM

| Composition ($\mu$g mg$^{-1}$ CWM) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Treatment | Neutral sugars | Rha | Fuc | Ara | Xyl | Man | Gal | Glc |
| 0 week + 1 days | 260.3a* | 16.2abc | 7.4a | 108.9a | 35.1cd | 6.8c | 54.7a | 31.1a |
| 0 week + 5 days | 245.1b | 15.8bc | 7.4a | 100.8b | 38.5b | 6.8c | 45.3c | 30.6a |
| 2 weeks + 1 days | 242.3b | 16.5ab | 7.5a | 96.7c | 36.4bc | 7.0bc | 47.4b | 30.9a |
| 2 weeks + 5 days | 223.0c | 16.8a | 7.0ab | 84.7d | 33.3de | 7.3a | 45.7c | 28.2d |
| 4 weeks + 1 days | 217.3d | 15.6cd | 6.9ab | 82.6d | 32.0e | 6.8c | 44.0d | 29.4b |
| 4 weeks + 5 days | 196.3e | 16.1bc | 6.9ab | 63.5f | 32.4e | 7.4a | 41.6e | 28.5bc |
| 6 weeks + 1 days | 227.5c | 16.0bc | 7.1ab | 78.1e | 44.4a | 7.2ab | 43.7d | 31.1a |
| 6 weeks + 5 days | 159.1f | 16.0d | 6.5b | 40.4g | 25.8f | 6.8c | 35.4f | 29.2bc |

Treatments were done after 0, 2, 4 and 6 weeks of cold storage of nectarine fruit (cv. "Caldesi 2000") and their maintenance at room temperature for 1 and 5 days, respectively.
CWM, cell wall material; Rha, rhamnose; Fuc, fucose; Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glc, glucose.
* Values within a column followed by the same letter are not significantly different to each other ($P < 0.05$).

(Dawson *et al.* 1992; Lurie *et al.* 1994). In addition, Ara content had a pronounced and dramatic loss during fruit ripening, which culminated after 6 weeks of cold storage and 5 days of shelf life, where it decreased by 63%.

Monosaccharide composition indicated that the losses were mainly attributed to a decrease in Ara residues. Our data are in agreement with those reported by Lurie *et al.* (1994), where a greater loss of Ara from the cell wall of fruits stored for a prolonged period than in normally ripened fruits after harvest was observed in another nectarine cultivar. Additionally, a significant correlation between the decrease of total neutral sugars ($r = 0.9832$, $P < 0.01$) and Ara content ($r = 0.9903$, $P < 0.01$) in relation to 5 days ripening at room temperature after harvest or after cold storage periods has been observed.

Galactose (Gal) decreased up to 36% as ripening progressed. Gal loss has been previously reported to occur in many fruits during their ripening (Redgwell *et al.* 1997a). However, the strong affinity between pectin solubilization and fruit softening cannot be correlated with Gal loss (Redgwell *et al.* 1997b). Redgwell *et al.* (1997a) observed that during the ripening of plum fruit, which softened markedly, there was no apparent Gal loss, but there was considerable pectin solubilization. Conversely, in the same study, Gal loss was marked by slight pectin solubilization in species that are characterized by a crisp fracturable texture (e.g., apple, nashi pear). Preferential loss of Gal moieties during ripening has also been reported for other species (Gross and Sams 1984; Koh and Melton 2002). Removal of galactan side chains from nectarine fruits may allow loosening of cell wall structure (Dawson *et al.* 1992;

Redgwell *et al.* 1997a), but whether galactan side chains is a prerequisite for pectin solubilization, as these two processes often occur concurrently during fruit softening, remains unclear.

Xylose and glucose accounted for a substantial portion of neutral sugar composition and their content fluctuated without any consistent trend throughout ripening. Rhamnose, fucose and mannose accounted for a relatively small percentage of noncellulosic neutral sugar composition, without any apparent differentiation during the progress of fruit ripening after harvest or after cold storage.

### Cellulose Content

Cellulose content accounted for 24–26% of CWM (data not shown) and its content remained relatively constant throughout ripening. Therefore, it does not seem to be directly implicated in the softening process of nectarine fruit. Lurie *et al.* (1994) also reported that the cellulose content of a nectarine cultivar remained rather constant. Furthermore, there has been no reported evidence for changes in the nature of cellulose crystallites of the polysaccharides adhering to crystallite surfaces even in CWM isolated from fruits in which cell wall dissolution was extreme (Newman and Redgwell 2002).

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