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Postharvest peel pitting at non-chilling temperatures in grapefruit is promoted by changes from low to high relative humidity during storage

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Abstract

'Marsh' white grapefruit are prone to develop postharvest peel pitting during storage at non-chilling temperatures. The disorder is characterized by sunken areas or 'pits' on the flavedo followed by browning and dryness of affected areas in severe stages. Despite its uncertain cause, coating fruit with commercial waxes coupled with warm-temperature storage has been reported to promote peel pitting. In this work, we examined the effect of changes in relative humidity (RH) and waxing at various times during storage on the incidence of postharvest peel pitting. Grapefruit stored at 20 °C and constant low (30%) or high (90%) RH developed low levels of peel pitting, even if waxed immediately after harvest. In contrast, when fruit stored at 30% RH were transferred to 90% RH, the incidence of peel pitting markedly increased, while transferring from 90 to 30% RH did not lead to increased pitting. This effect was greater in fruit exposed to prolonged periods of dehydration at 30% RH. Removal or redistribution of natural surface fruit waxes by washing followed by storage at low RH had little effect on peel pitting, but the transfer of washed fruits from low to high RH markedly increased peel damage. Waxing fruit enhanced severity of the damage only if there was a previous dehydration period. Light microscopy of damaged peel revealed no difference in morphology of affected tissues whether fruit were transferred from low to high RH, or after waxing following dehydration. Taken together, the results suggest that in 'Marsh' grapefruit the alteration of water relations in peel is a key factor in the incidence of postharvest peel pitting, and that preservation of a constant water status prior to and during postharvest handling may have an important role in maintaining peel quality.

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1. Introduction

Postharvest peel pitting of Marsh grapefruit at non-chilling temperatures has been described as a

severe postharvest disorder, and is characterized by the localized collapse of epidermal and subepidermal cells on the fruit surface (Petracek et al., 1995). Initially, the disorder begins as slight depressions on the peel in regions directly above oil glands. Within a few days after the first symptoms appear, depressions turn bronze in color. Postharvest peel pitting diminishes the external quality and consequently the

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value of fruit for fresh market (Petracek et al., 1995, 1998; Agusti et al., 2001). In general, peel disorders in citrus fruit are induced by a wide array of biotic and abiotic factors in the field or during postharvest handling and storage. Since different factors can induce similar symptomology in fruit (Grierson, 1986), it has been difficult to assign specific inductive factors to a postharvest disorder.

Efforts have been made to elucidate the primary factor triggering postharvest peel pitting, but the fundamental cause remains unknown. Waxing fruit with less permeable shellac-based waxes stimulates pitting (Petracek et al., 1998). Using more gas-permeable fruit waxes as well as cool-temperature storage of fruit coated with less permeable waxes was shown to alleviate postharvest peel pitting. Since marked reductions in internal O₂ and increases in internal CO₂ were found in peel-pitted fruit, it was suggested that the levels of these gases played a significant role in inducing the disorder (Petracek et al., 1998). Yet, very low correlations between the extent of pitting and internal CO₂ and O₂ levels in fruit were found (Petracek et al., 1995). These authors demonstrated a slight effect of low internal oxygen content on peel pitting, and suggested that susceptibility to low oxygen may be variable among fruit and that initiation of the disorder may depend on a wide range of factors including peel maturity (Petracek et al., 1998). These observations, taken together, strongly indicate that other factors besides internal CO₂ and O₂ are involved in postharvest peel pitting in ‘Marsh’ grapefruit.

Other peel disorders at non-chilling temperatures with similar morphology to ‘Marsh’ grapefruit peel pitting have been described. Among them, stem end rind breakdown in Valencia (Albrigo, 1972), peel pitting in ‘Navelina’ (Casas and Garcia-Bataller, 1986; Lafuente and Sala, 2002) and ‘Navelate’ (Agusti et al., 2001) and noxan in ‘Shamouti’ (Ben-Yehoshua et al., 2001) oranges have been related to some extent with stress from water loss. This water stress and subsequent dehydration of orange peel appeared to not be the only factor involved in some rind blemishes because loss of fruit turgor and softening were not enough to trigger peel pitting (Ben-Yehoshua, 1987).

In ‘Navelina’ and ‘Navelate’ oranges, a sharp increase in RH after episodes of fruit dehydration were responsible for peel pitting in the field (Agusti et al.,

2001) or during postharvest handling and storage (Alf rez et al., 2003). Such variation in RH changes the peel water status in fruit, alters turgor pressure of the flavedo and albedo cells, and ultimately leads to peel damage. We hypothesized that postharvest peel pitting described in Florida grapefruit was also related to peel water status. The objective of this study was to determine the effect of altering RH on the incidence of peel pitting at non-chilling temperatures during postharvest storage of ‘Marsh’ grapefruit. Two disparate RH conditions were selected for this study based on RH values that can occur during a spring day in Florida. The results demonstrate that grapefruit previously subjected to low RH conditions, followed by storage at high RH, develop postharvest peel pitting.

2. Material and methods

2.1. Plant material

Mature ‘Marsh’ white grapefruit (*Citrus paradisi* Macf.) used in experiments were harvested at a commercial grove in Vero Beach, Florida in March and April 2002 from ten 25 year-old trees on sour orange rootstock. Fruit were uniform in size and free of peel defects.

2.2. Conditions of storage

All postharvest treatments were performed in packinghouse facilities located at the Citrus Research and Education Center in Lake Alfred, Florida. In all cases, fruit were placed in perforated plastic bins during storage.

2.2.1. Field-run fruit

To study the effect of altering RH conditions of storage at constant temperature on the development of postharvest peel pitting in field-harvested grapefruit, fruit were randomly divided into five lots of 30 replicate fruit each. One lot was kept at 20 ± 1 °C and 90 ± 1% RH (VPD = 233 Pa), and four lots were stored up to 19 days at 20 ± 1 °C and 30 ± 1% RH (VPD = 1637 Pa). After 3, 6 or 10 days of storage at 30% RH, a single lot of 30 fruit was transferred from 30 to 90% RH until the end of the experiment.

2.2.2. Washed fruit

Eight additional lots of 30 uniform fruit were washed on conventional commercial brushes on a packingline. One lot was stored for 19 days at $20 \pm 1^\circ\text{C}$ and 90% RH. The remaining seven lots were stored for up to 19 days at $20 \pm 1^\circ\text{C}$ and $30 \pm 1\%$ RH. After 3, 6 or 10 days of 30% RH storage, two lots were transferred to 90% RH. One of these two lots was waxed with a commercially available shellac-based wax (Sta-Fresh 590 HS from FMC Food Tech) on conventional commercial brushes and air-dried prior to transfer to 90% RH.

2.2.3. Washed and waxed fruit

Four lots of 30 fruit each were washed and waxed immediately after harvest. One lot was stored at 90% RH and the remaining three lots at 30% RH. After 3, 6 or 10 days of storage at 30% RH, one lot was transferred to 90% RH until the end of the experiment.

2.3. Estimation of peel pitting index and cumulative weight loss

At various times during experiments, fruit were inspected and peel pitting quantified using a peel pitting index (PPI) estimate as previously described (Alférez et al., 2003). Briefly, fruit were rated on a scale from 0 (no pits) to 3 (severe pitting). The PPI was calculated according to the following formula previously reported for chilling injury and peel pitting by Lafuente et al. (1997) and Lafuente and Sala (2002):

$$\sum (\text{peel pitting scale (0–3)} \times \text{number of fruit in each class}) / \text{total number of fruit}$$

Cumulative percent weight loss was monitored during the experiments and daily % wt. loss rate was calculated. Data are presented as the mean \pm S.E. of 30 fruit.

2.4. Light microscopy

Peel samples for light microscopy were taken using a 5 mm diameter cork borer from healthy control fruit and from damaged areas of peel-pitted fruit. After fruit received 6 days of dehydration conditions at 30% RH, samples were taken 3, 5 or 8 days of storage at 90% RH. The peel tissue samples were fixed

in 3% glutaraldehyde in 0.2 M K_2HPO_4 (pH 7.0) for 3–4 h at room temperature, followed by post-fixation in buffered 2% OsO_4 for 2 h as described by Burns and Achor (1989). After dehydration in an ethanol series, the tissue was polymerized in L.R. White resin (Burns et al., 1992) overnight at 60°C for 24 h. Longitudinal sections ($1\ \mu\text{m}$) were cut with a microtome and stained with methylene blue—azure A/basic fuchsin.

2.5. Statistics

Means of percentage cumulative weight loss and PPI from all experiments were analyzed using the Regression Procedure available in SAS (Cary, NC, USA).

3. Results

3.1. Field-run fruit

Percentage of weight loss per day in fruit stored at 30% RH was about 0.4–0.5 during the storage period, whereas in fruit stored at 90% RH this rate was 0.3% (Fig. 1A). After 3, 6 and 10 days of storage at 20°C and 30% RH, cumulative percentage weight loss was about 1.5, 2.5 or 4%, respectively (not shown) and fruit were transferred to 90% RH. Transfer of fruit to high RH reduced the rate of weight loss (Fig. 1A). By the end of the experiment, weight loss of fruit stored at low RH was about two times greater than that of fruit stored at high RH (not shown), yet there was no difference in peel pitting between the two treatments (Fig. 1B). Transfer of fruit from low to high RH conditions increased postharvest peel pitting, even though the rate of dehydration was reduced to levels similar to fruit kept at high RH for 20 days. By the end of the experiment, PPI increased only slightly in fruit stored 3 days at 30% RH and then transferred to 90% RH but PPI was progressively higher in fruit stored for 6 and 10 days at low RH before transfer to high RH (Fig. 1B).

3.2. Washed fruit

Daily percent rate of weight loss in fruit stored at 30% RH was 1 after 6 days of storage, and decreased progressively thereafter to 0.6 by the end of the experiment. Weight loss rate in fruit stored at 90% RH

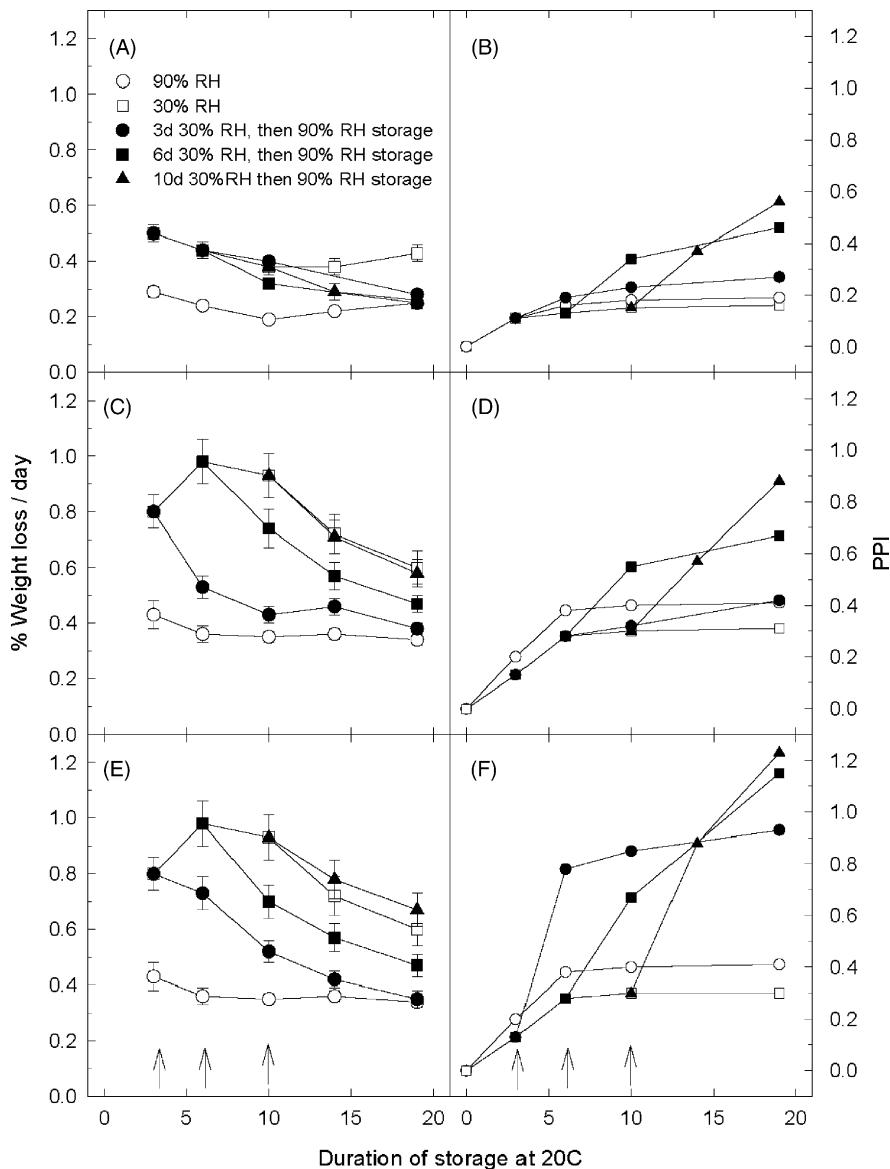


Fig. 1. Percentage weight loss per day (A, C and E) and PPI (B, D and F) of 'Marsh' grapefruit stored at 20°C. Fruit were either stored at 30% RH or 90% RH for 20 days, or fruit stored for 3, 6 and 10 days at 30% RH were transferred to 90% RH for the remainder of the experiment. Fruit were stored directly after harvest (A and B), washed immediately after harvest (C and D) or washed immediately after harvest and then waxed prior to transfer to 90% RH (E and F). Arrows indicate the point of transfer from 30 to 90% RH.

was around 0.4% during the storage period (Fig 1C). Weight loss of fruit stored at low RH was two times higher than that of fruit stored at 90% RH, and both rates were higher than in unwashed fruit used in the first experiment. By the end of the storage period, weight loss was about 12% for fruit stored at low RH

and 6% for those stored at high RH, as compared to 8 and 4%, respectively, in the first experiment. The transfer of fruit from 30 to 90% RH diminished the weight loss rate. By the end of the experiment, rate of weight loss in fruit stored at 30% RH for 3 days followed by storage at 90% RH was similar to that of

fruit stored at 90% RH. Rate of weight loss was also reduced in fruit stored at 6 and 10 days at 30% RH followed by transfer to 90% RH, but the rates were progressively higher (Fig 1C).

The PPI of fruit stored at constant low or high RH remained low (between 0.3 and 0.4 in both RH conditions), but transfer of fruit from 30 to 90% RH caused a marked increase in peel pitting (Fig. 1D). By the end of the experiment, PPI increased 0.3, 0.4 and 0.6 units in fruit stored for 3, 6 and 10 days at 30% RH and then transferred to 90% RH, respectively.

In those fruit washed immediately after harvest and stored at 30% RH, a decrease in the weight loss rate was observed when fruit were waxed and transferred to high RH after 3 and 6 days at low RH but not after 10 days at low RH (Fig. 1E). The change in RH conditions increased PPI in all cases, and values were higher than in the preceding experiments (Fig. 1F). By the end of the experiment, PPI increased 0.8, 0.85 and 0.9 units in fruit stored for 3, 6 and 10 days at 30% RH, respectively.

3.3. Washed and waxed fruit

Daily rate of weight loss in washed and waxed fruit stored at 30% RH decreased progressively from 0.7 to 0.5%, whereas this rate decreased from 0.4 to 0.2% in those fruit stored at 90% RH (Fig 2A). Weight loss of fruit stored at low RH was two times higher than that of fruit at high RH by the end of the experiment (not shown). After transfer to 90% RH, the weight loss rate of fruit kept for 3, 6 or 10 days at 30% RH diminished to equal the rate of the fruit kept at 90%. The PPI of fruit washed and waxed at the beginning of the experiment and stored at constant RH (30 or 90%) remained low. Transfer of fruit from low to high RH caused an increase in PPI (Fig. 2B). By the end of the experiment, PPI increased 0.44, 0.45 and 0.5 units in fruit stored for 3, 6 or 10 days at 30% RH, respectively.

Symptoms of postharvest peel pitting became apparent between 2 and 5 days after transfer of fruit from low to high RH (Fig. 3). Damage was more severe in washed and waxed fruit, followed by washed fruit and non-washed fruit. The PPI observed at the end of the experiment and the cumulative percentage weight loss at the point of transfer from low to high RH showed a significant positive correlation ($P < 0.05$; Fig. 4). In

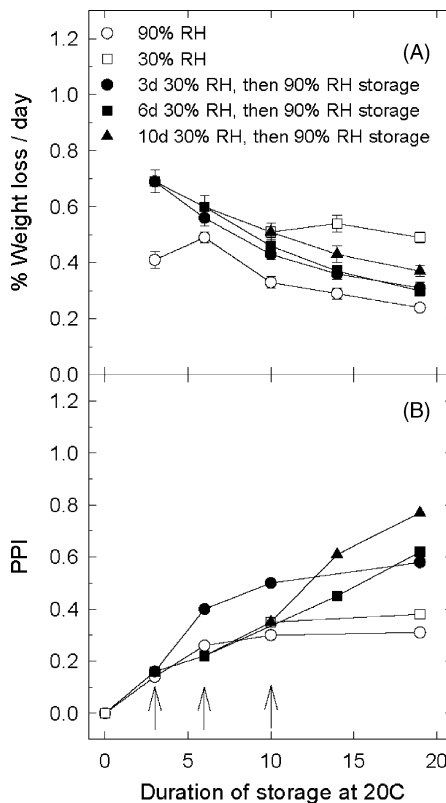


Fig. 2. Percentage weight loss per day (A) and PPI (B) of 'Marsh' grapefruit washed and waxed immediately after harvest. Fruit were either stored at 20°C and 30 or 90% RH for 20 days, or stored for 3, 6 and 10 days at 30% RH and then transferred to 90% RH for the remainder of the experiment. Arrows indicate the point of transfer from 30 to 90% RH.

general, the higher the weight loss during the dehydration event, the more pitting developed on the peel.

Light microscopy of healthy grapefruit flavedo revealed an epidermis with small, densely packed isodiametric cells. Below the epidermis, the oil gland was surrounded by oval hypodermal cells. Cells at the bottom of the oil glands were also spherical or oval. The size of these cells and the intercellular spaces among them increased deeper in the rind until the outer layers of albedo were reached (Fig. 5A). Initially, the disorder developed as a depression of small areas clustered randomly over the surface of the fruit, but mainly in equatorial areas. A few days later, new depressed clustered spots appeared randomly. Finally, some of the collapsed areas turned red and brown with progressive

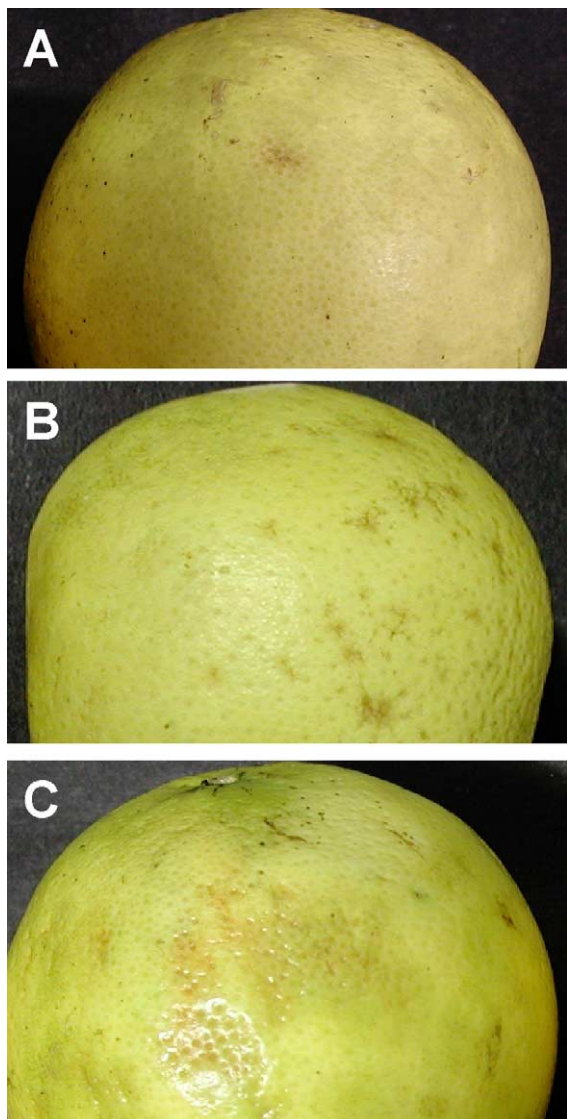


Fig. 3. Postharvest peel pitting in 'Marsh' grapefruit 10 days after transfer from 30 to 90% RH. Unwashed (A), washed (B) and washed and waxed (C) fruit.

drying. Observation of the damaged areas by light microscopy revealed the same ultrastructural abnormalities following every treatment assayed. Epidermal and subepidermal cells appeared strongly stained and crushed, indicating a collapse in these tissues, and some layers of enveloping cells of the oil glands had become flattened (Fig. 5B). Flattened cells were located surrounding the oil gland. Depressions in the

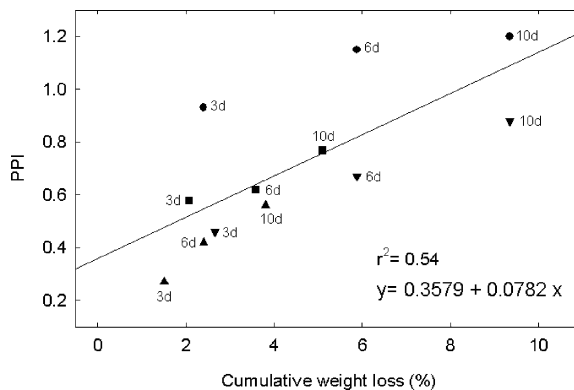


Fig. 4. Correlation plot between cumulative percentage weight loss and PPI in transferred fruit at the end of the storage period. Unwashed fruit stored for 3, 6 or 10 days at 30% RH and then transferred to 90% RH (▲). Washed fruit stored for 3, 6 or 10 days at 30% RH and then transferred to 90% RH (▼). Washed and waxed fruit stored for 3, 6 or 10 days at 30% RH and then transferred to 90% RH (■). Washed fruit kept at 30% RH for 3, 6 and 10 days and then waxed and transferred to 90% RH (●).

cuticle were coincident with areas of collapsed epidermal and subepidermal cells. As the disorder became more severe, layers of flavedo cells at the bottom of oil glands and adjacent deeper areas appeared twisted and wrinkled. Finally, coincident with browning of the flavedo, oil glands became deformed and began to collapse. At this stage, oil droplets could be visibly observed between albedo cells below the deformed oil glands.

4. Discussion

To our knowledge, these are the first data providing conclusive evidence for the involvement of changes in RH during postharvest handling in the development of postharvest peel pitting in 'Marsh' white grapefruit at non-chilling temperatures. The experimental conditions used in this study are not standard postharvest practices for citrus fruit, but as shown in previous work (Alférez et al., 2003), are useful to understand factors causing this disorder.

Fruit dehydrated at 30% RH for an increasing period of time (3, 6 or 10 days) followed by transfer to 90% RH storage, markedly increased PPI of 'Marsh' grapefruit in all treatments. PPI remained at low values in fruit in which storage RH was constant,

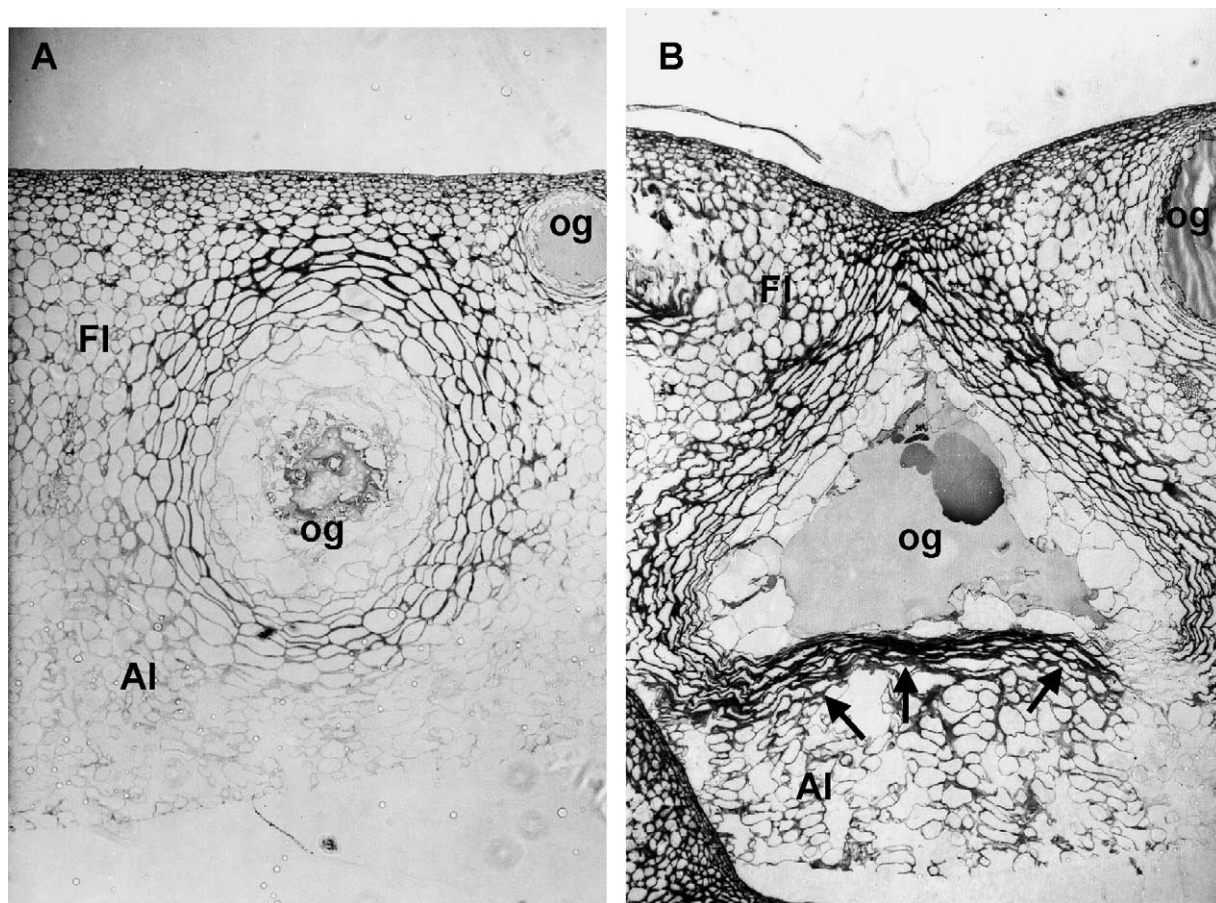


Fig. 5. Light micrographs of cross-sections of 'Marsh' grapefruit peel from healthy (A) and pitted (B) fruit. Fruit were washed immediately after harvest, stored for 6 days at 30% RH, waxed and then transferred to 90% RH. The sample was taken 10 days after transfer. Arrows show layers of collapsed flavedo cells at the bottom of the oil gland (B). Al: albedo; Fl: flavedo; og: oil gland.

regardless of whether RH was low or high from the beginning of the experiment. In addition, fruit stored at 90% RH for various periods of time and then transferred to 30% RH did not exhibit symptoms of postharvest peel pitting (data not shown) confirming previous observations by Alférez (2001).

The sudden change in RH from low to high resulted in a slower rate of dehydration in fruit followed by an increase in peel pitting several days later. Although a significant positive correlation ($P < 0.05$) was observed between PPI and percentage cumulative weight loss, the relatively low correlation coefficient suggests that the change in VPD may not be the sole triggering factor of this disorder. This agrees well with pre-

vious observations in 'Navel' oranges (Alférez, 2001; Alférez et al., 2003), which showed that changing VPD by holding RH constant while lowering storage temperature did not result in peel pitting.

Water potential within flavedo and albedo tissues was postulated to play an important role in peel pitting. During the process of dehydration, water potential decreases progressively in the peel as water moves from albedo through flavedo and then to the atmosphere. Water loss occurs mainly in the exocarp and mesocarp and the contribution of pulp to this process is minimal (Ben-Yehoshua, 1969).

Anatomical evidence suggests that water moving through a citrus fruit follows the vascular bundles in

the mesocarp, diffuses through the exocarp and evaporates. Since the vesicles of the endocarp are anatomically isolated, and there are no vascular connections with mesocarp, they are not involved in water transport during fruit transpiration (Kaufmann, 1970). In a situation of water stress, the flavedo largely draws water from the lower cell layers, generating a water deficit in the albedo layer (Alférez, 2001; Alférez et al., 2003). The transfer of fruit to high RH conditions reduces the VPD, and the water potential recovers faster in flavedo than in albedo cells. In this case, the newly hydrated flavedo can be a source of water for dehydrated albedo, and the high water demand of the albedo and the inability of albedo cells to hydrate cause areas of twisted and flattened cells between flavedo and albedo (Agusti et al., 2001). Our data with grapefruit corroborate these observations, as Fig. 5B shows that after transfer of fruit from low to high RH, collapsed subepidermal cells were visible and flattened layers of cells appeared in the deeper flavedo areas. Since plasmalemma and tonoplast functions are impaired in collapsed cells, their osmoregulatory capacity is affected as well, and in the absence of turgor, the walls of damaged cells collapse (Shomer and Erner, 1989; Agusti et al., 2001).

Several days after pitting was visible, peel depressions became brown and bronze in color. As affected cells became more and more compressed, cells appeared to lose cytoplasmic content. As cellular and oil gland contents were released into the intercellular spaces and surrounding areas (Fig. 5B), the subsequent enzymatic oxidation may be responsible for the brown peel staining as previously suggested (Agusti et al., 2001; Lafuente and Sala, 2002).

Other authors related peel pitting in grapefruit with coating formulation and gas permeability, suggesting that the development of postharvest pitting was caused by reduction in fruit internal O₂ and increase in CO₂ levels (Petracek et al., 1998). Using more permeable fruit coatings and/or storage at lower temperatures was shown to reduce postharvest peel pitting. Further, storage at constant relative humidities was shown not to cause peel pitting (Ben-Yehoshua, 1987; Alférez et al., 2003). We also found that storage at constant RH had little effect, but transferring fruit from low to high RH storage strikingly promoted peel pitting. Thus, it was the change in RH from low to high that led to pit-

ting. More permeable fruit coatings would not only increase O₂ and CO₂ exchange, but also increase water exchange. This increased water exchange would lessen the VPD gradient from the internal fruit tissues to the atmosphere, reducing the incidence of peel pitting. Lowering storage temperatures could also reduce the fruit/atmosphere VPD gradient and reduce the rate of water loss from the fruit to the atmosphere. When we used the less permeable shellac-based coating on grapefruit, peel pitting was enhanced but only if a previous water stress existed. In addition, fruit waxed at the beginning of the experiment that were subjected to increasing periods of low RH followed by high RH had increased amounts of peel pitting. In this case, the only postharvest condition that varied was the transition from low to high RH.

Petracek et al. (1995) found a very low correlation between internal CO₂, O₂ and peel pitting. In addition, lowering fruit O₂ and increasing CO₂ under controlled atmosphere conditions caused a variable amount of peel pitting (Petracek et al., 1998). They concluded that there is no critical level of O₂ triggering peel pitting, although altering gas exchange seemed to have an effect. Variation in peel pitting was attributed to sensitivity of cultivars to changes in internal gases. We also stored grapefruit at 5% CO₂ and 14% O₂ but were unable to induce peel pitting if there was no previous dehydration (data not shown). These results strongly suggest that exposure of fruit to low RH conditions followed by high RH storage, and not unfavorable internal CO₂ or O₂ concentration, promotes peel pitting.

One effect of washing fruit on a commercial packingline is the removal or redistribution of the natural fruit wax coating, which induces substantially greater water loss compared with non-washed fruit (Kaplan, 1986). Our results corroborate this observation, as fruit washed immediately after harvest and then kept at low RH for 18 days lost 50% more weight than fruit not washed but stored under the same conditions (Fig. 1A and C). Also, we observed a higher weight loss daily rate in the first 3 days of storage when fruit were stored at low RH after washing, followed by a decline until a constant transpiration rate was reached (Fig. 1C and E). This high initial weight loss is commonly observed as described (Smith, 1931, reviewed by Sastry et al., 1978) and may be due to injuries encountered during postharvest handling. It is interesting to note that the more prolonged the dehydration period, the

more peel damage was observed after rehydration. If dehydrated fruit were waxed prior to transfer to high RH, the PPI was even higher (Fig. 2). As Alf3rez et al. (2003) previously showed, longer dehydration periods resulted in higher PPI after rehydration due to impairment in the capacity of albedo to recover water potential. Waxing after dehydration could impede the water uptake by flavedo cells, thereby enhancing the water demand in dehydrated albedo.

In summary, this work indicates that water status plays a significant role in the ability of fruit to undergo postharvest peel pitting. Our work reported on the correlation between postharvest conditions and peel pitting, however, preharvest factors that alter fruit water status such as irrigation, rainfall, or time of day fruit are harvested may also be important. The critical storage condition that promoted pitting was the transition from low to high RH at non-chilling temperatures. Commercial waxes that alter the water status of the fruit can enhance peel pitting if fruit undergo a previous postharvest water stress. These observations have interesting implications for the citrus industry, namely that postharvest dehydrating conditions followed by rehydration should be avoided to minimize pitting. It will be important to determine a critical threshold dehydration time necessary to induce pitting after rehydration.

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