Postharvest Biology and Technology 123 (2017) 77–82

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

Susceptibility to postharvest peel pitting in Citrus fruits as related to albedo thickness, water loss and phospholipase activity

Paul J.R. Cronjé[a,*], Lorenzo Zacarías[b], Fernando Alférez[b,1]

[a] Citrus Research International, Department of Horticultural Science, Stellenbosch University, Private Bag X1, Matieland 7602, Stellenbosch, South Africa
[b] Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC), Catedratico Agustín Escardino 7, 46980 Burjasot, Valencia, Spain

ARTICLE INFO

Article history:
Received 17 March 2016
Received in revised form 15 August 2016
Accepted 23 August 2016
Available online 4 September 2016

Keywords:
Albedo
Citrus fruits
Peel pitting
Phospholipases
Water stress

ABSTRACT

To study the influence of albedo thickness and phospholipase activity on the incidence of postharvest peel pitting (PP) in citrus fruit the tangor Ortanique, mandarin, with a thin albedo and tolerant to peel pitting were compared to that of the sensitive Navelate Navel orange with a thick albedo. Fruit from both cultivars was subjected to identical postharvest practices consisting of washing and/or waxing on a commercial packline and thereafter the fruit was stored for 3 weeks at 30% or 90% relative humidity (RH). For comparison, other fruit lots were washed manually and stored as above. Periodically, water loss, water, osmotic and turgor potentials were monitored and peel pitting incidence was evaluated. For both cultivars fruit weight loss was higher in packline than in manually processed, however, only 'Navelate Navel' orange fruit developed peel pitting with higher incidence in the packline treatments compared with manually processed fruit. In addition, wax coating exacerbated this effect leading to higher pitting of 'Navelate Navel' orange. Accordingly, water potential variations were more pronounced in wax coated fruit from 'Navelate Navel' orange as compared to 'Ortanique' mandarin. Furthermore, Phospholipase D and A_2 (PLD and PLA_2) activities were higher in the peel from the pitting susceptible cultivar, suggesting their activation by sharper changes in peel water potential. Collectively, results suggest that water movement through cell layers in a thick albedo is related to postharvest peel pitting and support the notion that inability to properly adjust water status in peel tissue after prolonged water stress results in cell collapse and tissue damage. Moreover, enhanced phospholipase activity appears to be also a response of peel tissues to conditions causing peel pitting.

1. Introduction

Postharvest peel pitting at non-chilling temperatures is a physiological disorder affecting several varieties of citrus fruit (Alférez et al., 2005). In recent years' evidence has accumulated showing that peel water status plays a major role in its origin. Studies on water flux across peel tissues have suggested that albedo thickness and peel morphology may play an important role in determining susceptibility to develop peel pitting. In Marsh grapefruit, a cultivar with a thick albedo (±10 mm), early development of peel pitting after transferring fruit from low to high RH had a linear relationship with water loss before changing water conditions. However, as albedo thinned due to compaction following prolonged water stress, the linearity was lost resulting in a reduced pitting development rate (Alférez et al., 2005).

Pitting is influenced by maturation stage, the fruit being more susceptible to the disorder as the peel ages (Alférez and Zacarías, 2014). During fruit development and maturation, the albedo is comprised of the typical eight-lobed cells with abundant intercellular air spaces, which stretch thinner as the fruit grows. In mandarin cultivars it gradually degenerates and disappears, leaving only a netlike vascular element between the flavedo and pulp – this is referred to as "reticulata" for which the common mandarin has been named (Schneider, 1968). The pigmented flavedo layer consists of the cuticle and epidermis with plastids, oil glands and vascular trace endings (Ford, 1942; Scott and Baker, 1947; Alquézar et al., 2010). Mass transport of water vapour through the citrus peel involves movement over this complex cellular matrix of the pigmented flavedo as well as the spongy white layer of the albedo. In addition, these structures are anatomically isolated from the juice vesicles with an insignificant level of water transport between them (Kaufmann, 1970). Hence,

* Corresponding author.
E-mail address: paulcronje@sun.ac.za (P.J.R. Cronjé).

[1] Current address: Citrus Research and Education Centre, Institute of Food and Agricultural Sciences, University of Florida. 700 Experiment Station Rd, 33850 Lake Alfred, Fl, USA.

http://dx.doi.org/10.1016/j.postharvbio.2016.08.012
0925-5214/

morphological features of the different citrus fruit peel tissues and how water status can be regulated among them and the surrounding environment appear to determine susceptibility to develop peel pitting (Alférez et al., 2010; Alquézar et al., 2010).

There is a clear difference between albedo thickness amongst fruits of the major citrus cultivar groups of horticultural importance: i.e. oranges (*C. sinensis*) 5–10 mm, grapefruit (*C. paradisi*) >10 mm, lemons (*C. limon*) ±5 mm, and mandarin (*C. unshui* and *reticulata*) <3 mm. However, the influence of peel morphology and specifically the albedo thickness on physiological peel disorders is currently unknown. Previous studies have shown that peel water potential evolves differently in flavedo and albedo tissues, the changes of this parameter being exacerbated more in albedo as compared to the flavedo, as illustrated in the 'Marsh grapefruit' with a thick albedo (Alférez et al., 2010). These observations suggest that this spongy tissue may exert a buffering effect on sharp variations in water status, modulating the response of the peel to the stress. On the other hand, it has been suggested that water transport in peel occurs preferentially in an aqueous phase and is not inhibited by the natural wax layer or closed stomata (Ben-Yehoshua et al., 1985). However, postharvest processing involving wax application can effectively alter the water movement over the rind (Hagenmaier and Baker, 1994). The effect of packline processing on water status of citrus fruit peel has also been shown by Alquézar et al. (2010). Washing and waxing fruit through a rolling brush system enhanced peel water stress and significantly altered water potential and its components (i.e. osmotic and turgor potentials) in flavedo and albedo. These changes resulted in fractures in cell walls of the lobulated albedo cells of 'Navelate' orange and promoted an increase in peel pitting incidence (Alférez et al., 2010; Alquézar et al., 2010). Susceptibility to peel pitting by commercial processing may be due to the removal of the natural waxes on the fruit surface, as previously suggested (Kaplan, 2006). This may alter the water vapour exchange amongst peel tissues and the surrounding atmosphere, impeding an appropriate water potential in cells and creating conditions for pitting to develop. Maintaining appropriate water potential in plant cells depends on adequate regulation of turgor pressure, which prevents cells from absorbing water in excess. This requires some sort of water pressure and osmotic sensing (Sanchez-Diaz and Aguirreolea, 2000).

Phospholipases, a superfamily of enzymes linked to membranes that participate in membrane homeostasis, lipid turnover and/or membrane phospholipid degradation may act as mechanosensors in other biological systems (Lehtonen and Kinnunen, 1995). Phospholipid membrane degradation by activation of these enzymes in the development of peel pitting in citrus fruit has been suggested, since inhibition of PLA2 and PLD activities reduced peel pitting in 'Fallglo' tangerines subjected to water stress and rehydration (Alférez et al., 2008). In addition, the expression of genes encoding PLA_2 and PLD is altered by water stress in citrus fruit and ABA signalling appears to be involved the regulation of gene expression (Romero et al., 2013, 2014). For example, increased expression of PLD genes occurred in varieties with reduced levels of ABA and also in response to water deficit (Romero et al., 2014); and exogenous ABA overcome these effects. In general, response to ABA during water stress leading to peel disorders is regulated during fruit maturation and involves phospholipase activity (Romero et al., 2013). However, the mechanism by which alterations in water potential is related to phospholipases activation is not well understood.

Our hypothesis establishes that tissue thickness may modulate water flux across albedo. A previous study has shown that water potential evolves differently in different layers of a thick albedo, and that this effect is influenced by processing on a packline (Alférez et al., 2010). In this paper we have studied the effect of processing fruit from two cultivars with a distinct difference in albedo thickness on a commercial packline. We have selected 'Ortanique', a mandarin hybrid for the thin albedo (1–3 mm) and 'Navelate' orange as the thick albedo (5–10 mm) cultivar. Fruit from these varieties was subjected to identical postharvest manipulations. Our results show that 'Navelate Navel' orange fruit, known to be susceptible to peel pitting, underwent sharper variation in water stress and increase of phospholipase A_2 and D activities after processing and storage at high RH. These effects were not found in 'Ortanique' mandarin, which did not develop pitting. Our results link water stress in the peel, due to sharp modifications in water potential, with activation of phospholipases and peel pitting development. Furthermore, results strongly suggest that phospholipase activation depends on the ability of peel tissues to cope with the stress severity and the rapidity of water adjustment.

2. Material and methods

2.1. Plant material and postharvest treatments

Mature fruit of 'Navelate Navel' orange (*Citrus sinensis* L. Osbeck) and 'Ortanique' tangor, a natural hybrid between an orange (*C. sinensis*) and a mandarin (*C. reticulata* Blanco), referred to commercially as a mandarin with a leathery rind difficult to remove from the flesh (Saunt, 2000). Both cultivars were grafted on Carrizo citrange rootstock {[*Poncirus trifoliata* (L.) Raf.] x [*Citrus sinensis* (Osb.) L.]} and harvested at commercial maturity from a commercial orchard located in Lliria, Valencia (Spain). Fruit was free of peel defects, uniform in size and divided into five lots of 35 fruit each. Fruit were delivered the same day after harvesting to the packinghouse and submitted to the following treatments: one lot was stored continuously at 30% RH without any additional treatment as the control; a second lot was washed through a commercial packline and then stored at 90% RH; and a third lot was washed and waxed with commercially available shellac-based wax (Sta-Fresh 590 HS, FMC Food Tech) in the packline and then stored at 90% RH. The two remaining lots were stored at 30% RH for 4 d prior to being either washed only or washed and waxed as above and then stored at 90% RH for 17 d (21 days from harvest). All the treatments were stored at 20 °C for a total of 21 d. The day of the harvest 5 fruit per treatment lot were used for initial sampling of the peel water potential and the analysis of enzyme activity expression as described below. The remaining 30 fruit per treatment lot were divided into 3 replicates of ten fruit each from which one fruit per replicate were used for destructive sampling at 4, 7 and 14 days. The remaining fruit was evaluated for pitting as described below at day 21 before destructive sampling for analysis. Those two treatments being washed and waxed were regarded as commercial controls.

2.2. Weight loss and peel pitting estimation

Fruit weight was monitored after harvest and 14 and 21 days during storage and the peel inspected for the intensity and extension of pitting symptoms in order to rate on a scale from 0 (no pitting/damage) to 3 (severe pitting/damage). Results were expressed as peel pitting/damage index (PPI), calculated according to the formula:

PPI = $\sum$ (Peel pitting/damage scale (0–3) × number of fruit within each class)/total number of fruit.

2.3. Water potential measurement

To measure flavedo and albedo water potential, 5 mm disks from the equatorial region of the fruit were excised with a cork borer. Due to the thinness of the albedo in 'Ortanique' fruit, it was