Plant Science 210 (2013) 46–52

Contents lists available at SciVerse ScienceDirect

Plant Science

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

Cell wall modifications and ethylene-induced tolerance to non-chilling peel pitting in citrus fruit

Ariel R. Vicente[a,b,c], George A. Manganaris[c], Ioannis S. Minas[c], Vlasios Goulas[c], María T. Lafuente[d,*]

[a] Centro de Investigación y Desarrollo en Criotecnología de Alimentos, Facultad de Ciencias Exactas, CONICET-UNLP, 47 y 116, La Plata 1900, Argentina
[b] LIPA (Laboratorio de Investigación en Productos Agroindustriales), Facultad de Ciencias Agrarias y Forestales Universidad Nacional de La Plata, Calle 60 y 119, La Plata CP 1900, Argentina
[c] Cyprus University of Technology, Department of Agricultural Sciences, Biotechnology and Food Science, 3603 Lemesos, Cyprus
[d] Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC), Consejo Superior de Investigaciones Científicas, Av. Agustín Escardino 7, 46980 Paterna, Valencia, Spain

ARTICLE INFO

Article history:
Received 27 February 2013
Received in revised form 22 April 2013
Accepted 6 May 2013
Available online 13 May 2013

Keywords:
Albedo
Alcohol insoluble residue
Ethylene conditioning
Flavedo
Pectin
Physiological disorder

ABSTRACT

Non-chilling peel pitting (NCPP), a storage disorder resulting in the formation of depressed areas in the peel of many citrus cultivars, is reduced by ethylene treatments. We hypothesized that this effect may be associated with biochemical changes of cell wall components. Therefore, we extracted cell wall material from albedo and flavedo tissues of 'Navelate' oranges stored in air, conditioned with ethylene ($2\ \mu L\ L^{-1}$) for 4 days and subsequently transferred to air, or continuously stored in an ethylene-enriched atmosphere ($2\ \mu L\ L^{-1}$). Uronic acids and neutral sugars were extracted into five fractions enriched in specific wall polymers namely water-, CDTA-, Na_2CO_3-, and 1 and 4 M KOH-soluble fractions. Pectin insolubilization was found in control fruit at long storage times. Ethylene treatments, alleviating NCPP, increased polyuronide solubility in the albedo and had a slight effect on the flavedo. Ethylene-treated fruit showed greater content of water-soluble neutral sugars and a larger proportion of hemicelluloses readily extractable with 1 M KOH, with a concomitant reduction in the 4 M KOH-soluble fraction. This suggests that the protective role of ethylene on NCPP is associated with an increased solubilization of the wall of albedo cells.

1. Introduction

The plant hormone ethylene is usually associated with fruit ripening regulation, senescence and it is involved in the development of some physiological disorders such as blackheart of pineapple [1], toughening of asparagus [2] and apple scald [3]. However, it is well established that it may protect other horticultural commodities from stresses causing pathological and physiological disorders and that the responses to ethylene are affected by hormone concentrations and treatment durations and also by tissue susceptibility and by the organ physiological stage [4–7].

Mature citrus fruits, in which the chloroplast to chromoplast transition has been completed, tolerate ethylene levels that may reduce infection caused by *Penicillium digitatum* [8], chilling injury [9] as well as peel collapse occurring in fruit held under non-chilling conditions (22 °C, 90–95% RH) [7]. This disorder, manifested as collapsed areas of the flavedo and part of the albedo (Supplementary data, Fig. S1), is known as non-chilling peel pitting (NCPP). A transcriptomic approach on mature oranges highlighted the molecular basis of the ethylene-induced resistance of citrus fruits to *P. digitatum* infection [8]. However, the biological basis of the ethylene-induced tolerance to NCPP in citrus fruits is still unknown [7]. Water stress may favour this disorder [10,11], but NCPP may be also developed in harvested citrus fruit held under non-stressful environmental conditions [12]. Given that fruit detachment induces fast sucrose depletion [13,14] and changes in proteins related to starvation-induced ageing in citrus peel [15], it has been suggested that the lack of carbon sources originated by fruit detachment may be involved in peel collapse [16]. Moreover, on view of ultrastructural changes, it was suggested that ethylene-induced modifications in cell wall might participate in the beneficial effect of the hormone reducing NCPP [16].

The plant cell wall provides mechanical support to individual cells, tissues, and organs. However, its perception as a solely rigid structure providing mechanical support is long past and it is now accepted that it has a key role in a plant's interactions with pathogens and responses to abiotic factors. Dynamic changes in wall polymers occur during normal development and in response to hormonal and environmental conditions [17]. These modifications may have large effects on tissue biomechanical properties

* Corresponding author. Tel.: +34 963900022; fax: +34 963636301.
E-mail addresses: arielvicente@quimica.unlp.edu.ar (A.R. Vicente), george.manganaris@cut.ac.cy (G.A. Manganaris), iminas@agro.auth.gr (I.S. Minas), vlasios.goulas@cut.ac.cy (V. Goulas), mtlafuente@iata.csic.es (M.T. Lafuente).

0168-9452/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.plantsci.2013.05.001

[18,19]. In addition, wall turnover may generate biologically active oligogalacturonides (OGs) able to induce defensive and developmental responses [20] and to induce ethylene biosynthesis in climacteric fruits like tomato [21]. Solubilized pectin and/or other carbohydrate pools derived from the bulk degradation of cell wall have been considered as alternative sources for fuelling metabolism and maintaining cellular homeostasis under shortage conditions [17]. Although we have recently found that ethylene treatments reducing NCPP caused cell wall ultrastructural modifications [16], the cell wall changes induced by these treatments in the flavedo and albedo tissues of citrus fruit are still unknown. The aim of this study was to characterize compositional changes induced by ethylene treatments that reduce NCPP in cell wall of the flavedo and albedo of mature 'Navelate' (*Citrus sinensis*, L. Osbeck) sweet orange.

2. Materials and methods

2.1. Fruit material, ethylene treatments and storage

Mature 'Navelate' orange fruits were harvested by the end of February (2 months after fruit colour change) from a commercial orchard at Valencia, Spain and immediately delivered to the laboratory. A total of 540 oranges free of visual defects were sorted on the basis of uniform size and divided into three groups, each containing 180 fruit, which were stored under: (a) a continuous flow of air for up to 16 days (control); (b) a continuous flow of air after being conditioned for 4 days with air containing 2 $\mu L\,L^{-1}$ ethylene (ethylene conditioned, EC); and (c) a continuous flow of air containing 2 $\mu L\,L^{-1}$ ethylene (ethylene, E). All treatments were performed at 20 °C and 90–95% RH to avoid stressful environmental conditions. Calcium hydroxide was added to the storing trays to prevent the accumulation of respiratory CO_2. Three replicates of 20 fruit per treatment were used to determine the NCPP index. Additional replicate samples of 10 fruit per treatment and storage period were used for determining changes in cell wall materials (CWM) in both the flavedo and albedo tissues.

Flavedo and albedo samples were taken from the whole surface of fruit from each treatment at harvest, and after 4, 8, 12 and 16 days storage, and immediately frozen in liquid nitrogen, ground to a fine powder in a mill, and stored at −80 °C until use.

2.2. Estimation of non-chilling peel pitting

The NCPP symptoms were manifested as collapsed areas of the flavedo and part of the albedo (Supplementary data, Fig. S1). A visual rating scale from 0 (no damage) to 3 (severe damage), based on surface damage, was used to estimate NCPP severity. The average NCPP pitting index was calculated as previously reported [22] by using the following formula:

NCPP index = Σ(damage scale (0–3) × number of fruit in each class)/total number of fruit.

Three replicates of 20 fruits were used, and results were expressed as the mean NCPP index ± standard error (SE).

2.3. Isolation of cell walls

Five grams of albedo and flavedo tissues from either untreated (control) fruit or subjected to ethylene conditioning or ethylene treatments were placed in 95% (v/v) ethanol and subsequently were homogenized using a Polytron (Kinematica, Swiss) with 20 mL of 95% ethanol and boiled for 30 min to ensure the inactivation of cell wall modifying enzymes and the extraction of low molecular weight solutes. The insoluble material was vacuum filtered and sequentially washed with 40 mL of ethanol, 40 mL of chloroform:methanol (1:1, v/v), and 40 mL of acetone and dried at 37 °C, yielding the alcohol insoluble residue (AIR). The dried residue was weighed, and the yield of AIR was calculated. Three independent extractions were made from fruits exposed to each treatment and storage time.

2.4. Cell wall fractionation

Fractions of different cell wall components were obtained by sequential chemical extraction of the AIR as elsewhere described. [23]. Forty milligrams of AIR from each sample were suspended in 10 mL of water and stirred at room temperature for 3 h under continuous shaking, then centrifuged at 6000 × *g* and vacuum filtered. The filtrate was taken to 14 mL with water and designated as water-soluble fraction (WSF).

The residue was then extracted with 10 mL of 50 mM trans-1,2-diaminocyclohexane-tetraacetic acid (CDTA), pH 6.5, for 3 h under continuous shaking. The slurry was centrifuged and the supernatant was collected, taken to 14 mL with water and designated as CDTA-soluble fraction (CSF). The CDTA-insoluble pellet was then extracted with 10 mL of 50 mM Na_2CO_3 at 4 °C for 1 h. After centrifugation and volume adjustment (as mentioned above), the extracted solution was designated as Na_2CO_3-soluble fraction (NSF). Subsequently, the pellet was extracted with 10 mL of 1 M KOH at 4 °C for 1 h under continuous shaking. After centrifugation, the supernatant was adjusted to 14 mL with the addition of distilled water and designated as 1 M KOH-soluble fraction (1KSF). Finally the pellet was re-extracted with 4 M KOH to yield the 4 M KOH-soluble fraction (4KSF). Samples of the different fractions obtained were assayed in duplicate for uronic acids (UA) and neutral sugars (NS) contents as described below. The residue after extraction with 4 M KOH was subjected to three successive washes with ethanol 50% (v/v) in order to remove the KOH, leaving a residue which was dried at 60 °C, weighed and designated as α-cellulose.

2.5. Uronic acids

The UA contents were measured according to Blumenkrantz and Asboe-Hansen [24]. Aliquots of the different cell wall fractions were poured into test tubes and taken to 200 μL with water. Subsequently, 1 mL of 98% (w/w) H_2SO_4 containing 75 mM sodium borate was added in an ice water bath. Samples were shaken and incubated at 100 °C for 10 min. After boiling, the reaction mixtures were cooled in a water ice bath and 20 μL of 0.15% (w/v) *m*-phenylphenol in 0.5% (w/v) NaOH were added. The mixture was gently mixed and 300 μL of each sample was loaded in 96-well plates and the absorbances at 520 nm were measured in a plate reader (model Infinite 200 PRO, Tecan GmpH, Austria). The calibration curve was established using galacturonic acid in the range of 0–50 $\mu g\,mL^{-1}$ and results were expressed as milligrams of galacturonic acid equivalents per gram of AIR. Three independent samples were analysed for each treatment and storage condition and measurements were done in duplicate.

2.6. Neutral sugars

Anthrone method in 96-well plate format was used to measure NS [25]. Aliquots from the different cell wall fractions were pipetted into test tubes and diluted to 500 μL with distilled water. Subsequently, 1 mL of 2 $g\,L^{-1}$ anthrone (in 98%, w/w H_2SO_4) was added in a water-ice bath. The samples were then incubated for 10 min at 100 °C. The reaction mixtures were cooled in a water-ice bath; 300 μL of each sample was loaded in 96-well plates and the absorbances at 620 nm were measured. The calibration curve was established using glucose in the range of 0–30 $\mu g\,mL^{-1}$. Three independent samples were analysed for each treatment and

storage condition and measurements were done in duplicate. Results were expressed as mg of glucose equivalents per g of AIR.

2.7. Statistical analysis

Experimental data are the means ± SE of three replicates of the determinations for each sample. Data were subjected to analysis of variance (ANOVA) and mean comparisons were done independently in the albedo and flavedo tissues using the Duncan's test at a level of significance of $P \leq 0.05$. Statistical analysis was performed by using SPSS 17.0 for Mac OS X (SPSS, Chicago, IL, USA) and graphs were created using Prism v5.01 (Graph Pad Inc., San Diego, USA).

3. Results and discussion

3.1. Effect of ethylene on the susceptibility of 'Navelate' oranges to NCPP

'Navelate' sweet orange fruit harvested after fruit colour change can be considered as a model system to study NCPP because of its high susceptibility to this disorder and its tolerance to ethylene levels required for NCPP reduction [7]. Depressed peel areas (NCPP) affecting the inner and outer part of the peel were first observed by day 8 in fruit held at 20 °C and 90–95% RH and increased over time (Fig. 1). Treating the fruits for 4 days with ethylene (2 $\mu L L^{-1}$) considerably reduced NCPP. This effect was more marked when they were continuously stored in 2 $\mu L L^{-1}$ ethylene. The greatest increase in NCPP occurred between days 8 and 12 in air-stored fruit. By day 12, the peel pitting index of control fruit was 3 and 7 times higher than that of fruit conditioned or continuously stored in ethylene, respectively. Overall, the incidence of NCPP was considerably lower in the ethylene-treated fruit until the end of the experiment (<0.5 on a scale up to 3.0). Ethylene has been involved in diverse responses to abiotic stresses in fruit and vegetables [5]. While in some cases, it can trigger undesirable quality changes, increasing chilling injury [26], lignification [27], off-flavours [28], and surface scalds [3], it may also provide protection against stresses causing physiological disorders. In citrus, ethylene production markedly increased in response to different biotic [29] and abiotic stress conditions [10,30]. We have previously shown that ethylene treatments reduced citrus pitting under chilling and non-chilling conditions [7,9]. Multiple responses triggered by ethylene have been suggested to participate in stress acclimation of plants and horticultural crops [5]. Examination of fruit surface by scanning electron microscopy suggested that ethylene induces epicuticular wax formation in sweet oranges [12]. Moreover, ethylene treatments reduced damage and up-regulated phenylpropanoid metabolism [7]. The cell wall plays important roles both affecting texture and at fruit-pathogen interfaces [31–34]. A role for pectic substances in the acclimation of fruits to stresses causing peel collapse has been also suggested [35]. However, changes in cell wall components in response to postharvest treatments are poorly studied in citrus fruit [36]. In terms of structural and histochemical analysis we have reported that ethylene treatments that reduced NCPP induced changes in cell wall appearance and composition [16]. In order to further characterize this response, we evaluated the changes in the content and solubility of the main cell wall components.

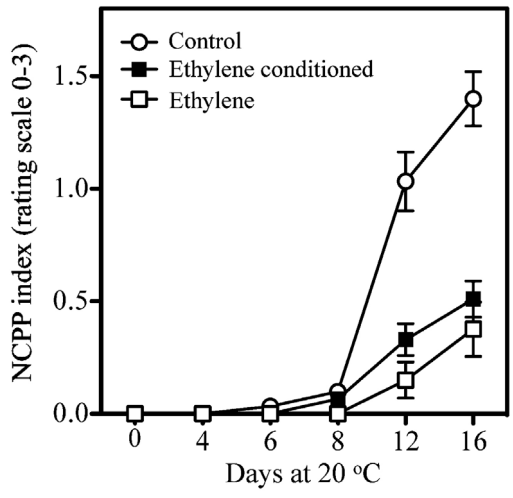

Fig. 1. Effect of ethylene on changes in NCPP index in mature 'Navelate' oranges stored under non-stressful environmental conditions (20 °C and 90–95% RH) for 16 days. Fruits were held in: (a) air (control); (b) air after being conditioned for 4 days with 2 $\mu L L^{-1}$ ethylene (ethylene conditioned) and (c) air containing 2 $\mu L L^{-1}$ ethylene (ethylene). A rating scale from 0 (no damage) to 3 (severe damage) was used. Results are presented as means of three replicates of 20 fruits each ± SE.

3.2. Effect of ethylene on the AIR content and on the relative distribution of uronic acids and neutral sugars in the albedo and flavedo tissues

The AIR content in the albedo (ca. 15%) of 'Navelate' oranges was greater than that in the flavedo (ca. 12%) (data not shown). Baluška et al. [37] reported that pectin material could be recycled by endocytosis and used for *de novo* formation of the cell wall. Moreover, it is well known that carbon starvation may increase the activity of hydrolases, leading to released sugar residues from cell wall polysaccharides to sustain respiration and other metabolic processes [38–40]. In the present study, the cell wall content decreased by 7% in the flavedo of fruit treated continuously with ethylene. In the albedo, a 10% reduction of the AIR was recorded by the end of the storage. Our results also showed that the decrease in the AIR was similar in control and ethylene-treated fruit (data not shown). This suggests that if ethylene treatments increase the recycling and usage of wall sugars as energy sources, the effect is not substantial. Plant walls can adapt to the highly dynamic conditions at which cells are exposed [41], while pectins are considered as a source of signalling molecules that could be generated in response to specific environmental cues [20,42]. Thus, rather than focusing on energy metabolism in the peel tissues, it could be of interest to determine whether the identified decrease in flavedo and albedo wall material indicated by our analysis has contributed to the generation of biologically active pectin-derived oligosaccharide signals in the ethylene-treated fruit. If so, these could serve to promote the observed cell wall changes or an increase in the fruit's production of ethylene or other responses that contribute to the reduction in NCPP. Most of the UA were either loosely (WSF) or ionically bound (CSF) to the cell wall in both the albedo and flavedo tissues. The tightly associated UA (NSF) represented in both tissues less than 10% of the total UA (Fig. 2A). A higher UA content was found in the WSF fraction of the albedo, while in the flavedo CDTA-soluble fraction was the more prominent. In both tissues, such proportions represented ca. 60% of the total UA content (Fig. 2A). The UA distribution remained nearly constant in the albedo during fruit storage and was not affected by the ethylene treatments. Ethylene had also little effect on the relative UA content in the flavedo cell wall where a slight decrease in the water-soluble UA and a slight increase in the ionically bound UA were measured after 12 days. In this tissue, a slight decrease in the loosely bound, together with a slight increase in the ionically bound UA, was observed after 12 days and this effect was slightly higher in ethylene-treated fruit. The relative contents of NS in the flavedo and the albedo of freshly harvested fruit were similar in the WSF, CSF and NSF fractions (Fig. 2B). Neutral sugars increased in the WSF of ethylene-treated fruit by day 12,

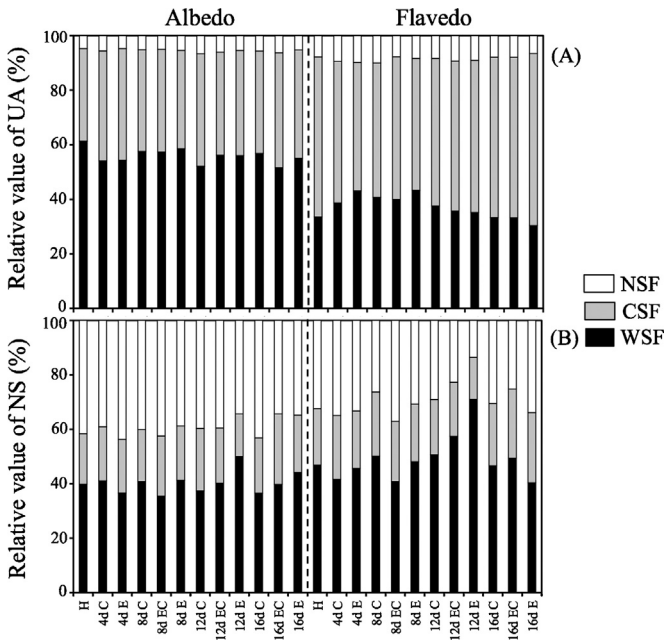

Fig. 2. Relative distribution of uronic acids (UA, A) and neutral sugars (NS, B) from the pectin rich fractions [(water-(WSF), CDTA-(CSF) and Na2CO3 -soluble (NSF)] in albedo and flavedo tissues of 'Navelate' orange at harvest (H) and during fruit storage at 20 °C in air (control, C), in air after being conditioned with ethylene (ethylene conditioned (EC)), or in ethylene (E).

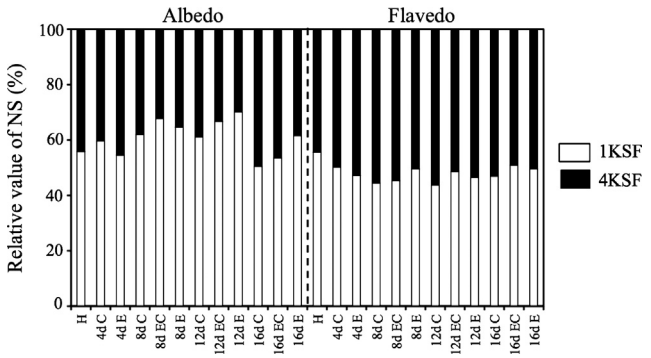

Fig. 3. Relative distribution of neutral sugars from the hemicellulose rich fractions [(1 M KOH-(1KSF) and 4 M-KOH soluble (4KSF)] in albedo and flavedo tissues of 'Navelate' orange at harvest (H) and during fruit storage at 20 °C in air (C), in air after being conditioned with ethylene (EC) or in ethylene (E).

and this effect was more marked in the fruit continuously treated with ethylene. The changes in the WSF were mirrored when examining changes in NS in the NSF fraction, but they were lost by 16 days when the NCPP manifestation was advanced (Fig. 2B).

In the hemicellulose fractions, NS in both the albedo and flavedo were almost equally extracted with 1 M and 4 M KOH (Fig. 3). The proportion of hemicelluloses extracted with 1 M KOH was greater in the albedo than in the flavedo and slight changes were observed in both control and ethylene-treated fruit during their storage. A previous ultrastructural and histochemical study revealed an

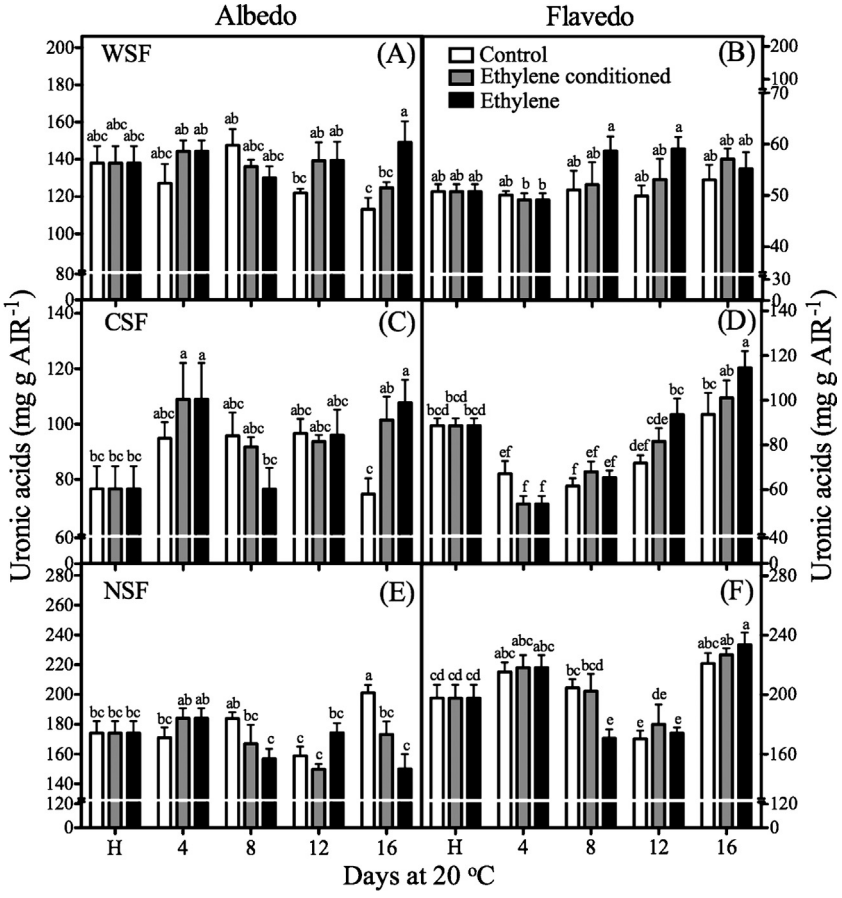

Fig. 4. Pectin content of water- (WSF, A and B), CDTA- (CSF, C and D) and Na_2CO_3-soluble (NSF, E and F) fractions in albedo and flavedo tissue of 'Navelate' orange at harvest (H) and during storage at 20 °C in air (control), in air after being conditioned with ethylene (EC) or in ethylene (E). Means ± SE labelled with the same letter did not differ significantly ($P \leq 0.05$) according to Duncan's test.

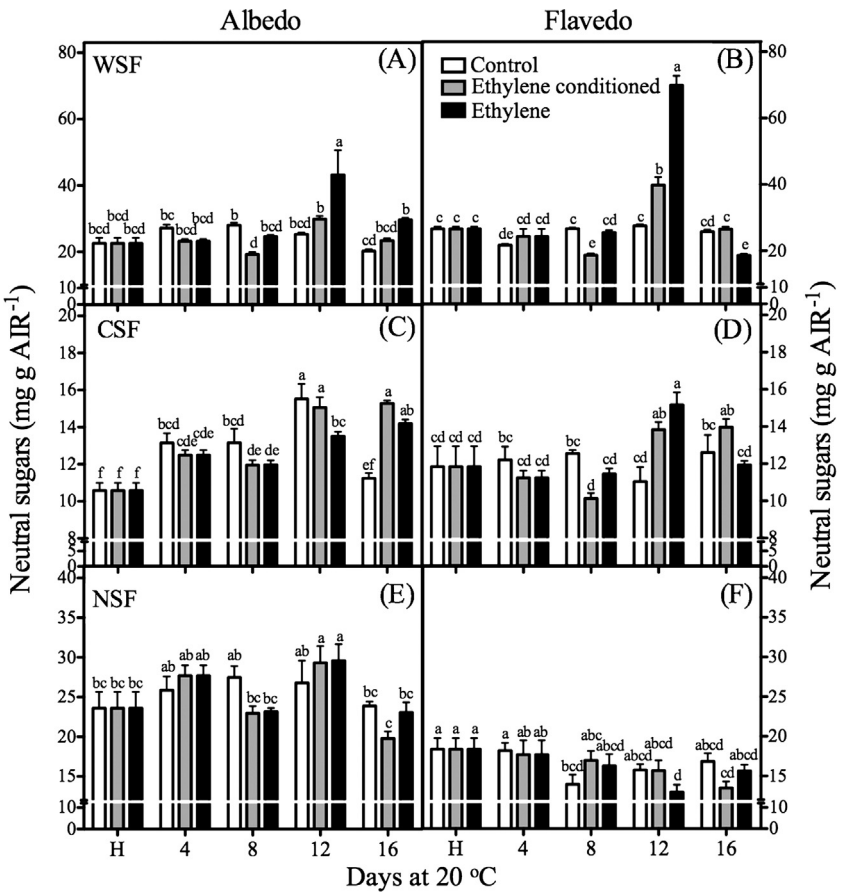

Fig. 5. Neutral sugars content of water-soluble (WSF, A and B), CDTA-soluble (CSF, C and D) and Na_2CO_3-soluble (NSF, E and F) fractions in albedo and flavedo tissue of 'Navelate' orange fruit at harvest (H) and during storage at 20 °C in air (control), in air after being conditioned with ethylene (EC) or in ethylene (E). Statistical analysis was performed as indicated in Fig. 4 legend.

increased staining of CWM in fruit treated with ethylene that could have resulted from increased deposition of pectic exudates [16]. Nevertheless, analysis of cell wall composition in the present study indicated that changes in the relative abundance of all pectin fractions compared to hemicelluloses on an AIR basis were not significant in the ethylene-treated fruit (data not shown).

3.3. *Effect of ethylene on uronic acid and neutral sugar contents in the albedo and flavedo tissues*

The UA content in the albedo WSF was approximately 3-fold greater than in the flavedo (Fig. 4A and B). Treating the fruit for 4 days with ethylene induced a slight increase in UA content of the CSF fraction in the albedo. Such differences were statistically significant by day 16 in this tissue (Fig. 4C and D). The largest changes were found in the albedo tissue and after prolonged storage (16 days). At this time point, ethylene-treated fruit presented greater content of water-soluble UA than the control, which instead had higher level of tightly bound pectin. This trend is usually observed in fruits presenting more advanced stages of wall turnover [43]. Ethylene has been shown to induce several enzymes involved in pectin degradation [31]. It is noteworthy, that changes induced by ethylene in the flavedo tissue were more restricted than in the albedo but still significant in the CSF at the end of the storage period. Overall, the results suggested that ethylene increased the solubility of pectins in the albedo. The difference in the solubility of albedo pectins between control and ethylene-treated fruit at long storage (16 days) was not only due to higher polyuronide extractability in the ethylene-treated fruit, but also to a partial insolubilization in control fruit. Intermediate levels of pectin solubility were found in fruit treated with ethylene for 4 days and then transferred to air. The moderate modification in cell wall solubilization in fruit discontinuously treated with ethylene, in which NCPP was also reduced; confirm that the responses induced by ethylene are influenced by treatment duration [7]. In addition, it suggests that, besides the wall changes, other mechanisms may contribute to the protective effect of ethylene reducing NCPP.

The WSF and NSF were the richest fractions in NS in both the albedo and the flavedo (Fig. 5). The levels in the NSF remained nearly constant in both peel tissues during storage. A transient increase in the NS levels was found in the WSF of the albedo and flavedo of fruit maintained in ethylene after 12 days of storage (Fig. 5A and B) when differences in NCPP incidence between control and ethylene-treated fruit were evident. Such effect was also found in the flavedo of fruit conditioned for 4 days with the hormone. After 16 days, ethylene-treated fruit maintained higher levels of water and CDTA soluble NS in the albedo than the control fruit. These results may indicate an effect of ethylene on endo-enzymes cleaving arabinans and galactans from rhamnogalacturan I; whereas the subsequent decrease found by 16 days might be due to the potential hydrolysis of this partially degraded polymer to monosaccharides caused by exo-acting enzymes. Future work would be useful to identify specific wall neutral sugars catabolized by ethylene exposure in citrus peel.

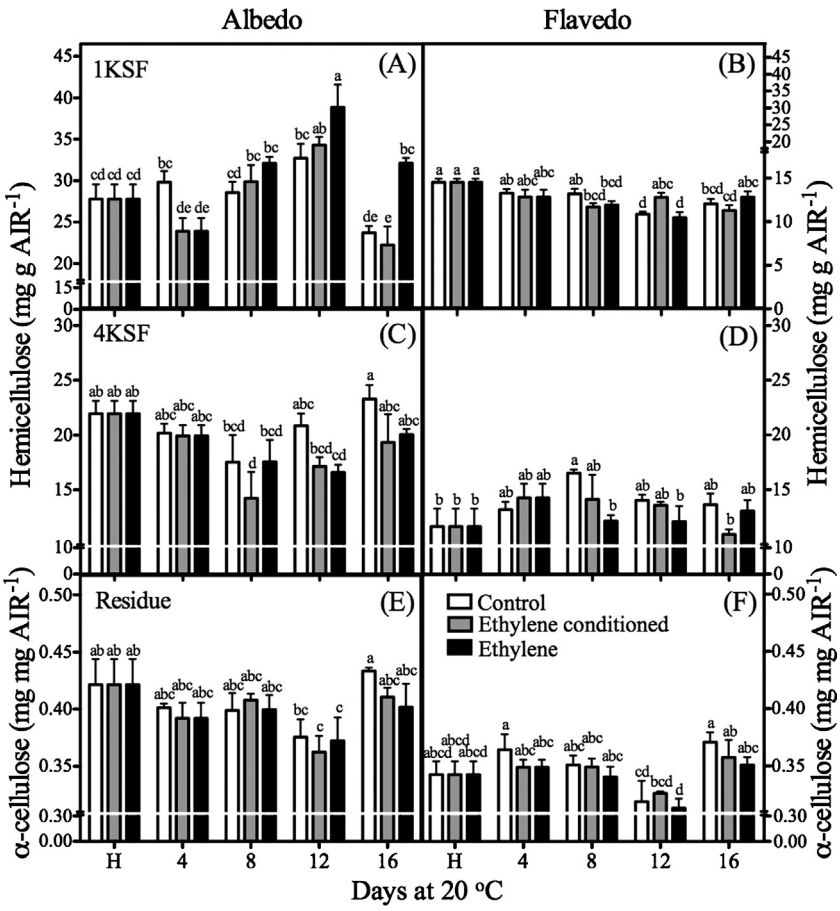

Fig. 6. Hemicellulose content of 1 M KOH-soluble (1KSF, A and B), 4 M KOH-soluble (4KSF, C and D) and α-cellulose (E and F) in albedo and flavedo tissue of 'Navelate' orange fruit at harvest (H) and during storage at 20 °C in air (control), in air after being conditioned with ethylene (EC) or in ethylene (E). Statistical analysis was performed as indicated in Fig. 4 legend.

3.4. Effect of ethylene on hemicellulose and α-cellulose contents in the albedo and flavedo tissues

Relative content in the albedo was twice that of the flavedo both at harvest and throughout the storage period (Fig. 6E and F). The hemicelluloses of the 1 M and 4 M KOH-soluble fractions did not change significantly in the flavedo during storage either in the control or ethylene-treated fruit (Fig. 6B and D). In contrast, ethylene induced changes in the hemicelluloses in the albedo. The NS content in the 1KSF increased in the albedo of fruit held in ethylene for 12 and 16 days, relative to fruit maintained in air (Fig. 6A). Likewise, NS levels showed an initial decreasing tendency in the 4 M KOH soluble fraction (4KSF) in the albedo, although significant differences were not found between the control and the ethylene-treated fruit (Fig. 6C). On the other hand, the cell wall residue showed a decreasing trend in the albedo of both control and ethylene-treated fruits until day 12. Afterwards, a sudden increase of its content was monitored in the albedo and flavedo of fruits exposed to any treatment showing different NCPP index. Such increase could result from the insolubilization of polymers.

4. Conclusions

Ethylene exposure decreased NCPP in 'Navelate' oranges. Continuous storage under ethylene (2 $\mu L L^{-1}$) did not increase pectin deposition, but affected the cell wall composition and disassembly, increasing the solubility of both pectin and hemicelluloses, and also the UA content of the CSF, after long storage periods. The effect was more marked in the albedo than in the flavedo. The moderate modification in cell wall solubilization in fruit discontinuously treated with ethylene, in which NCPP was also controlled, suggests that additional mechanisms might be involved in the protection of cell collapse by ethylene. Further research is necessary to determine whether or not the changes in wall disassembly or in qualitative composition are involved in the reduction of NCPP but certainly there is a temporal association between the protection against the disorder and cell wall remodelling. Overall results encourage future research aimed to identify the generation of active OGs as well as the changes induced in cell wall structural proteins.

Acknowledgements

We gratefully acknowledge the technical assistance of Mrs. D. Arocas for helping us with the ethylene treatments and sampling. This work was supported by the Spanish Ministry of Science and Technology (research grant AGL2009-11969) and by the Generalitat Valenciana (grant PROMETEO/2010/010). This study was partially supported by an internal grant (EX-038) of Cyprus University of Technology.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.plantsci.2013.05.001.

References

[1] S. Selvarajah, A.D. Bauchot, P. John, Internal browning in cold-stored pineapples is suppressed by a postharvest application of 1-methylcyclopropene, Postharvest Biol. Technol. 23 (2001) 167–170.
[2] Z.Y. Liu, W.B. Jiang, Lignin deposition and effect of postharvest treatment on lignification of green asparagus (*Asparagus officinalis* L.), Plant Growth Regul. 48 (2006) 187–193.
[3] Z. Du, W.J. Bramlage, Roles of ethylene in the development of superficial scald in 'Cortland' apples, J. Am. Soc. Hort. Sci. 119 (1994) 516–523.
[4] N. Lisker, L.E. Cohen, E. Chalutz, Y. Fuchs, Fungal infections suppress ethylene-induced phenylalanine ammonia-lyase activity in grapefruits, Physiol. Plant Pathol. 22 (1983) 331–338.
[5] M.E. Saltveit, Effect of ethylene on quality of fresh fruits and vegetables, Postharvest Biol. Technol. 15 (1999) 279–292.
[6] S. Lurie, C.H. Crisosto, Chilling injury in peach and nectarine, Postharvest Biol. Technol. 37 (2005) 195–208.
[7] J.F. Cajuste, M.T. Lafuente, Ethylene-induced tolerance to non-chilling peel pitting as related to phenolic metabolism and lignin content in 'Navelate' fruit, Postharvest Biol. Technol. 45 (2007) 193–203.
[8] L. González-Candelas, S. Alamar, P. Sanchez-Torres, L. Zacarías, J.F. Marcos, A transcriptomic approach highlights induction of secondary metabolism in citrus fruit in response to *Penicillium digitatum* infection, BMC Plant Biol. 10 (2010) 194.
[9] M.T. Lafuente, J.M. Sala, L. Zacarias, Active oxygen detoxifying enzymes and phenylalanine ammonia-lyase in the ethylene-induced chilling tolerance in citrus fruit, J. Agric. Food Chem. 52 (2004) 3606–3611.
[10] F. Alférez, M. Agusti, L. Zacarías, Postharvest rind staining in Navel oranges is aggravated by changes in storage relative humidity: effect on respiration, ethylene production and water potential, Postharvest Biol. Technol. 28 (2003) 143–152.
[11] P. Romero, M.J. Rodrigo, A. Alférez, A.R. Ballester, L. González-Candelas, L. Zacarías, M.T. Lafuente, Unravelling molecular responses to moderate water stress in sweet orange (*Citrus sinensis* L. Osbeck) by using a fruit-specific ABA-deficient mutant, J. Exp. Bot. 63 (2012) 2753–2767.
[12] J.F. Cajuste, L. González-Candelas, A. Veyrat, F.J. García-Breijo, J. Reig-Armiñana, M.T. Lafuente, Epicuticular wax and morphology as related to ethylene and storage performance of 'Navelate' orange fruit, Postharvest Biol. Technol. 55 (2010) 29–35.
[13] N. Holland, H.C. Menezes, M.T. Lafuente, Carbohydrates as related to the heat-induced chilling tolerance and respiratory rate of "Fortune" mandarin fruit harvested at different maturity stages, Postharvest Biol. Technol. 25 (2002) 181–191.
[14] N. Holland, H.C. Menezes, M.T. Lafuente, Carbohydrate metabolism as related to high temperature conditioning and peel disorders occurring during storage of citrus fruit, J. Agric. Food Chem. 53 (2005) 8790–8796.
[15] I. Lliso, F.R. Tadeo, B.S. Phinney, C.G. Wilkerson, M. Talon, Protein changes in the albedo of citrus fruits on postharvesting storage, J. Agric. Food Chem. 55 (2007) 9047–9053.
[16] J.F. Cajuste, F.J. García-Breijo, J. Reig-Armiñana, M.T. Lafuente, Ultrastructural, histochemical analysis reveals ethylene-induced responses underlying reduced peel collapse in detached citrus fruit, Microsc. Res. Technol. 74 (2011) 970–979.
[17] R. Sasidharan, L.A.C.J. Voesenek, R. Pierik, Cell wall modifying proteins mediate plant acclimatization to biotic and abiotic stresses, Crit. Rev. Plant Sci. 30 (2011) 548–562.
[18] T. Hamann, L. Denness, Cell wall integrity maintenance in plants: lessons to be learned from yeast? Plant Signal Behav. 6 (2011) 1706–1709.
[19] D. Cantu, A.R. Vicente, L.C. Greve, F.M. Dewey, A.B. Bennett, J.M. Labavitch, A.L.T. Powell, The intersection between cell wall disassembly, ripening, and fruit susceptibility to *Botrytis cinerea*, Proc. Natl. Acad. Sci. U.S.A. 105 (2008) 859–864.
[20] K. Hématy, C. Cherk, S. Somerville, Host-pathogen warfare at the plant cell wall, Curr. Opin. Plant Biol. 12 (2009) 406–413.
[21] E. Melotto, L.C. Greve, J.M. Labavitch, Cell wall metabolism in ripening fruit. VII. Biologically active pectin oligomers in ripening tomato (*Lycopersicon esculentum* Mill.) fruits, Plant Physiol. 106 (1994) 575–581.
[22] M.T. Lafuente, J.M. Sala, Abscisic acid levels and the influence of ethylene, humidity and storage temperature on the incidence of postharvest rindstaining of Navelina orange (*Citrus sinensis* L. Osbeck) fruit, Postharvest Biol. Technol. 25 (2002) 49–57.
[23] G.A. Manganaris, A.R. Vicente, C.H. Crisosto, J.M. Labavitch, Cell wall modifications in chilled-injured plum fruit (*Prunus salicina*), Postharvest Biol. Technol. 48 (2008) 77–83.
[24] N. Blumenkrantz, G. Asboe-Hansen, New method for quantitative determination of uronic acids, Anal. Biochem. 54 (1973) 484–489.
[25] E.W. Yemm, A.J. Willis, The estimation of carbohydrates in plant extracts by anthrone, Biochem. J. 57 (1954) 508–514.
[26] E. Pesis, M. Ackerman, R. Ben-Arie, O. Feygenberg, Q.X. Feng, A. Apelbaum, R. Goren, D. Prusky, Ethylene involvement in chilling injury symptoms of avocado during cold storage, Postharvest Biol. Technol. 24 (2002) 171–181.
[27] P. Wang, B. Zhang, X. Li, C. Xu, X. Yin, L. Shan, I. Ferguson, K. Chen, Ethylene signal transduction elements involved in chilling injury in non-climacteric loquat fruit, J. Exp. Bot. 61 (2010) 179–190.
[28] M.T. Lafuente, G. López-Gálvez, M. Cantwell, S.F. Yang, Factors influencing ethylene-induced isocoumarin formation and increased respiration in carrots, J. Am. Soc. Hort. Sci. 121 (1996) 537–542.
[29] A.R. Ballester, M.T. Lafuente, J. Forment, J. Gadea, C.H.R. De Vos, A.G. Bovy, L. González-Candelas, Transcriptomic profiling of citrus fruit peel tissues reveals fundamental effects of phenylpropanoids and ethylene on induced resistance, Mol. Plant Pathol. 12 (2011) 879–897.
[30] M.T. Lafuente, L. Zacarias, M.A. Martínez-Téllez, M.T. Sanchez-Ballesta, E. Dupille, Phenylalanine ammonia-lyase as related to ethylene in the development of chilling symptoms during cold storage of citrus fruits, J. Agric. Food Chem. 49 (2001) 6020–6025.
[31] A.B. Bennett, J.M. Labavitch, Ethylene and ripening-regulated expression and function of fruit cell wall modifying proteins, Plant Sci. 175 (2008) 130–136.
[32] D.P.F. Almeida, D.J. Huber, In vivo pectin solubility in ripening and chill-injured tomato fruit, Plant Sci. 174 (2008) 174–182.
[33] D. Goudeau, S.L. Uratsu, K. Inoue, F.G. daSilva, A. Leslie, D. Cook, R.L. Reagan, A.M. Dandekar, Tuning the orchestra: selective gene regulation and orange fruit quality, Plant Sci. 174 (2008) 310–320.
[34] G. D'hallewin, M. Schirra, A.L.T. Powell, L.C. Greve, J.M. Labavitch, Properties of polygalacturonase-inhibiting protein isolated from 'Oroblanco' grapefruit, Physiol. Plant 120 (2004) 395–404.
[35] R.R. Balandrán-Quintana, A.M. Mendoza-Wilson, G. Alvarez-Manilla, C.W. Bergmann, I. Vargas-Arispuro, M.A. Martínez-Téllez, Effect of pectic oligomers on physiological responses of chilling injury in discs excised from zucchini (*Cucurbita pepo L.*), Biochem. Biophys. Res. Commun. 29 (2002) 577–584.
[36] M. Schirra, M. Mulas, A. Fadda, I. Mignani, S. Lurie, Chemical and quality traits of 'Olinda' and 'Campbell' oranges after heat treatment at 44 or 46 °C for fruit fly disinfestation, LWT 38 (2005) 519–527.
[37] F. Baluška, A. Hlavacka, J. Šamaj, K. Palme, D.G. Robinson, T. Matoh, D.W. McCurdy, D. Menzel, D. Volkmann, F-sctin-dependent endocytosis of cell wall pectins in meristematic root cells. Insights from brefeldin A-induced compartments, Plant Physiol. 130 (2002) 422–431.
[38] S.M. Yu, Cellular and genetic responses of plants to sugar starvation, Plant Physiol. 121 (1999) 689–693.
[39] A.P. Alonso, H. Vigeolas, P. Raymond, D. Rolin, M. Dieuaide-Noubhani, A new substrate cycle in plants. Evidence for a high glucose-phosphate-to-glucose turnover from *in vivo* steady-state and pulse-labeling experiments with [^{13}C] glucose and [^{14}C] glucose, Plant Physiol. 138 (2005) 2220–2232.
[40] E.J. Lee, Y. Matsumura, K. Soga, T. Hoson, N. Koizumi, Glycosyl hydrolases of cell wall are induced by sugar starvation in Arabidopsis, Plant Cell Physiol. 48 (2007) 405–413.
[41] A.R. Vicente, L.C. Greve, J.M. Labavitch, Recent findings in plant cell wall structure and metabolism: future challenges and potential implications on softening, Stewart Postharvest Rev. 1 (2006) 1–8.
[42] E.E. Farmer, T.D. Moloshok, M.J. Saxton, C.A. Ryan, Oligosaccharide signaling in plants. Specificity of oligouronide-enhanced plasma membrane protein phosphorylation, J. Biol. Chem. 266 (1991) 3140–3145.
[43] D.A. Brummell, Cell wall disassembly in ripening fruit, Funct. Plant Biol. 33 (2006) 103–119.