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Development and control of scald on wonderful pomegranates during long-term storage

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Abstract

Scald of the husk surface is an important factor limiting long-term storage of pomegranates and little information is available about its cause and methods of control. We evaluated the efficacy of prestorage treatment with diphenylamine and/or 1-methylcyclopropene and of atmospheric modification during storage on scald incidence and severity on Wonderful pomegranates.

Scald incidence and severity were greater on pomegranates harvested during late season than on those harvested during mid season, indicating that this disorder may be associated with senescence. All pomegranates from both harvests that were kept in air exhibited some scald after 4–6 months at 7 °C. Neither diphenylamine, at 1100 or 2200 μL L^{-1}, nor 1-methylcyclopropene at 1 μL L^{-1}, alone or together reduced scald incidence and severity. In contrast, the three controlled atmosphere (CA) storage conditions tested (1 kPa O_2, 1 kPa O_2 + 15 kPa CO_2 and 5 kPa O_2 + 15 kPa CO_2) significantly reduced scald incidence and severity on pomegranates from both harvest dates for up to 6 months at 7 °C. However, the two CA treatments with 1 kPa O_2 resulted in greater accumulation of fermentative volatiles (acetaldehyde, ethanol, and ethyl acetate) than the CA treatment with 5 kPa O_2, especially in the mid-season-harvested pomegranates. In addition to its fungistatic effects, 15 kPa CO_2 appears to be critical for inhibition of scald development on pomegranates. These results confirm recommendation by Hess-Pierce and Kader (2003) of 5 kPa O_2 + 15 kPa CO_2 (balance N_2) as the optimal CA for pomegranates at 7 °C and 90–95% relative humidity. Since very little if any α-farnesene or its conjugated trienol oxidation products were found in the peel of pomegranates, it appears that the biochemical basis of scald in pomegranates is different from that in apples. CA storage (5 kPa O_2 + 15 kPa CO_2) decreased or prevented changes in carotenoid, acyl lipid, and phenylpropanoid metabolism that were associated with scald development in stem-end peel tissue of air-stored fruit and are indicative of stress and/or senescence.

Keywords: *Punica granatum*; Physiological disorder; Controlled atmosphere; 1-MCP; DPA

1. Introduction

Appearance, especially red color, is an important quality factor for marketing fresh pomegranates. Many factors affect appearance, including bruising, water loss, decay, and the development of physiological disorders during storage (Elyatem and Kader, 1984). In general, the major cause limiting the storage potential of pomegranates is the development of decay, which is often caused by the presence of fungal inoculum in the blossom end of the fruit (Hess-Pierce and Kader, 2003). This problem is aggravated at temperatures higher than 5 °C, which are recommended for pomegranates to avoid chilling injury (internal tissue browning). For long-term storage, scald of the husk surface is another factor limiting storage life (Ben-Arie and Or, 1986). Scald symptoms appear as a superficial (skin) browning, similar to superficial scald of apples, and generally develop from the stem end of the fruit, spreading toward the blossom end as the severity increases. Moreover, husk scald increases the susceptibility of the fruit to decay.

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Several postharvest conditions have been evaluated for long-term storage of pomegranates, including low temperature, delayed harvest (Ben-Arie and Or, 1986), intermittent warming (Artés et al., 1998) and controlled atmosphere (CA) (Ben-Arie and Or, 1986; Artés et al., 1996; Hess-Pierce and Kader, 2003). Among these procedures, the most successful in reducing decay and physiological disorders is the use of CA storage, which, with a combination of 5 kPa O_2 and 15 kPa CO_2, has been shown to extend pomegranate postharvest life for up to 5 months at 7 °C (Hess-Pierce and Kader, 2003). This combination also avoids the accumulation of high levels of ethanol, observed under CA conditions with lower levels of oxygen, which limits the marketability of the fruit (Ben-Arie and Or, 1986).

Despite the importance of husk scald, little information is available about its origin and mechanisms of control during long-term storage of pomegranates. Studies performed by Ben-Arie and Or (1986) suggested that scald symptoms may be caused by the enzymatic oxidation of *o*-dihydroxyphenols during storage, but the biochemical changes that conclude with enzymatic browning remain unclear. The similarities between pomegranate scald and apple scald, in terms of symptomatology and occurrence, suggest that the two disorders may be similar in the biochemical causes and mechanism of control. It is generally accepted that apple scald is an oxidative stress disorder involving conjugated triene oxidation products of the sesquiterpene α-farnesene (Whitaker, 2004). The disorder has long been controlled by treatment with the antioxidant diphenylamine (DPA) and/or low oxygen CA storage (Ingle and D'Souza, 1989), and more recently by treatment with the blocker of ethylene action 1-methylcyclopropene (1-MCP) (Watkins et al., 2000; Zanella, 2003).

The development of more sensitive analytical tools and the availability of new products that can control scald in apple (Whitaker et al., 1997; Zanella, 2003) led us to re-examine this disorder in pomegranates. Therefore, the objective of this work was to evaluate the efficacy of DPA, 1-MCP (SmartFresh™, AgroFresh Inc., Spring House, PA), and low oxygen atmospheres in controlling incidence and severity of scald on 'Wonderful' pomegranates during long-term storage.

2. Materials and methods

2.1. Plant material

Freshly harvested, sorted, and packed 'Wonderful' pomegranates from a packinghouse near Fresno, California were brought to the Postharvest Laboratory at the University of California at Davis. Two harvest dates were evaluated; a mid-season harvest on 20 October, and a late-season harvest on 18 November (2003) (Table 1). Before postharvest treatments, the pomegranates were sorted and those with surface blemishes and other defects were discarded.

Table 1
Comparison of maturity indices (soluble solids, pH, titratable acidity and color) between mid- and late-season harvested 'Wonderful' pomegranates

Quality parameters	Harvest time	
	Mid season	Late season
Soluble solids content (%)	15.7 ± 0.3	17.0 ± 0.3
Titratable acidity (%)	1.2 ± 0.1	1.2 ± 0.1
pH	3.2 ± 0.0	3.3 ± 0.1
Aril color		
L value	47.8 ± 1.2	44.7 ± 4.1
Chroma	48.4 ± 1.0	48.6 ± 3.6
Hue angle	27.7 ± 1.1	24.5 ± 2.4

Data shown are means of three replicates ± S.E.

2.2. Treatments tested

The eight treatments tested for scald control are listed in Table 2. For each CA treatment, 6 boxes (30 fruit each) of pomegranates were placed into a 0.3 m^3-stainless steel container that was ventilated with either humidified air or the desired gas mixture for each CA treatment at 7 °C. The ethylene action inhibitor, 1-MCP (SmartFresh™, AgroFresh Inc., Philadelphia, PA), was applied in 0.3 m^3 gas-tight containers at 7 °C for 24 h. 1-MCP was generated with "Light Orange SmartFresh™ Research Tablets" with a Blue Activator Tablet and Activator Solution as suggested by Agrofresh. Pomegranates treated with DPA were dipped in the solution for 3 min and air dried at 20 °C before storage. Pomegranates were evaluated after 2, 3, 4, 5 and 6 months to determine their marketability based on visual external and internal quality. After removal from storage, fruit were kept at 20 °C for 4 days to simulate marketing conditions before final quality evaluations.

2.3. Maturity and quality parameters

At harvest, 3 replicates of 10 fruit each were evaluated for compositional analysis. Arils were squeezed through cheesecloth and pH, titratable acidity (TA) and soluble solids content (SSC) were measured. SSC was measured using a refractometer (Abbe refractometer model 10450, American Optical, Buffalo, NY). Four grams of juice diluted with 20 mL of distilled water were titrated to pH 8.1 with 0.1N NaOH

Table 2
Treatments tested for control of scald on pomegranates

Treatment	Details
Control	No treatment, stored in air
CA-1	1 kPa oxygen + 99 kPa nitrogen
CA-2	1 kPa oxygen + 15 kPa carbon dioxide + 84 kPa nitrogen
CA-3	5 kPa oxygen + 15 kPa carbon dioxide + 80 kPa nitrogen
DPA-1	1100 μL L^{-1} DPA for 3 min, stored in air
DPA-2	2200 μL L^{-1} DPA for 3 min, stored in air
1-MCP	1 μL L^{-1} 1-MCP for 24 h at 7 °C, then stored in air
1-MCP + DPA	1 μL L^{-1} 1-MCP for 24 h at 7 °C and dipped in 1100 μL L^{-1} DPA for 3 min, stored in air

to measure TA (as citric acid) and pH using an automatic titration system (Radiometer, Copenhagen, Denmark). Moreover, pomegranates were initially marked at three equatorial points on each fruit so that subsequent external color readings could be taken at exactly the same spots on the fruit. Color readings were then taken on 100 fruit with their average considered as the initial value. After each storage duration, external color measurements were taken on 15 pomegranates per treatment using a hand-held Minolta colorimeter (model CR-300; Minolta, Ramsey, NJ) and expressed as $L^*a^*b^*$ color values. Hue angle and Chroma value were also calculated.

2.4. Fermentative metabolites

Three replicates of five fruit each were used for determination of fermentative metabolites. At each sampling time, 5 mL of juice were collected into a 10-mL vial containing 2 g of NaCl. The vial was immediately sealed and kept at −80 °C until analysis. After thawing in a cool water bath at about 20 °C, the juice was incubated at 37 °C for 15 min and a 1-mL headspace gas sample was withdrawn with a 1-mL gas-tight glass syringe and injected into a Hewlett Packard Model 5890 gas chromatograph equipped with a flame ionization detector and a 1.8 m × 2 mm i.d. glass column packed with Supelco 60/80 Carbopack B/5% Carbowax 20 M (Ke et al., 1994). Acetaldehyde, ethanol and ethyl acetate were identified based on their retention times and quantified based on integration of the peaks and comparison with the standard curves. The ranges of calibration curves (with a $R^2 \geq 0.95$) used for quantification of individual compounds were: acetaldehyde (0–100 $\mu L\,L^{-1}$), ethanol (0–20,000 $\mu L\,L^{-1}$) and ethyl acetate (0–10 $\mu L\,L^{-1}$).

2.5. Evaluation of visual quality and scald

After each storage duration, all 30 pomegranates per treatment were individually evaluated for overall visual quality and for scald incidence and severity. External appearance including decay, bruising and scald was evaluated following the hedonic scale: 1 = unusable, 3 = poor, 5 = good, 7 = very good, and 9 = excellent. Scald incidence was expressed as percentage of the fruit affected by scald. Severity considers the percentage of fruit surface affected with scald (brown discoloration) and was evaluated following the hedonic scale: 1 = no scald, 2 = 1–25%, 3 = 26–50%, 4 = 51–75%, and 5 = 75–100% of the surface affected.

2.6. Sample preparation and HPLC analysis of α-farnesene and conjugated trienols

Peel tissue from scalded and sound late-season-harvested fruit was carefully separated avoiding any contamination by juice from the arils that might impart extraneous isoprenoid, lipid, and phenolic constituents. Tissue samples were taken from control (untreated) and CA-3 (5 kPa O_2 + 15 kPa CO_2) fruit at 0, 2, 3, 4, 5, and 6 months, and from fruit of all other treatments except CA-1 and CA-2 (Table 1) at 0, 2, and 4 months. Separate stem-end and blossom-end tissue samples from 4 to 6 fruit were pooled. The tissue was frozen in liquid N_2, packaged with dry ice, and sent to the USDA-ARS Produce Quality and Safety Laboratory in Beltsville, MD overnight by courier.

Individual tissue samples from the variously treated fruit were briefly immersed in liquid N_2 and 15-g portions were weighed, fractured with a mallet, and then ground to ∼0.5 cm^2 pieces using a mortar and pestle. The ground, frozen tissue was transferred to 50-mL screw-cap culture tubes and immersed in 25 mL of hexane, after which the tubes were flushed with N_2 and sealed with Teflon-lined caps. The tubes were agitated on a rotary shaker at 13.1 rad s^{-1} at 4 °C overnight. Following centrifugation at 500 × g for 2 min, the hexane extracts were decanted and vacuum filtered through a sintered glass funnel lined with a glass fiber filter. Using a gentle stream of N_2, the filtered extracts were reduced in volume to 15 mL. Aliquots (1.5 mL) of the concentrated extracts were transferred to 2-mL glass vials, followed by evaporation of the hexane with a gentle stream of N_2 (without heating) and addition of 0.5 mL of methanol. The vials were flushed with N_2, capped, vortexed, and centrifuged 2 min at 1000 × g. The 0.5 mL methanolic samples were then passed through 0.4 μm PTFE syringe filters into amber HPLC vials, which were flushed with N_2 and capped.

Samples were analyzed by high-performance liquid chromatography (HPLC) using a Hewlett-Packard Series 1100 HPLC system (Agilent Technologies) with a quaternary pump, autosampler, and photodiode array detector (PDA). The vials were loaded in the autosampler and 80-μL aliquots were injected onto a Luna 5 μm $C_{18}(2)$ column (250 mm long, 4.6 mm i.d.) from Phenomenex (Torrance, CA) and eluted with isocratic methanol:water:acetonitrile, 90:5:5 (v/v/v), at a flow rate of 13.3 $\mu L\,s^{-1}$. PDA monitoring at 232 and 269 nm was used to determine levels of α-farnesene and its conjugated trienol oxidation products. HPLC-purified α-farnesene and conjugated trienols isolated from peel tissue of apple fruit were used as external standards (Whitaker et al., 1997), and eluted at 15.8 and 6.0 min, respectively.

2.7. Spectrophotometric measurement of total carotenoids

It was observed that yellow pigments in the hexane extracts prepared for HPLC analysis of α-farnesene increased with the duration of storage. These were presumed to be carotenoids because of their solubility in hexane. Two replicate extracts from 15-g peel tissue samples were pooled, the solvent evaporated with a stream of N_2, and the residue dissolved in 2 mL of hexane for UV spectrophotometric analysis using a Shimadzu model UV160U spectrophotometer. The concentrated extracts were diluted as required to keep the maximum absorbance below 1.0 AU. All samples showed a series

of six absorbance maxima (A_{max}) at about 348, 367, 400, 425, 447, and 473 nm, which varied up to ±3 nm with storage duration and atmosphere (particularly the two maxima above 440 nm). These A_{max} are typical of commonly occurring carotenoids, e.g., the two major A_{max} of phytofluene are 348 and 367 nm, A_{max} of ζ-carotene are 400 and 425 nm, and those of α- and β-carotene are close to 447 and 473 nm (Rodriguez-Amaya, 2001). Hence, the approximate concentration of total carotenoids in each extract was calculated as follows: published absorption coefficients ($A^{1\%}_{1cm}$ in hexane; Rodriguez-Amaya, 2001) for phytofluene (1577 at 348 nm), ζ-carotene (2550 at 400 nm), and α-carotene (2710 at 445 nm) were used to calculate the concentrations of carotenoids with major A_{max} at 348 and 367 nm, 400 and 425 nm, and 447 and 473 nm, respectively. These three values were then summed to give an estimate of total carotenoids.

2.8. Neutral lipid and phospholipid extraction, fractionation, and fatty acid analysis

Total lipids were extracted from three 5 g samples of peel tissue from the stem end of late-harvested fruit that were processed shortly after harvest (initials) or after 6 months of storage at 7 °C in air (controls) or CA (5 kPa O_2 + 15 kPa CO_2). Tissue was pulverized in liquid N_2 with a mortar and pestle and extracted with 30 mL of chloroform:methanol, 2:1 (v/v) by vortexing 10 min in 50-mL screw-cap culture tubes. After a 3-min centrifugation at 1000 × *g*, the extracts were decanted, vacuum-filtered through a glass fiber disk, and a phase separation effected by addition of 8 mL of 0.8% NaCl. The chloroform phase was washed twice with 8 mL of methanol:water, 1:1 (v/v), then evaporated under a stream of N_2. Total lipids were re-dissolved in 2 mL of chloroform and loaded on a pipette column containing a 0.8 cm diameter × 10 cm long bed of 100–200-mesh silicic acid prewashed with chloroform. Neutral lipids (NL) were eluted with 6 mL chloroform plus 6 mL chloroform:acetone, 1:2 (v/v), followed by elution of polar lipids with 10 mL of methanol:water, 20:1 (v/v). After N_2 evaporation of the solvents, NL were dissolved in 1 mL of hexane:ethanol, 2:1 (v/v), and polar lipids in 1 mL of chloroform:methanol, 1:1 (v/v). Aliquots (25 μL) of the two lipid fractions were used for determination of total phospholipids (PL) by the method of Ames (1966).

UV absorbance spectra of NL from peel of fruit stored 6 months in air had three prominent maxima at 264, 273, and 285 nm typical of conjugated trienes, whereas UV spectra of NL from peel of initials or 6-month CA-stored fruit had a less intense single broad maximum at 312 nm with a shoulder at 296 nm. After N_2 evaporation of the hexane:ethanol, 2:1, NL were re-dissolved in 1 mL of hexane, which left an insoluble residue. The conjugated triene in the 6-month control NL samples, presumed to be punicic acid (9*Z*,11*E*,13*Z*-octadecatrienoic acid; Hornung et al., 2002), was hexane soluble, whereas the 312 nm-absorbing compounds were not. Hexane-soluble NL were used for fatty acid analysis. The hexane-insoluble material was dissolved in methanol and subsequently analyzed by HPLC and liquid chromatography–mass spectrometry (LC–MS) (see Section 2.9).

Fatty acid methyl esters (FAME) prepared from the NL and PL fractions were quantified by GC-FID using a Hewlett-Packard 5890 gas chromatograph fitted with a Supelco 15 m × 0.25 mm i.d. SP2330 fused silica capillary column (0.20 μm film thickness). FAME were identified by co-retention with authentic standards from Sigma–Aldrich, with the exception of punicic acid methyl ester, which was obtained from Larodan Fine Chemicals (Malmö, Sweden). Identification of FAME, particularly several minor constituents, was confirmed by gas chromatography–mass spectrometry (GC–MS) over the range of *m/z* 40–400 using an Agilent Technologies 6890N network GC system and 5973N mass selective detector equipped with a 50 m × 0.2 mm i.d. Agilent Ultra 2 capillary column (0.33 μm film thickness). Solvents were N_2 evaporated from the NL and PL samples after addition of 50 μg of heptadecanoic acid methyl ester (17:0 ME) as an internal standard. NL were initially transesterified by mild alkaline methanolysis in 0.6 M methanolic KOH at 70 °C for 20 min. This yielded free fatty acids in addition to FAME but avoided degradation of punicic acid (Hernández et al., 2000). Both NL and PL fatty acids were subsequently methyl esterified in 14% boron trifluoride in methanol at 75 °C for 20 min.

2.9. HPLC and LC–MS analysis of lipophilic *p*-coumaric acid conjugates

Four closely related compounds in the hexane-insoluble fraction from peel tissue NL with an absorbance maximum at ∼312 nm were identified as lipophilic *p*-coumaric acid conjugates (LPCAC) by HPLC and electrospray negative ionization LC–MS analysis with photodiode array detection. Instrumentation and conditions were as described in Whitaker and Stommel (2003). LPCAC were separated on a 250 mm × 4.6 mm i.d. 5 μm Luna $C_{18}(2)$ column (Phenomenex). The mobile phase gradient consisted of aqueous 0.01% phosphoric acid (HPLC) or 0.05% formic acid (LC–MS) in methanol at 16.7 μL s^{-1}, with the percentage of methanol varied as follows: 0–4 min, 80%; 4–18 min, 80–90% (linear); 18–22 min, 90%; 22–25 min, 90–95% (linear); 25–28 min, 95%; 28–31 min, 95–80% (linear); 31–34 min, 80%. LPCAC isolated by HPLC and then refluxed in 14% boron trifluoride in methanol at 75 °C for 30 min yielded a 313 nm-absorbing product that coeluted with, and had the same UV absorbance spectrum as, the methyl ester of *trans p*-coumaric acid (*E*-*p*CA-ME) prepared by methyl esterification of the free acid (from Sigma–Aldrich) in 14% methanolic BF_3. LPCAC in the peel tissue NL fractions were subsequently quantified by HPLC-UV using *E*-*p*CA-ME as an external standard.

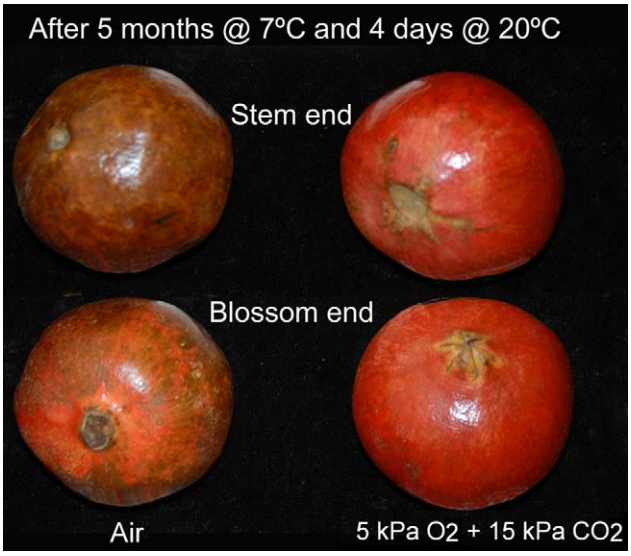

Fig. 1. Symptoms of scald (more severe on the stem-end than the blossom-end) on 'Wonderful' pomegranates kept in air for 5 months at 7 °C. Storage in 5 kPa O_2 + 15 kPa CO_2 prevented scald development.

3. Results and discussion

3.1. Incidence and severity of scald

Scald symptoms developed mainly from the stem end of the fruit, characterized by a brown discoloration covering up to 60% of the skin's surface area, and generally without affecting the arils and surrounding pericarp tissues (Fig. 1). Symptoms developed in the mid-season harvest control fruit after 6 months of storage, and as early as 4 months in the late-season harvest control fruit (Table 3). By the end of storage, scald incidence reached maximum levels of 30% and 100% on fruit from the mid-season and late-season harvests, respectively. A high incidence of scald in fruit from the late-season harvest, close to 70%, was already evident after 4 months of storage. These results differ from those in a prior report, which indicated that a delay in harvest time reduces the incidence of the disorder (Ben-Arie and Or, 1986). However, in our experiments using fruit harvested later in the season, the delay in harvest not only increased susceptibility to scald but also to decay, as observed in our previous study (Hess-Pierce and Kader, 2003). Among the seven treatments evaluated for scald control, only CA treatments were able to effectively control or delay scald development, especially the atmospheres including 15 kPa CO_2, in which the disorder was completely controlled for up to 6 months at 7 °C. In general, there was an increase in scald incidence and severity after shelf-life simulation (4 days at 20 °C). The CA treatment with 1 kPa O_2 only gave effective scald control for fruit from the mid-season harvest, while in pomegranates from the late-season harvest it only delayed the appearance of symptoms and scald incidence was close to 40% by the end of storage. In scalded fruit from the 1 kPa O_2 treatment, levels of severity were lower (<25% of surface area) than those observed in other treatments with similar levels of incidence (Table 4).

The evaluation of treatments that have shown an effective control of scald in apple and pear did not show the same effectiveness in reducing scald in pomegranate. Fruit treated with DPA, especially at the higher concentration, reached similar or higher levels of scald (incidence and severity) relative to the untreated controls. This was aggravated by the high incidence of decay observed, which was probably due to incomplete drying of the blossom end (calyx area) after DPA treatment. Although the antioxidant action of DPA did not control the disorder, we cannot exclude the possibility that an oxidation process is involved in symptom development (Ben-Arie and Or, 1986). Use of the ethylene action inhibitor 1-MCP had only a partial effect in reducing scald in fruit from the late-season harvest; relative to control fruit, scald incidence was reduced by 60% and 43% after 4 and 5 months of storage, respectively. No major effect of 1-MCP on scald control was observed after 6 months of storage. A similar trend was observed for the 1-MCP and DPA combination, suggesting that DPA at 2200 μL L^{-1} only aggravated expression of the disorder. Despite the non-climacteric nature of the fruit, the partial effect of 1-MCP in controlling or delaying scald symptoms suggests that ethylene is involved but is not solely responsible for triggering the biochemical mechanism(s) leading to scald (Watkins et al., 1993, 2000). Because 1-MCP and DPA do not control superficial scald in pomegranate as they do in apple (Zanella, 2003; Whitaker,

Table 3
Incidence of scald on mid-season and late-season-harvested 'Wonderful' pomegranates stored at 7 °C for up to 6 months plus 4 days at 20 °C

Treatment	Scald incidence (%) after indicated storage duration				
	Mid-season harvest		Late-season harvest		
	5 months	6 months	4 months	5 months	6 months
Control	0	30	70	100	90
1 kPa O_2	0	0	17	10	40
1 kPa O_2 + 15 kPa CO_2	0	0	0	0	0
5 kPa O_2 + 15 kPa CO_2	0	0	0	0	0
DPA (1100 μL L^{-1})	0	27	57	63	87
DPA (2200 μL L^{-1})	0	33	100	93	100
1-MCP (1 μL L^{-1}, 24 h)	0	17	10	57	93
1-MCP + DPA (1100 μL L^{-1})	0	30	23	57	100

Table 4
Severity of scald on mid-season and late-season harvested 'Wonderful' pomegranates stored at 7 °C for up to 6 months plus 4 days at 20 °C

Treatment	Scald severity score[a] after indicated storage duration				
	Mid-season harvest		Late-season harvest		
	5 months	6 months	4 months	5 months	6 months
Control	1	1.7 ± 0.6[b]	3.5 ± 1.0	4.4 ± 0.5	4.3 ± 0.9
1 kPa O_2	1	1	1.2 ± 0.2	1.4 ± 0.7	1.6 ± 0.6
1 kPa O_2 + 15 kPa CO_2	1	1	1	1	1
5 kPa O_2 + 15 kPa CO_2	1	1	1	1	1
DPA (1100 μL L^{-1})	1	1.6 ± 0.6	2.4 ± 0.8	4.0 ± 1.2	4.2 ± 0.8
DPA (2200 μL L^{-1})	1	1.7 ± 0.8	2.5 ± 0.5	4.7 ± 0.6	5.0 ± 0.8
1-MCP (1 μL L^{-1}, 24 h)	1	1.6 ± 0.9	1.2 ± 0.4	2.4 ± 0.8	3.0 ± 0.6
1-MCP + DPA (1100 μL L^{-1})	1	2.8 ± 0.7	1.5 ± 0.5	3.3 ± 1.2	3.4 ± 1.8

[a] Hedonic scale based on percentage of area affected: 1 = no scald, 2 = 1–25%, 3 = 26–50%, 4 = 51–75% and 5 = 75–100%.
[b] Mean ± S.E. (n = 3).

2004), we conclude that the biochemical processes that cause the disorder must be different in these two fruit.

3.2. External color

In general, no major differences in external color among treatments were noted during the first 3 months of storage for both harvest times (data not shown). However, after 5, and especially 6 months of storage, CA treatments kept a lighter red color relative to the control and other treatments for both harvests. This difference may be due to the delayed synthesis of anthocyanins and other phenolics responsible for the red color of the skin (Holcroft et al., 1998). This visual evaluation was supported by the higher L value (lightness) measured in the fruit under CA treatments (Fig. 2). The 1-MCP and DPA treatments showed a pattern similar to that of the untreated controls, which was expected due to the mode of action of both compounds and the non-climacteric nature of the fruit (Elyatem and Kader, 1984).

Since the development of scald was manifested mainly on the stem end of the fruit (Fig. 1), we performed color measurements in order to differentiate objectively a scalded fruit from a sound fruit. Fruit from CA treatments had a higher Chroma value than scalded fruit (untreated controls for example), indicating an increase in browning of the affected area caused by scald as discussed above (Fig. 2).

3.3. Visual quality

In general there was a reduction in visual quality with storage duration, being greater in fruit from the late-season harvest (Table 5). Only CA treatments, especially 1 kPa O_2 + 15 kPa CO_2 and 5 kPa O_2 + 15 kPa CO_2, were able to maintain a very good quality until 6 months of storage. Neither DPA treatments nor 1-MCP could keep the visual quality at acceptable levels, and only the combination of both treatments kept the levels of visual quality close to the CA treatments for up to 5 months at 7 °C, but only in fruit from the mid-season harvest. Decay was one of the most important factors affecting visual quality, and was successfully con-

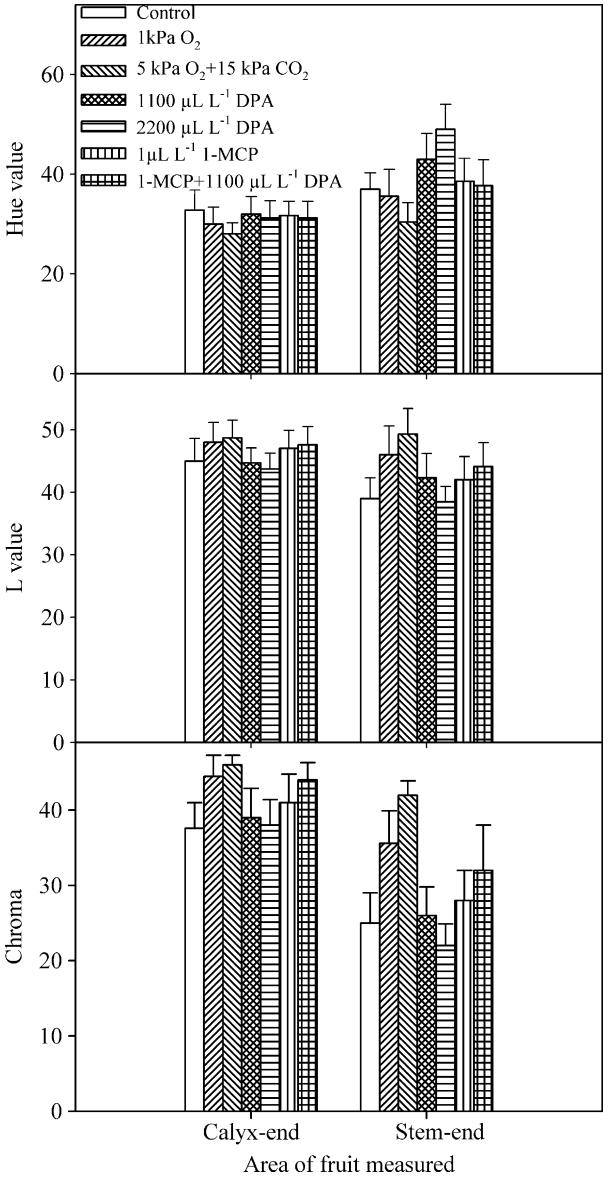

Fig. 2. Differences in skin color between the calyx end and stem end of late-harvested 'Wonderful' pomegranates after 6 months at 7 °C.

Table 5
Visual quality of mid-season and late-season-harvested 'Wonderful' pomegranates stored at 7 °C for up to 6 months plus 4 days at 20 °C[a]

Treatment	Visual quality[a] after indicated storage durations							
	Mid-season harvest				Late-season harvest			
	3 months	4 months	5 months	6 months	3 months	4 months	5 months	6 months
Control	8.7 ± 0.4[b]	6.2 ± 0.0	6.0 ± 1.4	3.1 ± 0.4	5.7 ± 1.4	2.9 ± 0.7	1.4 ± 0.2	1.4 ± 0.8
1 kPa O_2	6.9 ± 0.4	7.0 ± 0.0	7.0 ± 0.0	6.2 ± 0.9	6.9 ± 0.3	6.6 ± 0.9	5.9 ± 1.2	2.7 ± 0.9
1 kPa O_2 + 15 kPa CO_2	9.0 ± 0.0	6.8 ± 0.6	6.8 ± 0.6	7.0 ± 0.0	6.4 ± 1.1	7.0 ± 0.0	6.8 ± 0.6	6.8 ± 0.6
5 kPa O_2 + 15 kPa CO_2	9.0 ± 0.0	6.9 ± 0.2	7.0 ± 0.0	7.0 ± 0.0	8.2 ± 1.4	7.0 ± 0.0	6.8 ± 0.6	7.0 ± 0.0
DPA (1100 μL L^{-1})	9.0 ± 0.0	6.1 ± 1.5	6.1 ± 0.6	4.6 ± 0.7	4.9 ± 1.4	2.5 ± 2.3	2.3 ± 1.2	3.3 ± 1.2
DPA (2200 μL L^{-1})	9.0 ± 0.0	7.0 ± 0.0	5.8 ± 1.3	5.4 ± 1.5	4.6 ± 1.3	3.1 ± 1.3	1.3 ± 0.6	1.0 ± 0.0
1-MCP (1 μL L^{-1}, 24 h)	8.6 ± 0.6	5.0 ± 1.4	3.7 ± 1.5	2.3 ± 1.1	5.9 ± 1.2	6.4 ± 2.5	2.5 ± 0.5	1.4 ± 0.7
1-MCP + DPA (1100 μL L^{-1})	9.0 ± 0.0	5.7 ± 1.1	4.4 ± 1.2	1.0 ± 0.0	6.6 ± 0.9	5.5 ± 0.4	4.9 ± 0.4	1.0 ± 0.0

[a] Hedonic scale: 1 = unusable, 3 = poor, 5 = good, 7 = very good and 9 = excellent.
[b] Mean ± S.E. (n = 3).

trolled by CA treatments including 15 kPa CO_2, as previously shown (Hess-Pierce and Kader, 2003). In general, the main factors reducing visual quality for the mid-season-harvested fruit were decay, scald and physical damage. For the late-season harvest, scald was the most important skin disorder contributing to the reduced visual quality of the fruit.

3.4. Off-flavor

Since CA treatments were very effective in controlling scald and reducing decay levels, we evaluated the presence of fermentative metabolites that have been associated with the use of CA conditions (Ben-Arie and Or, 1986; Hess-Pierce and Kader, 2003). The most abundant compound among fermentative metabolites was ethanol, the maximum concentrations of which were about 200- and 3000-fold higher than those of acetaldehyde and ethyl acetate, respectively (Fig. 3). There was a general increase in all three volatiles throughout storage, especially for CA treatments including 1 kPa O_2, in which levels were 3–12-fold higher than those in the air-stored controls, depending on the compound. Increasing the level of O_2 to 5 kPa during CA storage significantly reduced the levels of these volatile compounds, especially for the mid-season harvest, to below the threshold of detection for off-flavors in fruit (Ke et al., 1991), as observed in prior experiments (Hess-Pierce and Kader, 2003). Interestingly, the differences in concentrations of fermentative metabolites among treatments are less significant in fruit from the late-season harvest than in fruit from the mid-season harvest.

3.5. Biochemical basis of pomegranate scald

Peel tissue samples from the variously treated, late-season-harvested fruit were analyzed for α-farnesene and conjugated trienol contents after 0, 2, and 4 months of storage. Most of the tissue extracts included a trace component that eluted at about the same time as the authentic α-farnesene standard from apple peel. However, only two, those from 1100 μL L^{-1} DPA-treated fruit stored 2 months and from control fruit stored 4 months, had enough of the compound to generate PDA UV absorbance spectra which closely resembled that of α-farnesene (single broad maximum at 232 nm). The calculated α-farnesene concentrations (Whitaker et al., 1997) in these two samples were 0.3 and 1.0 mg kg^{-1}, respectively. This is below the basal level in most apple fruit at harvest, and typically α-farnesene concentration in peel tissue of scald-susceptible apples increases as much as 100-fold during the initial 2–3 months of storage, reaching a maximum ≥150 mg kg^{-1} (Whitaker, 2004). Not surprisingly, the conjugated trienol oxidation products of α-farnesene that begin to accumulate in apple peel after several weeks of storage, and often correlate closely with the incidence and severity of superficial scald (Whitaker et al., 1997; Whitaker, 2004), were not detected in any of the pomegranate peel tissue extracts. These findings indicate that α-farnesene synthesis and oxidation, hypothesized to play an integral role in the induction of superficial scald in apple, are not involved in scald development in pomegranate.

Visual inspection of the concentrated hexane extracts from 30 g of peel tissue suggested that carotenoid biosynthesis was much more active in fruit stored in air than in those stored in CA (5 kPa O_2 + 15 kPa CO_2). Spectrophotometric analysis showed that during 6 months of storage total carotenoids in stem-end peel tissue increased more than 10-fold in controls and less than 3-fold in CA fruit (Table 6). In addition, carotenoid accumulation was much slower in blossom-end than in stem-end tissue in air-stored fruit. On a gram fresh weight basis, the peel tissue carotenoid concentration was quite low, reaching a maximum of just under 2 mg kg^{-1} in air-stored fruit after 6 months. This probably reflects the fact that carotenoids are localized in a few cell layers of the epidermis, the bulk of the pericarp being largely devoid of pigments. UV absorbance of peel tissue hexane extracts at 348 and 367 nm, attributed to the precursor carotenoid phytofluene (Rodriguez-Amaya, 2001), was generally highest after 2–3 months of storage, then declined later in storage with a coincident increase in absorbance maxima at about 449 and 475 nm. The series of desaturation steps in carotenoid biosynthesis require molecular oxygen and involve the plastid terminal oxidase (Kuntz, 2004). Moreover, it has been shown that

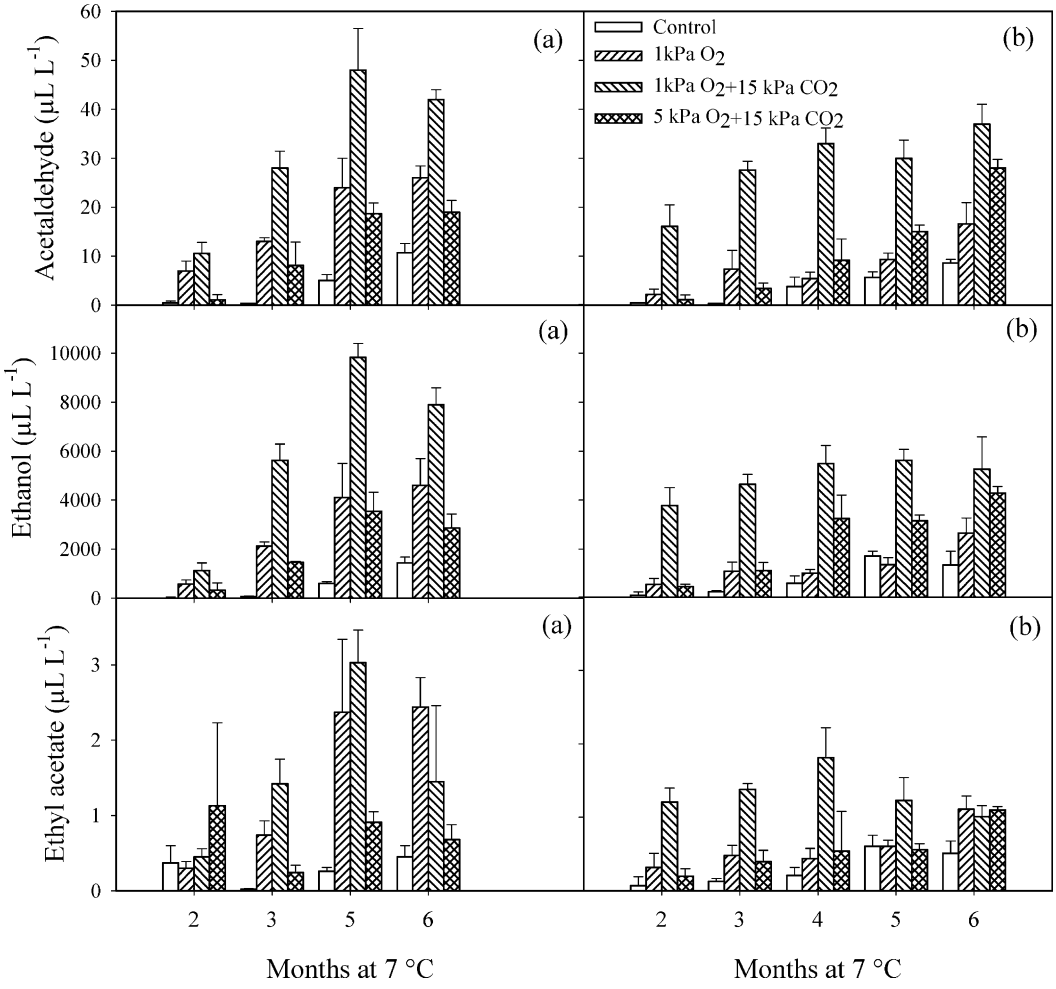

Fig. 3. Effect of storage duration at 7 °C and controlled atmosphere treatments on concentrations of fermentative metabolites in arils of: (a) mid-season and (b) late-season harvested 'Wonderful' pomegranates.

plastid transformation and carotenogenesis are induced by oxidative stress and free radicals in plant tissues such as bell pepper pericarp (Bouvier et al., 1998). It is therefore noteworthy that the highest rate of carotenoid synthesis occurred in stem-end peel tissue of air-stored fruit, which also developed the most severe symptoms of superficial scald (Fig. 1).

Substantial changes in stem-end peel tissue neutral lipid (NL) and phospholipid (PL) fatty acid content and composition occurred over 6 months of storage (Table 7). In both lipid fractions there was an increase in linoleate [18:2(9,12)] and one or more saturated fatty acids at the expense of oleate [18:1(9)] and linolenate [18:3(9,12,15)]. These changes were more pronounced in air control than in CA fruit. A change in NL fatty acids unique to the controls was the occurrence of about 8% punicic acid [18:3(9*Z*,11*E*,13*Z*)], an unusual conjugated trienoic acid that composes about 70–80% of the total fatty acids in seed oil from pomegranate (Hernández et al., 2000). The presence of punicic acid was associated with a >4-fold increase in total NL fatty acids. In contrast, total NL fatty acids declined 2.4-fold in peel tissue of CA-stored fruit. Although it is possible that the punicic acid in air control NL was derived from bits of seed or contaminant seed oil in the pulverized peel tissue, this could not account for the dramatic increase in total NL. Moreover, GC–MS detected trace levels of punicic acid isomers among the fatty acids of PL from air-stored fruit, whereas none was detected in

Table 6
Total carotenoid concentration (mg kg^{-1}) in stem-end (SE) and blossom-end (BE) peel tissue from late-season-harvested 'Wonderful' pomegranates at harvest (Initial) and after storage at 7 °C for up to 6 months plus 4 days at 20 °C

Treatment	Initial	2 months	3 months	4 months	5 months	6 months
Control (SE)	0.18	0.19	0.48	0.85	1.86	1.94
5 kPa O_2 + 15 kPa CO_2 (SE)	0.18	0.17	0.21	0.38	0.45	0.53
Control (BE)	0.16	n.d.	n.d.	0.18	0.32	0.47

Table 7
Composition and concentration of neutral lipid and phospholipid fatty acids in stem-end peel tissue from late-season-harvested 'Wonderful' pomegranates at harvest (initial) and after storage at 7 °C for 6 months in air (control) or under controlled atmosphere (CA = 5 kPa O_2 + 15 kPa CO_2) plus 4 days at 20 °C

Fatty acid	Neutral lipids			Phospholipids (PL)		
	Initial	Control	CA	Initial	Control	CA
12:0	0.7 ± 1.2	0.1 ± 0.1	0.7 ± 0.1	–	–	–
14:0	1.6 ± 0.3	1.1 ± 0.1	2.7 ± 0.2	–	–	–
16:0	18.4 ± 2.1	18.5 ± 1.0	22.6 ± 1.8	19.7 ± 0.2	24.8 ± 1.0	20.4 ± 1.7
18:0	3.9 ± 0.2	7.0 ± 1.7	6.6 ± 1.0	1.6 ± 0.1	2.3 ± 0.1	2.2 ± 0.1
18:1(9)	33.8 ± 0.3	23.6 ± 0.8	29.0 ± 1.2	24.6 ± 0.8	12.6 ± 0.2	20.4 ± 0.2
18:1(11)	3.0 ± 0.9	1.0 ± 0.4	2.9 ± 1.6	1.0 ± 0.0	0.8 ± 0.1	1.3 ± 0.1
18:2(9,12)	29.2 ± 0.5	38.1 ± 1.7	30.1 ± 0.7	32.1 ± 1.3	45.6 ± 0.9	39.0 ± 0.5
18:3(9,12,15)	9.3 ± 0.8	2.3 ± 0.2	5.5 ± 0.2	17.1 ± 1.0	8.3 ± 0.6	11.5 ± 0.2
18:3(9,11,13)	–	8.2 ± 3.7	–	–	0.2 ± 0.1	–
20:0	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	1.5 ± 0.2	1.7 ± 0.1	1.5 ± 0.1
22:0	–	–	–	2.1 ± 0.3	3.5 ± 0.5	2.7 ± 0.2
Total (mg kg^{-1})	185 ± 31	783 ± 130	77 ± 15	143 ± 16	107 ± 11	75 ± 9
PL (μmol kg^{-1})	0	0	0	210 ± 30	156 ± 18	124 ± 14

Values indicate the mean ± S.D. from three replicate extractions.

PL from initial or CA-stored fruit. A substantial loss of PL occurred during 6 months of storage and, somewhat surprisingly, the decline was greater in CA-stored than in air-stored fruit (about 41% and 26% PL loss, respectively). The aberrant lipid metabolism in peel of air-stored fruit could result in membrane destabilization and increased lipid peroxidation.

The four lipophilic conjugates of *p*-coumaric acid (LPCAC) isolated from the NL fraction of peel tissue lipid extracts and separated by C_{18} HPLC were shown to have the same molecular mass (1258) and yielded very similar fragmentation patterns with the same base ion (*m*/*z* 617) when analyzed by electrospray negative ion LC–MS. Thus, they appear to be isomers of the same compound, although the UV absorbance spectra of the first pair (retention times 20.3 and 21.1 min) differ slightly from those the second pair (retention times 22.3 and 23.1 min). The proportions of the four LPCAC isomers were remarkably similar in NL isolated from initial, 6-month air control, and 6-month CA tissue samples (Table 8). Compounds (HPLC peaks) 1, 2, 3, and 4 composed about 12.5%, 22.5%, 23.5%, and 41.5% of the total, respectively. However, the total concentration of LPCAC in the air-stored controls was nearly two-fold higher than that in the initials and nearly three-fold higher than that in the CA-stored fruit. Other than inclusion of one or more *p*-coumaric acid moieties, structures of the four LPCAC isomers are currently unknown, but their hydrophobicity suggests that they are cutin- or suberin-like oligomers (Moire et al., 1999). The increase in LPCAC in air control peel tissue exhibiting scald symptoms may reflect a response to oxidative stress and/or water loss, and also indicates an increase in production of hydroxycinnamic acids that may contribute to the browning reactions involved in scald development (Ben-Arie and Or, 1986).

Table 8
Composition and concentration of four lipophilic *p*-coumaric acid conjugates (LPCAC) in stem-end peel tissue from late-season-harvested 'Wonderful' pomegranates at harvest (initial) and after storage at 7 °C for 6 months in air (Control) or under controlled atmosphere (CA = 5 kPa O_2 + 15 kPa CO_2) plus 4 days at 20 °C

HPLC Peak	LPCAC concentration (μmol kg^{-1})			
	Rtn time (min)	Initial	Control	CA
1	20.3	21 ± 6	44 ± 2	15 ± 2
2	21.1	39 ± 12	75 ± 3	27 ± 4
3	22.3	42 ± 13	78 ± 3	27 ± 4
4	23.1	74 ± 23	134 ± 4	50 ± 7
Total LPCAC		176 ± 27	331 ± 10	119 ± 17

Values indicate the mean ± S.D. from three replicate extractions and are calculated on the basis of *p*-coumaric acid (*p*CA) equivalents using $A_{312\ nm}$.

4. Conclusions

Scald symptoms developed mainly on the stem end of the fruit as brown discoloration on up to 60% of the skin without affecting the internal tissues. Scald is a physiological disorder that limits long-term storage of pomegranate fruit. Among treatments tested, CA was the only treatment that successfully controlled this disorder. The best CA combination was 5 kPa O_2 + 15 kPa CO_2, which resulted in a lower accumulation of fermentative metabolites than the other CA treatments. CA storage (5 kPa O_2 + 15 kPa CO_2) also decreased or prevented changes in carotenoid, acyl lipid, and phenylpropenoid metabolism that were associated with scald development in stem-end peel tissue of air-stored fruit.

References

Ames, B.N., 1966. Assay of inorganic phosphate, total phosphate and phosphatases. Meth. Enzymol. 8, 115–118.

Artés, F., Marin, J.G., Martinez, J.A., 1996. Controlled atmosphere storage of pomegranate. Z. Lebensm. Unters. Forsch. 203, 33–37.

Artés, F., Tudela, J.A., Gil, M.I., 1998. Improving the keeping quality of pomegranate fruit by intermittent warming. Z. Lebensm. Unters. Forsch. 207, 316–321.

Ben-Arie, R., Or, E., 1986. The development and control of husk scald on 'Wonderful' pomegranate fruit during storage. J. Am. Soc. Hort. Sci. 111, 395–399.

Bouvier, F., Backhaus, R.A., Camara, B., 1998. Induction and control of chromoplast-specific carotenoid genes by oxidative stress. J. Biol. Chem. 273, 30651–30659.

Elyatem, S.M., Kader, A.A., 1984. Postharvest physiology and storage behaviour of pomegranate fruits. Sci. Hort. 24, 287–298.

Hernández, F., Melgarejo, P., Olías, J.M., Artés, F., 2000. Fatty acid composition and total lipid content of seed oil from three commercial pomegranate cultivars. In: Melgarejo-Moreno, P., Martínez-Nicolás, J.J., Martínez-Tomé, J. (Eds.), Production, Processing and Marketing of Pomegranate in the Mediterranean Region: Advances in Research and Technology. CIHEAM-IAMZ, Zaragoza, Spain, pp. 205–209.

Hess-Pierce, Kader, A.A., 2003. Responses of 'Wonderful' pomegranates to controlled atmospheres. Acta Hort. 600, 751–757.

Holcroft, D.M., Gil, M.I., Kader, A.A., 1998. Effect of carbon dioxide on anthocyanins, phenylalanine ammonia lyase and glucosyltransferase in the arils of stored pomegranates. J. Am. Soc. Hort. Sci. 123, 136–140.

Hornung, E., Pernstich, C., Feussner, I., 2002. Formation of conjugated Δ^{11} Δ^{13}-double bonds by Δ^{12}-linoleic acid (1, 4)-acyl-lipid-desaturase in pomegranate seeds. Eur. J. Biochem. 269, 4852–4859.

Ke, D., Rodriguez-Sinobas, L., Kader, A.A., 1991. Physiology and prediction of fruit tolerance to low-oxygen atmospheres. J. Am. Soc. Hort. Sci. 116, 253–260.

Ke, D., Yahia, E., Mateos, M., Kader, A.A., 1994. Ethanolic fermentation of 'Barlett' pears as influenced by ripening stage and atmospheric composition. J. Am. Soc. Hort. Sci. 119, 976–982.

Kuntz, M., 2004. Plastid terminal oxidase and its biological significance. Planta 218, 896–899.

Moire, L., Schmutz, A., Buchala, A., Yan, B., Stark, R.E., Ryser, U., 1999. Glycerol is a suberin monomer. New experimental evidence for an old hypothesis. Plant Physiol. 119, 1137–1146.

Rodriguez-Amaya, D.B., 2001. A Guide to Carotenoid Analysis in Foods. ILSI Press, Washington, DC, 15-19.

Watkins, C.B., Barden, C.L., Bramlage, W.J., 1993. Relationships between α-farnesene, ethylene production and superficial scald development of apples. Acta Hort. 343, 155–160.

Watkins, C.B., Nock, J.F., Whitaker, B.D., 2000. Responses of early, mid and late season apple cultivars to postharvest application of 1-methylcyclopropene (1-MCP) under air and controlled atmosphere storage conditions. Postharv. Biol. Technol. 19, 17–32.

Whitaker, B.D., 2004. Oxidative stress and superficial scald of apple fruit. HortScience 39, 933–937.

Whitaker, B.D., Stommel, J.R., 2003. Distribution of hydroxycinnamic acid conjugates in fruit of commercial eggplant (*Solanum melongena* L.) cultivars. J. Agric. Food Chem. 51, 3448–3454.

Whitaker, B.D., Solomos, T., Harrison, D.J., 1997. Quantification of α-farnesene and its conjugated trienol oxidation products from apple peel by C18-HPLC with UV detection. J. Agric. Food Chem. 45, 760–765.

Zanella, A., 2003. Control of apple superficial scald and ripening—a comparison between 1-methylcyclopropene and diphenylamine postharvest treatments, initial low oxygen stress and ultra low oxygen storage. Postharv. Biol. Technol. 27, 69–78.