

Responses of ‘Clemenules Clementine’ and ‘W. Murcott’ mandarins to low oxygen atmospheres

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Abstract

The potential of controlled atmospheres (CA) of 1, 3, and 5 kPa oxygen (balance N₂) to extend the storage life and maintain the quality of two mandarin cultivars was investigated. Low oxygen (O₂) atmospheres at 5 °C for 8 weeks of storage slightly decreased respiration rates but had no effect on ethylene production rates, soluble solids content (SSC), titratable acidity (TA), or flavor compared to the air control after being transferred to air at 20 °C for 3 days of simulated marketing conditions. ‘Clemenules Clementine’ and ‘W. Murcott Afourer’ mandarins (*Citrus reticulata*) kept in 1, 3, and 5 kPa O₂ generally had higher ethanol and acetaldehyde concentrations than those kept in air. However, fruit kept in 3 and 5 kPa O₂ showed similar amounts of ethyl acetate compared to fruit kept in air. The 1 kPa O₂ atmosphere reduced decay incidence in ‘Clemenules Clementine’ fruit during storage at 5 °C for up to 4 weeks but enhanced the decay incidence in ‘W. Murcott’ fruit. Based on these findings, ‘Clemenules Clementine’ and ‘W. Murcott’ mandarins (waxed and fungicide-treated) could best be stored in air at 5 °C and 90–95% relative humidity for up to 5 and 7 weeks, respectively.

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Keywords: *Citrus reticulata*; Mandarin; Low oxygen atmospheres; Fermentative metabolites; CA storage

1. Introduction

During the last 5 years, mandarin production has rapidly increased in California. The cultivars ‘Clementine’, and ‘W. Murcott’ dominate the majority of mandarin producing areas in California with ‘Clementine’ being the number one cultivar produced (Roger, 2004; CASS, 2004). With a 34% increase from 2002 to 2003, there were 12,288 ha of ‘Clementine’ planted (Roger, 2004; CASS, 2004). The planted area of the ‘W. Murcott Afourer’ (W. Murcott) cultivar reached 8389 ha in California in 2004, a 47% increase from 2003 (Roger, 2004; CASS, 2004).

Despite increased production, only limited information is available about postharvest physiology of ‘Clemenules Clementine’ and ‘W. Murcott’ mandarins produced in California. The optimal postharvest handling conditions for

mandarins in general are 5 °C and 90–95% RH and could be stored for 2–6 weeks (Kader, 2002). The limitations to use of controlled atmosphere (CA) storage for citrus fruits are mainly fermentative metabolism and physiological stress that result in the development of off-flavors and induced fungal decay after return to air (Grierson et al., 1966; Chace et al., 1967; Harding, 1969; Hatton et al., 1972; Davis, 1973; Kader, 2002). Nevertheless, information relative to the optimum concentrations of oxygen and carbon dioxide in the internal atmosphere could be used to evaluate various CA treatments and also as a basis for developing new treatments and handling procedures, such as waxing, film wrapping and modified atmosphere packing (Porat et al., 2005).

The objectives of this study were to evaluate the potential of controlled atmospheres to extend the storage life and maintain the quality of two mandarin cultivars, and to determine the minimum O₂ concentration that can be tolerated by mandarins based on the incidence of objectionable off-flavors.

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2. Materials and methods

2.1. Plant material

'Clemenules Clementine' and 'W. Murcott Afourer' (Delite) mandarins (*Citrus reticulata*) that were grown on Carrizo citrange (*Citrus sinensis* [L.] Osb. × *Poncirus trifoliata* [L.] Raf.) rootstock in Ventura, California were acquired on February 14 and March 12, 2004, respectively, from a commercial packinghouse, where they were treated with a 'Pace' shellac-based wax (Pace International, Visalia, California) containing 2000 $\mu\text{L L}^{-1}$ imazalil (residue level of imazalil in fruit was 2–3 $\mu\text{L L}^{-1}$) as fungicide and transported to the Department of Pomology's Postharvest Laboratory at the University of California, Davis. The mandarins were sorted to eliminate defects and stored for up to 8 weeks at 5 °C in air (control), 5, 3 and 1 kPa O₂ (balance N₂). Three replications of 10 fruit each were used for each storage atmosphere and duration. A total of 240 fruit (from 10 fruit × 3 replications × 8 weeks) were placed in a 296-L metal tank ventilated with humidified air or the desired O₂ level at 10 mL s⁻¹ flow rate. Oxygen levels were checked regularly and kept within ±10% of the desired concentrations.

2.2. Gas measurement

At weekly intervals, 3 fruit per replicate were used for measuring respiration and ethylene production rates. Each fruit was placed into a 275-mL jar that was sealed for 10 min before 20 mL of gas was withdrawn from the head-space atmosphere using a plastic syringe. A 10 mL sample was used to measure CO₂ production with an infrared gas analyzer (Model PIR-2000 R, Horiba Instruments, Irvine, CA) and the other 10 mL sample was used to measure C₂H₄ production with a gas chromatograph (Model Carle 211, Hach Carle, Loveland, CO) equipped with a flame ionization detector.

2.3. Internal CO₂

Internal atmosphere samples were withdrawn from 3 fruit per replication immediately after opening the CA chamber with a 1-mL syringe with the needle inserted through the blossom end of individual fruit. Fruit with punctures or peel injuries through which gas could freely diffuse were not used for these measurements. The internal CO₂ concentrations were measured by an infrared gas analyzer.

2.4. Quality assessment

Fruit quality was assessed weekly and after holding in air at 20 °C for 3 additional days to simulate marketing conditions. At each evaluation time, the fruit were checked for decay incidence, overall appearance quality, sol-

uble solids content (SSC), pH, titratable acidity (TA), and flavor.

2.5. Soluble solids content and titratable acidity

The 2–3 segments taken from each of 8–10 fruit per replication were squeezed with a hand-press juicer. The juice was checked for soluble solids content with a refractometer (Abbé Model 10450, American Optical Co. Buffalo, NY). Four grams of juice from each replicate diluted with 20 mL of distilled water were used for titratable acidity and pH measurements. Titration was conducted with 0.1N NaOH to a pH 8.2 end point and TA was calculated as [H⁺] using an automatic titrator (Radiometer, Copenhagen, Denmark) equipped with a pH meter (PHM85 Precision), an autoburette (AUB 80), a printer (PRS 12 Alpha), and a sample changer (SAC80).

2.6. Ethanol, acetaldehyde and ethyl acetate determination

Fermentative metabolites were determined using the procedure of Pelayo et al. (2003) with a modification as described. From each replicate, 10 mandarin segments were crushed to extract the juice. Samples of 5 mL juice were placed in crimp-sealed 10 mL vials containing 2 g of NaCl, immediately sealed with a plastic cap and stored at -80 °C for subsequent analysis of ethanol, acetaldehyde, and ethyl acetate concentrations.

Frozen juice was thawed at room temperature and incubated in a water bath at 37 °C for 15 min. A headspace sample was taken with a 1-mL gas-tight glass syringe and injected into a gas chromatograph (Model HP5890, Hewlett Packard, Palo Alto, CA) equipped with a flame ionization detector and a glass column (2 mm × 1.8 m) containing 5% Carbowax 20M on 60/80 Carbopack (Supelco, Bellefonte, PA). The oven temperature was maintained at 85 °C for 7 min, then programmed to rise by 10 °C/min to the final temperature of 125 °C and held for 3 min. The injector and detector temperatures were 115 and 200 °C, respectively. Concentrations were calculated by using standard aqueous solutions of every analysis and by preparing the corresponding standard curves under the same conditions as those used for the mandarin samples.

2.7. Sensory evaluation of flavor

Five to six judges conducted a sensory panel evaluation of the flavor of the mandarins kept in various atmospheric compositions for various durations. The judges rated the flavor and overall visual quality on a subjective scale of 1–9, where 9 = excellent, 7 = very good, 5 = good (limit of acceptability), 3 = poor, and 1 = very poor; 24–30 fruit per treatment with three segments from each fruit were coded with a three-digit random number. The judges tasted these segments, in random order, without knowing their identities.

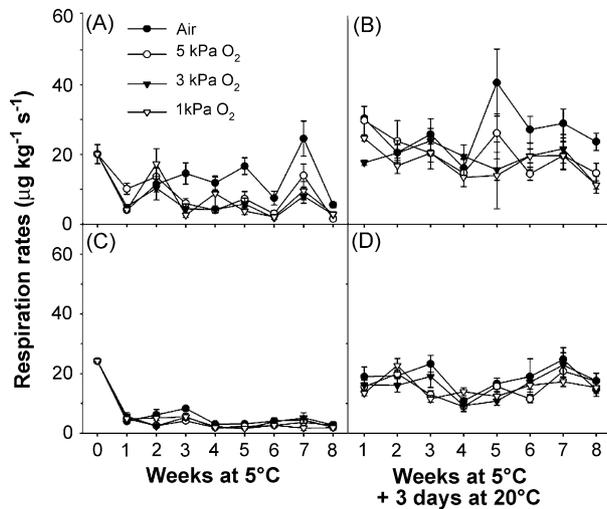


Fig. 1. Respiration rates (means of three replicates \pm S.E.) of 'Clemenules Clementine' (A and B), and 'W. Murcott' (C and D) mandarins kept in air or 5, 3 or 1 kPa O_2 at 5 °C for up to 8 weeks (A and C) followed by holding in air at 20 °C for 3 days (B and D).

3. Results

3.1. Respiration

Low oxygen (O_2) atmospheres reduced respiration rates of 'Clemenules Clementine' mandarins by 50–75% during the first 6 weeks at 5 °C and respiration rates remained below $14 \mu\text{g kg}^{-1} \text{s}^{-1}$ for the remainder of the test. No significant difference among the three O_2 levels used in this experiment was found (Fig. 1A). After the fruit were transferred from low O_2 atmospheres to air at 20 °C for 3 days, respiration rates greatly increased with a range of Q_{10} values from 2.5 to 4.0 (Fig. 1B). In general, the respiration rate was lower in the mandarins previously kept in low O_2 than those previously kept in air (Fig. 1B).

In 'W. Murcott' fruit, a 77–84% reduction in respiration rate from 24 to 4–6 $\mu\text{g kg}^{-1} \text{s}^{-1}$ was observed during the first week of storage at 5 °C in all treatments and it remained low for the rest of the storage period. There were no significant effects of the low- O_2 atmospheres on the respiration rate after 3 weeks of storage at 5 °C as compared with that of the control fruit kept in air (Fig. 1C). Upon transfer to air for 3 days at 20 °C, the respiration rate of 'W. Murcott' mandarins increased 3–4-fold with a Q_{10} of 2–3 and no difference between fruit stored under air or low- O_2 atmospheres (Fig. 1D).

3.2. Internal CO_2

Internal CO_2 concentrations of 'Clemenules Clementine' mandarins held in low- O_2 atmospheres at 5 °C declined from 2.0 kPa to a range of 0.6–1.6 kPa during the first three weeks of storage. In the 5 kPa O_2 atmosphere, the internal CO_2 concentration declined after the first week, but increased over 4 weeks of storage. A relatively small increase of internal CO_2

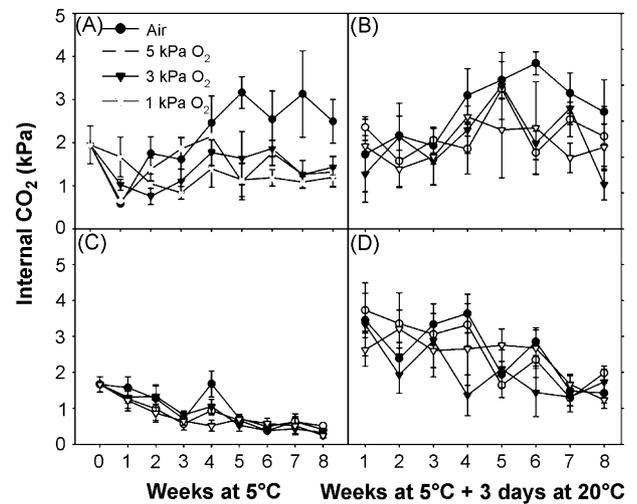


Fig. 2. Internal CO_2 (means of three replicates \pm S.E.) of 'Clemenules Clementine' (A and B), and 'W. Murcott' (C and D) mandarins kept in air or 5, 3 or 1 kPa O_2 at 5 °C for up to 8 weeks (A and C) followed by holding in air at 20 °C for 3 days (B and D).

levels was observed for the rest of the storage period, but was still 50–70% lower than that of the control (Fig. 2A). 'Clemenules Clementine' fruit showed only a slight increase in internal CO_2 concentrations under all storage conditions after subsequent transfer to air at 20 °C for 3 days (Fig. 2B).

In general, 'W. Murcott' fruit exhibited no significant differences in internal CO_2 level during the course of the experiment at 5 °C, regardless of the storage conditions (Fig. 2C). The level of internal CO_2 doubled when the mandarins were transferred to air at 20 °C for an additional 3 days and tended to decline during that storage period as well. The 3 kPa O_2 atmosphere lowered the internal CO_2 of the fruit significantly at week 4 compared to the control fruit kept in air; otherwise no significant difference was found in internal CO_2 between the low- O_2 atmosphere and air (Fig. 2D).

3.3. Ethylene production

The 'Clemenules Clementine, and 'W. Murcott' mandarins exposed to low- O_2 atmosphere produced very low levels of ethylene similar to the control (data not shown). In general, ethylene production by both cultivars remained below 52 and 104 $\text{ng kg}^{-1} \text{s}^{-1}$ at 5 and 20 °C, respectively.

3.4. Fermentative metabolites

Fermentative metabolism did not induce rind injury in any of the fruit held under 5, 3, or 1 kPa O_2 concentrations. At the beginning of storage for 'Clemenules Clementine' mandarins, the levels of acetaldehyde and ethyl acetate were low while the level of ethanol was 944 $\mu\text{L L}^{-1}$ (Fig. 3A, C, and E). When 'Clemenules Clementine' mandarins were stored in air, the levels of ethanol and ethyl acetate did not change for 7 weeks of storage followed by 3 days at 20 °C. Acetaldehyde levels of fruit kept in air, however, increased over 3 weeks

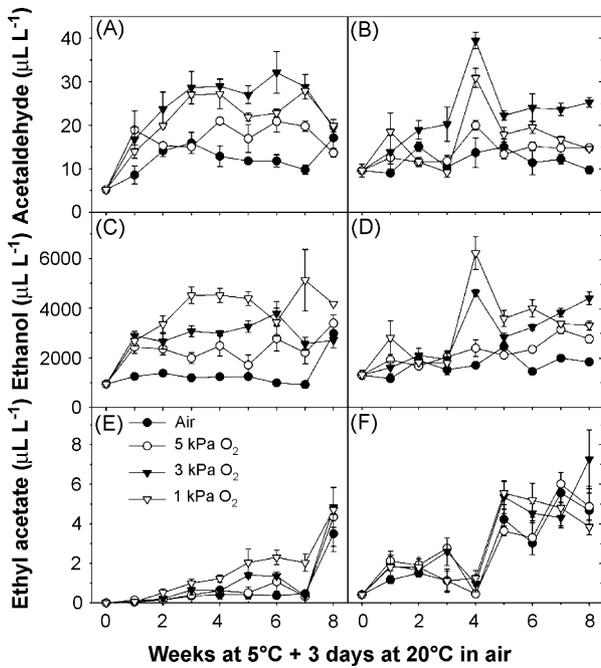


Fig. 3. Changes (means of three replicates \pm S.E.) in concentrations of fermentative metabolites in 'Clemenules Clementine' (A, C, and E), and 'W. Murcott' (B, D, and F) mandarins kept in air or 5, 3 or 1 kPa O₂ at 5 °C for up to 8 weeks and followed by holding in air at 20 °C for 3 days.

and then declined through week 7. In contrast, mandarins exhibited a proportional increase in ethanol and acetaldehyde content to the reduction in O₂ level during storage. At least, the doubling of acetaldehyde content was established in 1 and 3 kPa O₂ atmospheres after 4 weeks and remained at those levels until week 7 of storage (Fig. 3A). Fruit stored in low-O₂ atmosphere for 1–7 weeks accumulated more ethanol than fruit kept in air. The ethanol content of the fruit held in 5 kPa O₂ was lower than for those held in 3 or 1 kPa O₂ atmospheres but was still usually 2 times higher than the control except at week 5. The fruit kept in 3 and 1 kPa O₂ atmospheres had nearly 3000 and 4500 $\mu\text{L L}^{-1}$ ethanol contents, respectively (Fig. 3C). Generally, the ethyl acetate contents were very low (below 2.3 $\mu\text{L L}^{-1}$) and did not change during 7 weeks of storage, but increased 2–10-fold during week eight. Fruit kept in 1 kPa O₂ had a higher level of ethyl acetate than fruit stored in 3, 5 kPa O₂, or air during the first 7 weeks at 5 °C (Fig. 3E). After 8 weeks at 5 °C followed by 3 days at 20 °C, 'Clemenules Clementine' fruit from all storage conditions had similar acetaldehyde, ethanol, and ethyl acetate contents (Fig. 3A, C and E).

There were no significant changes in the acetaldehyde or ethanol content of 'W. Murcott' fruit kept in air at 5 °C for up to 8 weeks followed by 3 days at 20 °C (Fig. 3B and D). In contrast, mandarins kept in low C-atmospheres showed an increase in ethanol content generally proportional to the lower O₂ levels but not always significant (Fig. 3D). As compared with control fruit, a 1.5–2-fold increase in acetaldehyde content was obtained when the fruit were stored under 3 kPa O₂ and a slight increase was induced in fruit stored in 1 kPa O₂

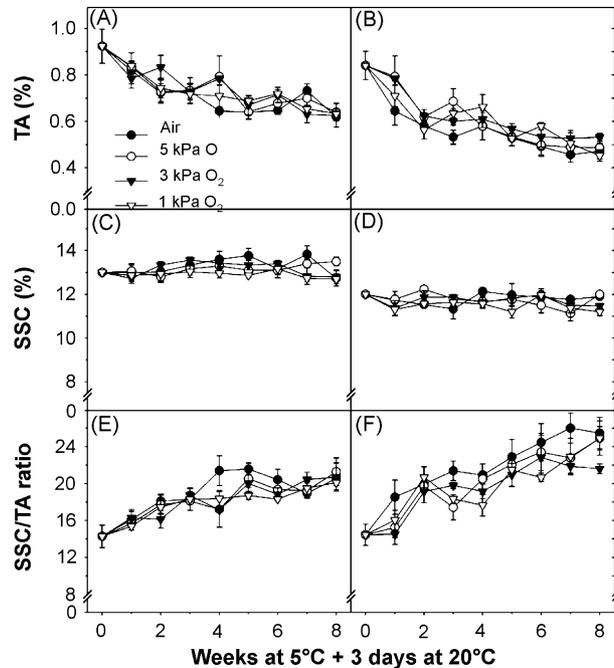


Fig. 4. Changes (means of three replicates \pm S.E.) in soluble solids content (SSC), titratable acidity (TA) and SSC/TA ratio of 'Clemenules Clementine' (A, C, and E), and 'W. Murcott' (B, D, and F) mandarins kept in air or 5, 3, and 1 kPa O₂ at 5 °C for up to 8 weeks followed by holding in air at 20 °C for 3 days.

during 8 weeks of storage. However, acetaldehyde production by 'W. Murcott' mandarins stored in air or 5 kPa O₂ did not change and was not significantly different (Fig. 3B). After 4 weeks of storage, fruit stored in 1 and 3 kPa O₂ atmospheres had about 6200 and 4500 $\mu\text{L L}^{-1}$ ethanol, respectively, and then rapidly decreased for the subsequent week. Ethanol content from fruit kept in 1 kPa O₂ treatment gradually decreased for the remainder of the storage periods whereas the ethanol content from the fruit kept in 3 kPa O₂ atmosphere steadily increased through week 8. The ethanol content of the fruit held in 5 kPa O₂ was lower than those in fruit kept in 3 or 1 kPa O₂ atmospheres, and was not significantly different from that in fruit stored in air up to 5 weeks of storage (Fig. 3D). The ethyl acetate contents were very low (below 2 $\mu\text{L L}^{-1}$) and did not change during the first 4 weeks at 5 °C, but gradually increased 4–6-fold during the subsequent storage periods. There was no effect of low-O₂ atmosphere on ethyl acetate content in 'W. Murcott' fruit during 8 weeks of storage at 5 °C followed by 3 days at 20 °C (Fig. 3F).

3.5. TA, SSC and SS:TA ratio

The two cultivars had similar Titratable acidity (TA) at the beginning of storage. Mandarins stored at 5 °C, followed by 3 days at 20 °C exhibited decrease in TA during 8 weeks of storage but there were no significant differences among mandarins from the various atmospheres (Fig. 4A and B). By the end of the storage period, TA, in general, decreased by 33%, and 40% in 'Clemenules Clementine', and 'W. Mur-

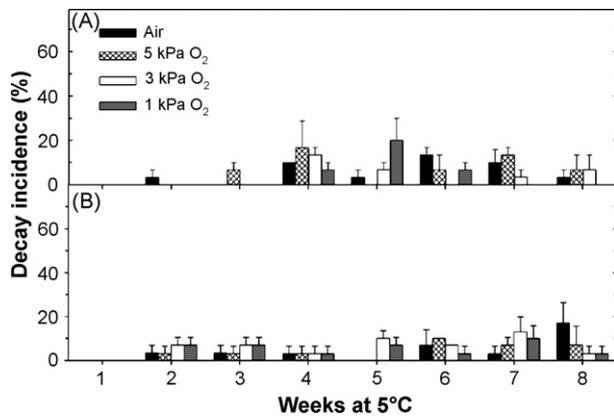


Fig. 5. Changes (means of three replicates \pm S.E.) in decay incidence of 'Clemenules Clementine' (A), and 'W. Murcott' (B) mandarins kept in air or 5, 3 or 1 kPa O_2 at 5 °C for up to 8 weeks.

cott', respectively (Fig. 4A and B). In contrast, SSC did not change significantly with duration or atmosphere of storage. 'Clemenules Clementine' had higher SSC than 'W. Murcott' mandarins: 12.7–13.8% versus 11.1–12.2% (Fig. 4C and D). Since the decrease in TA was accompanied by no change in SSC, it resulted in a 51 and 79% increase in SSC:TA ratio in 'Clemenules Clementine' and 'W. Murcott' juices, respectively, after 8 weeks of storage (Fig. 4E and F).

3.6. Decay

A major cause of deterioration of mandarins was green and blue molds (*Penicillium digitatum* and *P. italicum*, respectively) invading the surface peel and rendering the fruit unmarketable. In general, a 1 kPa O_2 atmosphere reduced decay incidence on 'Clemenules Clementine' during 4 weeks of storage at 5 °C (Fig. 5A). However, for both cultivars decay incidence was significantly higher in fruit kept in 1 kPa O_2 atmosphere at week 5 of storage but not significantly different at week 6 of storage compared to control fruit kept in air (Fig. 5A and B). After the mandarins had been transferred to air for 3 days at 20 °C, decay incidence was reduced in 'Clemenules Clementine' kept in 1 kPa O_2 atmosphere for up to 4 weeks, but was significantly greater in 1 kPa O_2 treatment stored fruit at week 5 (Fig. 6A). In contrast, decay incidence was higher in 'W. Murcott' fruit previously stored in low- O_2 atmospheres up to 5 weeks of storage but appeared not significantly different from fruit kept in air (Fig. 6B).

3.7. Flavor

The flavor scores of 'Clemenules Clementine' and 'W. Murcott' mandarins held at 5 °C under low- O_2 and air storage atmosphere were evaluated after transferring the fruit to air for 3 days at 20 °C and did not show a consistent effect of atmosphere (Fig. 7A and B). In general, low O_2 -treated mandarins for both cultivars exhibited no significantly different flavor compared to air stored fruit during 8 weeks of storage.

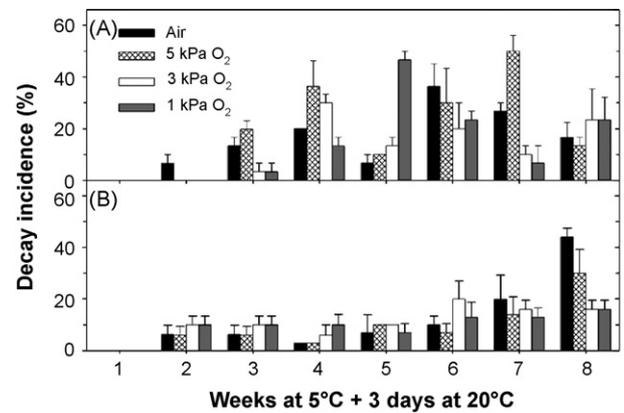


Fig. 6. Changes (means of three replicates \pm S.E.) in decay incidence of 'Clemenules Clementine' (A), and 'W. Murcott' (B) mandarins kept in air or 5, 3 or 1 kPa O_2 at 5 °C for up to 8 weeks followed by holding in air at 20 °C for 3 days.

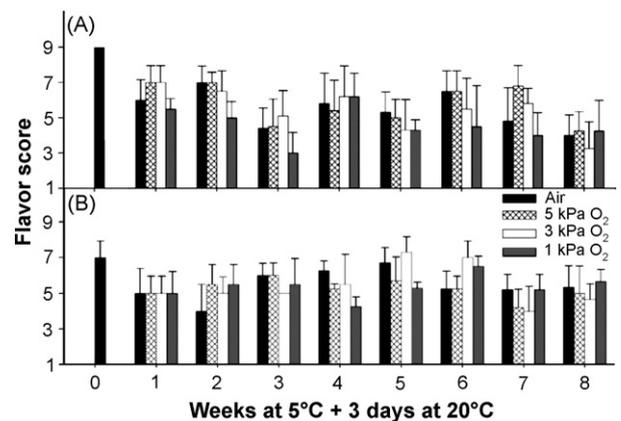


Fig. 7. Changes (means of three replicates \pm S.E.) in flavor scores of 'Clemenules Clementine' (A) and 'W. Murcott' (B) mandarins kept in air or 5, 3 or 1 kPa O_2 at 5 °C for up to 8 weeks followed by holding in air at 20 °C for 3 days. Flavor was assessed by sensory evaluation using the following rating scale: 9=excellent, 7=very good, 5=good (limit of acceptability), 3=poor and 1=very poor.

4. Discussion

Mandarins are non-climacteric fruit and do not exhibit a rise in respiration and ethylene production rates associated with ripening and senescence. The results from this study showed that low- O_2 atmospheres at 5 °C for 8 weeks followed by 3 additional days at 20 °C had no effect on ethylene production and resulted in slightly lower respiration rates in 'Clemenules Clementine' but no effect on 'W. Murcott' mandarins compared to those kept in air. These results suggested that anaerobic respiration had not occurred.

Although the internal CO_2 level depends on the rate of respiration, the respiratory quotient (RQ), and resistance to gas diffusion (Ben-Yehoshua, 1969; McDonald et al., 1993), our results indicated that the low- O_2 atmosphere storage had a slight residual and not clear effect on the internal CO_2 concentration of 'Clemenules Clementine' and 'W. Murcott', respectively. Such information on internal CO_2 and the respi-

ration rate of mandarins under low-O₂ conditions can be used in the development of improved methods of handling fruit, such as wax and film wrapping applications, transportation, and storage.

Changes in fermentative metabolites were dependent upon storage time, storage atmosphere and cultivar. Until the end of postharvest life in air (8 weeks), the levels of these compounds, except ethyl acetate, did not change in fruit of both cultivars. In contrast, low-O₂ atmospheres induced the accumulation of fermentative metabolites; however this induction was not accompanied by elevated CO₂ production in those mandarin cultivars. Acetaldehyde is a product of anaerobic respiration, but it may be reduced to ethanol and react further to form ethyl acetate (Larsen and Watkins, 1995; Smagula and Bramlage, 1977). Our results indicate that ethyl acetate and ethanol content might be used to indicate anaerobic respiration for mandarin fruit. In addition, fermentative metabolism in fruit can also be enhanced by several stress factors including environmental (chilling injury temperatures, hypoxic conditions), biotic (microbial infections) and internal factors such as ripening and senescence (Purvis, 1997).

Ethanol is a product of sugar and acid metabolism under anaerobic conditions. Citrus is able to metabolize glucose and malic acid to pyruvate, resulting in accumulation of acetaldehyde, ethanol, and ethyl acetate (Davis, 1973). However, our results showed no association between changes in either SSC or TA and ethanol or acetaldehyde accumulation. Thus, other compounds may have contributed to the fermentative metabolites pool.

The postharvest life of mandarins stored in air at 5 °C followed by 3 days at 20 °C, defined as the maximum storage period with 75% or higher marketable fruit, was a maximum of 5 and 7 weeks for 'Clemenules Clementine' and 'W. Murcott' mandarins, respectively. The postharvest life in this study was at least partially due to the imazalil in the wax, which provides reliable control of *P. digitatum* sporulation by arresting spore development resulting in only white fungal growth (Smilanick et al., 1997; Peter et al., 2000). Anti-sporulant activity is important because it reduces airborne inoculum around the citrus fruit (Eckert et al., 1994). Since neither respiration nor ethylene production rates were correlated with postharvest life, other factors might play a role in the duration of storage life in mandarins (Ben-Yehoshua, 1969; Chervin et al., 1996). For example, the direct and indirect effect of low temperature with high relative humidity on defense mechanism of mandarins or on the growth of microorganisms, or fungistatic effect of low O₂ atmospheres may also influence the storage life of these fruit.

The off-flavor of citrus fruit is related to the accumulation of the products of anaerobic respiration, i.e. ethanol and acetaldehyde and the threshold levels of those volatiles, and varies considerably among different types of fruit. For example, ethanol content above 1000 μL L⁻¹ induced off-flavors in Valencia oranges (Ke and Kader, 1990). The relationship between fermentative metabolite content and sensory evaluation were calculated in an attempt to obtain a com-

bined value that better reflected the sensory perception of mandarins. However, none of these relationships reflected the preferences for the mandarins by the panelists. Besides, the panelists could not detect any differences among fruit kept in low O₂ atmosphere and air. The ethanol levels of the mandarins in our study, even those kept in air, were constant at 1000 μL L⁻¹ for 8 weeks of storage and did not result in off-flavors. Although 3 and 1 kPa O₂-treated mandarins accumulated very high ethanol (up to 4500 μL L⁻¹) and acetaldehyde concentrations (up to 30 μL L⁻¹), they had similar flavor to air stored fruit during 8 weeks of storage. Ethanol, acetaldehyde and ethyl acetate, in certain amounts, appear to be important components of flavor quality for fresh citrus fruit (Norman and Craft, 1971; Moshonas and Shaw, 1974; Hagenmaier and Shaw, 2002). Cohen et al. (1990) reported that a certain amount of acetaldehyde improved the flavor of Murcott tangerine fruit. However, other components, including SSC and TA, are also important to citrus fruit flavor and can influence perception of off-flavors (Ke and Kader, 1990).

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