



# Continuous exposure to ethylene in the storage environment adversely affects ‘Afourer’ mandarin fruit quality



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## ABSTRACT

‘Afourer’ mandarins were stored in air containing ethylene at 0.001, 0.01, 0.1, and 1  $\mu\text{L L}^{-1}$  at 20, 10, 5 and 0 °C and changes in a range of external and internal quality parameters were examined for up to 10 weeks in storage. At all storage temperatures, reducing ethylene concentration in the storage environment decreased the rate of respiration, visible deterioration of the calyx region, ethanol accumulation in the juice, loss of eating quality, and at chilling temperatures reduced rind pitting. The quality attributes limiting mandarin storage life differed between the different storage temperatures but retention of mandarin quality was always optimised by maintaining the lowest possible ethylene atmosphere around fruit. Thus, the primary target should be to ensure the ethylene levels are  $\leq 0.01 \mu\text{L L}^{-1}$ , as loss of quality was accelerated above this concentration.

## 1. Introduction

The consumption of mandarins has substantially increased in recent years but these fruit generally have a shorter postharvest life than other citrus fruits such as oranges (Kader, 2002). While there are many causes of deterioration during storage, a major factor of mandarins is their susceptibility to develop off-flavours which has been ascribed to a lower peel permeability to gas exchange and higher alcohol dehydrogenase activity that lead to ethanol and acetaldehyde accumulation in the juice (Shi, Goldschmidt, Goren, & Porat, 2007). An ethanol concentration of 1.5–2 g  $\text{L}^{-1}$  has been proposed as a threshold level for off-flavour detection in citrus juice (Cohen, Shalom, & Rosenberger, 1990; Hagenmaier, 2002; Ke & Kader, 1990; Marcilla, Martinez, Carot, Palou, & Rio, 2009).

As with other citrus fruit, the long-term storage of mandarin fruit can be limited due to their susceptibility to physiological breakdown resulting in the development of various rind disorders (Porat, Weiss, Cohen, Daus, & Aharoni, 2004). Ethylene has been reported to play a role in rind pitting in citrus. Cronje, Barry, and Huysamer (2011) reported that an ethylene degreening treatment enhanced rind breakdown of mandarin during subsequent storage at 7.5 °C and this was attributed to ethylene enhancing senescence of the peel which led to subsequent desiccation of peel cells. Brown and Burns (1998) reported

that ethylene degreening induced calyx senescence in oranges by enhancing the activity of abscission enzymes, while enhanced stem-end rot of oranges has been reported following treatment with 10  $\mu\text{L L}^{-1}$  ethylene for 60 h and four weeks storage at 20 °C (Porat et al., 1999).

Most published reports on ethylene effects on citrus fruits have been associated with degreening where this research had been conducted at high temperatures (15–25 °C) with high ethylene levels (> 1  $\mu\text{L L}^{-1}$ ) for a short treatment time before storage and/or marketing (Mayuoni, Tietel, Patil, & Porat, 2011; Porat, 2008). However, ethylene can also accumulate in the marketing chain with levels of ethylene in the ambient air in wholesale markets and produce distribution centres reported to average 0.06  $\mu\text{L L}^{-1}$  with a range of 0.03–0.09  $\mu\text{L L}^{-1}$  (Wills, Warton, & Ku, 2000). Wills, Ku, Shohet, and Kim (1999) stored ‘Valencia’ oranges at 2.5 °C in a wide range of ethylene concentrations down to < 0.005  $\mu\text{L L}^{-1}$  and showed that the appearance of chilling injury was progressively delayed as the ethylene concentration was reduced.

There is no reported study on the effect of low concentrations of ethylene on mandarin fruit quality during storage at ambient or refrigerated temperatures. Mandarins are often degreened with high ethylene levels before marketing and then sold in a range of different markets that vary from immediate consignment to nearby markets, to longer-term storage or export to capture higher returns. The various

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marketing options mean that mandarins can be exposed to a range of temperatures for prolonged periods and different types of deterioration in quality can be encountered over these ranges of storage temperatures. The present study examined the effect on external and internal quality of 'Afourer' mandarins (also known as 'Nadorcott' or 'W. Murcott') stored in exogenous ethylene concentrations from 0.001 to 1.0  $\mu\text{L L}^{-1}$  at temperatures of 20°, 10°, 5° and 0 °C. While most citrus are chilling sensitive below 5 °C, cold treatment (1–3 °C) is often necessary for quarantine pest cold disinfestation treatment for market access (De Lima, Jessup, Cruickshank, Walsh, & Mansfield, 2007). Therefore assessing the response of mandarins to ethylene below 5 °C could provide an insight into the anticipated physiological behavior and quality following cold disinfestation. This is the first study to report on the interaction of storage temperature and ethylene in the storage environment on mandarin quality.

## 2. Materials and methods

### 2.1. Produce and treatments

Mandarins (*Citrus reticulata* Blanco cv. 'Afourer') were obtained from a local market on two occasions. Fruit had been waxed with commercial carnauba wax (morpholine-free) as per the standard commercial practice in Australia. The fruit had a uniform orange skin colour and a brix : acid maturity ratio of 18.2, which is considered commercial maturity (Australian Citrus Standard, 2015). Fruit were sorted for uniformity of size and freedom from visible defects then randomly distributed into 48 treatment units each consisting of 20 fruit. The 20 fruit in a unit were distributed into four groups of five fruit with each group placed into a separate net bag. The four net bags in each unit were placed into a 10 L plastic container that was fitted with inlet and outlet airflow ports. Twelve containers were placed into a temperature-controlled cabinet held at 0, 5, 10 and 20 °C and within each cabinet three containers were ventilated with humidified air (100 mL  $\text{min}^{-1}$ ) containing ethylene at 0.001, 0.01, 0.1 or 1  $\mu\text{L L}^{-1}$ . The desired concentrations of ethylene were obtained by mixing ethylene from a gas cylinder (BOC Gases, Sydney, Australia) with compressed air that was made ethylene-free by passing through a jar containing potassium permanganate pellets, and humidified to approximately 90% RH by bubbling through water. The rate of ventilation exchanged one volume of atmosphere per empty container in 1.5 h. After storage for four weeks in continuous ethylene, 10 fruit from each unit held at 0, 5 and 10 °C from each ethylene concentration were transferred to 20 °C and stored in air. Five fruit were assessed after 24 h and the other five fruit were assessed after a total of five days at 20 °C to simulate market and retail conditions. After a total of 7 and 10 weeks in storage, a further five fruit from each treatment were transferred to 20 °C in air and assessed after five days. Quality assessment at each time point was by visual inspection and physico-chemical analysis.

### 2.2. Respiration

Fruit respiration was measured as  $\text{CO}_2$  production after 4, 7 and 10 weeks storage just before removal to 20 °C. At each assessment period, the five fruit from each treatment unit at 0, 5, 10 and 20 °C were sealed in a 2 L glass jar for 5, 4, 3 and 2 h, respectively. A 1 mL gas sample was then collected from the headspace for  $\text{CO}_2$  analysis by thermal conductivity gas chromatography (Li, Wills, Golding, & Huque, 2015).

### 2.3. Assessment of rind quality

Visual assessment was conducted on the exterior appearance of fruit. The presence of microbial growth on or near the calyx (button) was assessed by determining the percentage of fruit showing visual mould. The colour of the calyx region was rated on a 5-point scale

where 1 = green, 2 = slightly yellow, 3 = moderately yellow, 4 = totally yellow and 5 = brown and the mean score of all fruit in the sample was calculated. The number of calyxes that had detached from the fruit was recorded and the percentage of fruit with calyxes still attached was calculated. Rind pitting was rated on a 5-point scale where 1 = no pitting, 2 = < 5%, 3 = 5–15%, 4 = 15–30% and 5 = > 30% of the rind showing pitting and the mean score of all fruits in the sample was calculated.

### 2.4. Assessment of internal quality

Internal quality was assessed on fruit that had been held for one day or five days at 20 °C after storage. The five fruit in a treatment removal unit were peeled and half of the segments from each fruit were hand juiced through two layers of cheesecloth. The collected juice was analysed for total soluble solids (TSS), titratable acidity (TA), ethanol and acetaldehyde. TSS in the juice was determined with a digital refractometer (PR-32 Atago, Japan) and expressed as % Brix. TA was determined by titrating juice (5 mL) with 0.1 mol  $\text{L}^{-1}$  NaOH to pH 8.2 with an automatic titrator (Mettler Toledo, Switzerland) and data were expressed as citric acid equivalents ( $\text{g L}^{-1}$ ). The TSS:TA ratio was calculated as % Brix: g  $100^{-1}$  acid. Ethanol and acetaldehyde were determined by transferring juice (10 mL) into 20 mL glass vials with silicone septum seals that were then immersed in a 30 °C water bath for 15 min. A headspace gas sample (1 mL) was withdrawn with a syringe and injected in a gas chromatograph fitted with a flame ionization detector (Model 580, Gow-Mac, Bethlehem PA, USA). The column, injector and detector temperatures were 65, 190 and 190 °C, respectively, with gas flow rates of 30, 30 and 300 mL  $\text{min}^{-1}$  and for nitrogen, hydrogen air, respectively. Data were expressed as g ethanol and mg acetaldehyde per L of juice based on calibration curves obtained by injecting known volumes of the respective chemical standards.

The eating quality of fruit at each removal was evaluated by a small panel of four untrained consumers using the remaining half of fruit segments which were separated and distributed into different plastic cups. Each cup contained segments from five different fruit. Panelists rated the fruit on a five point scale for overall taste preference where 1 = excellent, 2 = good, 3 = fair, 4 = poor 5 = very poor. Distilled water was provided to cleanse the palate between samples.

### 2.5. Statistical analysis

Statistical procedures were performed using SPSS for Microsoft version 18.0 software package (SPSS, Chicago, IL, USA). ANOVA analysis of variance was conducted and least significant differences (LSD) at  $P=0.05$  for means were calculated to determine significant differences between treatments.

## 3. Results

### 3.1. Change in external quality

#### 3.1.1. Appearance of the calyx area

For fruit stored at 20 °C, the first change in fruit quality was the appearance of mould on the calyx and the surrounding area. Table 1 shows that a substantial proportion of fruit stored at 20 °C were mouldy after 4 and 7 weeks storage, but the incidence of mould was reduced significantly as the concentration of ethylene was reduced ( $P < 0.001$ ). The incidence of mould more than doubled in the presence of 0.1 or 1  $\mu\text{L L}^{-1}$  ethylene compared with fruit held in 0.001  $\mu\text{L L}^{-1}$  ethylene. It was considered that the high proportion of fruit with mould precluded meaningful observation of any change in other quality parameters and storage at 20 °C was terminated at 7 weeks.

For fruit stored at 10, 5 and 0 °C, mould did not occur but there was a significant change in calyx condition during storage. Table 2 shows

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