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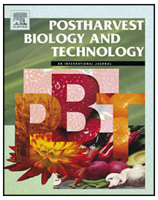

Postharvest ethylene conditioning as a tool to reduce quality loss of stored mature sweet oranges

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ABSTRACT

Ethylene is related to senescence but also induces protective mechanisms against stress in plants. The citrus industry only applies the hormone to induce fruit degreening. The aim of this work was to determine the effect of ethylene on the quality of colored citrus fruit stored under commercial conditions to extend postharvest life, since it protects them from stress causing postharvest disorders such as chilling injury (CI) and non-chilling peel pitting (NCPP). The effect of conditioning mature Navelate and Lane Late sweet oranges (*Citrus sinensis* L. Osbeck) for 4 days with 2 $\mu L\,L^{-1}$ ethylene at 12 °C, rather than at higher temperatures used for degreening, on the quality of fruit stored at 2 or 12 °C, was examined. The ethylene conditioning (EC) treatment did not increase color but reduced calyx abscission and NCPP in fruit of both cultivars stored at 12 °C, and also CI in Navelate fruit at 2 °C. Lane Late fruit did not develop CI but showed a new disorder in EC fruit held at 2 °C. This disorder began as scalded areas around the fruit stem end and extended over the fruit surface during storage. EC had no deleterious effect on the quality of Navelate oranges stored at either 2 or 12 °C. Similar results were found in Lane Late fruit although EC slightly increased off-flavor perception at 2 °C and the maturity index at 2 and 12 °C. Moreover, EC slightly increased the content of bioactive flavonoids in the pulp of Navelate fruit but significant differences between control and EC fruit were only found after prolonged storage at 2 °C. In Lane Late fruit, EC avoided the initial decrease in flavonoid content found in control samples. Results show, therefore, that EC at 12 °C may be a tool to extend postharvest life of NCPP and CI-sensitive oranges, and that the tolerance of citrus cultivars to the combined effect of EC and non-freezing low temperature (2 °C) should be tested to select the proper storage temperature.

1. Introduction

Several types of postharvest physiological disorders affecting peel quality have been described in citrus fruit but factors causing them and how they are related to each other is not understood (Grierson, 1986; Lafuente and Zacarías, 2006; Magwaza et al., 2013). Efforts have been made to reveal factors responsible for different postharvest citrus fruit disorders and to develop new strategies to control them. However, currently no proven methodologies exist to reduce their incidence. Most studies on reduction of physiological peel disorders have been related to methodologies aiming at reducing chilling injury (CI), and the effect on citrus fruit quality has been well characterized (Schirra et al., 2005; Mulas and Schirra, 2007).

CI and non-chilling peel pitting (NCPP), also known as rind-staining, are two main postharvest physiological disorders in citrus fruit (Lafuente and Zacarías, 2006). NCPP develops at temperatures higher than those causing CI, which in citrus fruit may occur at temperatures ranging between 1 and 12 °C. Sweet oranges from the Navel group may develop these physiological disorders (Lafuente and Sala, 2002; Alférez et al., 2003; Holland et al., 2005). Navelate orange (*Citrus sinensis* (L.) Osbeck) is prone to develop NCPP as well as CI after prolonged cold storage. In this cultivar, NCPP is characterized by depressed areas in the peel affecting both the albedo (inner part of the peel) and the flavedo (outer part of the peel), which eventually turn bronze in color; while CI is manifested as superficial scalding or flavedo bronze non-depressed areas (Alférez et al., 2005). The postharvest performance of other late maturing sweet Navel orange cultivars, which have expanded very quickly in many countries to extend the citrus season, is less known. This is the case

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of the late-maturing Lane Late orange (*C. sinensis* (L.) Osbeck) that has good fruit quality characteristics.

Ethylene is usually related to senescence but it plays also a role protecting plants from stress causing damage (Yang and Hoffman, 1984). Although this hormone may enhance a senescence-related physiological disorder, rind breakdown in Nules Clementine mandarin (Cronjé et al., 2011), or other peel defects such as zebra skin or horseshoe/green rings in other citrus cultivars (Krajewsky and Pittaway, 2002), it also protects citrus fruit from stresses causing CI and NCPP (Lafuente et al., 2001; Lafuente and Sala, 2002). The industry only applies this hormone to early-maturing citrus fruit for degreening purposes although it may reduce physiological disorders in mature fruit harvested after color break (Lafuente and Sala, 2002; Lafuente et al., 2004; Cajuste and Lafuente, 2007). The effect on the quality of citrus fruit of postharvest degreening at 90% RH and 20–22 °C using 2–4 $\mu L\,L^{-1}$ ethylene, has been documented (Carvalho et al., 2008; Mayuoni et al., 2011a; Sdiri et al., 2012a,b; Moscoso-Ramírez and Palou, 2014). Previous data also show that conditioning mature citrus fruit with ethylene concentrations ranging between 1 and 10 $\mu L\,L^{-1}$ considerably reduces NCPP in fruit stored afterwards at 20–22 °C and increases peel phenolic content (Lafuente and Sala, 2002; Cajuste and Lafuente, 2007). Moreover, it is known that physiological responses of citrus fruit to ethylene may vary with maturation stage (Lisker et al., 1983; John-Karuppiah and Burns, 2010). However, there is no information on the effect of ethylene on the quality of fully mature fruit stored for prolonged periods at lower temperatures under commercial conditions required to extend postharvest life. Experiments performed to evaluate the effect of degreening have been limited to examining changes occurring after a single quarantine treatment (1 °C for 16 days) in fruit of citrus cultivars that did not develop physiological disorders (Sdiri et al., 2012a), while storage duration and temperature affects citrus fruit quality (Mulas and Schirra, 2007; Obenland et al., 2008). Therefore, the aim of this work was to evaluate the effect of treating full colored mature sweet Navelate and Lane Late orange cultivars, which are not degreened for commercialization, for 4 days with 2 $\mu L\,L^{-1}$ ethylene at 90–95% relative humidity (RH) on the quality of fruit stored for up to 60 days at 2 or 12 °C and 90–95% RH. This ethylene conditioning (EC) treatment was performed at 12 °C rather than at the temperature used for commercial degreening (20–21 °C) (Mayuoni et al., 2011a; Sdiri et al., 2012a,b). The degreening temperature is recommended to enable color development after ethylene treatment. Fruit in this study were already fully colored, and therefore a lower temperature (12 °C) was used to reduce fruit quality loss. The effects of the treatment on color, firmness, weight loss and internal quality of the fruit, as well as the incidence of calyx abscission, decay and physiological disorders were evaluated. Considering that ethylene increases the activity of the enzyme phenylalanine ammonia-lyase (PAL) in citrus fruit, especially in the more mature fruit (Lisker et al., 1983), that this is a key enzyme at the entry point of phenylpropanoid metabolism, and the health benefits of phenolics (Tripoli et al., 2007), we have also evaluated in the fruit edible portion the effect of the EC treatment on the accumulation of total phenos and flavonoids, which are the most abundant phenolics in citrus fruit (Nogata et al., 2006).

2. Materials and methods

2.1. Plant material and postharvest treatments

Fully mature Navelate and Lane Late sweet oranges (*C. sinensis* (L.) Osbeck) were harvested in March from the same orchard at Lliria (Valencia, Spain), and immediately delivered to the laboratory. Fruit were surface-sterilized with a commercial bleach solution (Ballester et al., 2013) and then randomly divided into 2 groups that were treated with ethylene (group 1) or held in air (control fruit, group 2). These groups were further subdivided into two subgroups to evaluate the effect of the storage temperature (2 and 12 °C). Fruit in each subgroup were divided into two lots. Three replicates of 10 fruit were included in the first lot to estimate periodically the incidence and severity of physiological disorders and of calyx abscission. The second lot contained 3 replicates of 10 fruit per storage period since they were used to determine changes in peel color but also in other quality attributes in the pulp requiring destructive methods. Fruit within the first group were conditioned for 4 days with 2 $\mu L\,L^{-1}$ ethylene at 90–95% RH and 12 °C (EC fruit), and then stored at 2 (subgroup 1) or 12 °C (subgroup 2). Control fruit were maintained for 4 days in air at 90–95% RH and 12 °C (AC fruit) before being stored at 2 or 12 °C. EC and AC fruit were kept for 8 weeks at 2 °C or for 6 weeks at 12 °C and then transferred to 20 °C for 4 days to simulate a shelf-life (SL) period.

2.2. Estimation of postharvest physiological disorder severity and incidence

Three different physiological disorders were identified under the above mentioned storage conditions. Since their symptoms were different, severity was evaluated independently during holding of AC and EC fruit at 2 and 12 °C, and after the SL period. Depending on the cultivar, postharvest conditioning treatment or storage condition, fruit showed: (1) NCPP, manifested as collapsed surface areas that became dark brown with time (Supplemental Fig. S1A); (2) CI, manifested as bronzed non-depressed areas (scalding) of the fruit surface (Supplemental Fig. S1B); and/or (3) a physiological disorder, which as far as we know has not been previously described in citrus fruit, that begins as scalded areas around the stem end of the fruit (Supplemental Fig. S1C) and extends through the fruit surface during storage (Supplemental Fig. S1D). This disorder, named SECI (from stem end chilling injury), shows non-depressed areas in the fruit stem end, and therefore it is different to stem-end-rind-breakdown (SERB). A rating scale from 0 (no damage) to 4 (severe damage) (Vicente et al., 2013) was used to determine the average severity damage index of each physiological disorder. In this scale, only fruit showing a damage score higher than 1 would be rejected by consumers. The same fruit were used at the various evaluation dates. The severity indexes were calculated by adding up the products of the number of fruit in each category multiplied by its score, and then dividing the total obtained by the number of fruit, evaluated as described by Lafuente et al. (1997). Moreover, the commercial incidence of each physiological disorder was estimated by calculating the percentage of fruit showing a damage score higher than 1. The results were the means of 3 replicate samples containing 10 fruit each ± S.E.M.

Supplemental Fig. S1 related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.postharvbio.2014.03.011.

2.3. Analysis of quality attributes

Changes in fruit quality were assessed by measuring the acidity and SSC in the pulp, peel fruit color, and fruit firmness and weight loss as described by Holland et al. (1999) and Lafuente et al. (2011). Soluble solids (°Brix) were determined from fruit juice with an Atago/X-1000 digital refractometer (Atago Co. Ltd., Tokyo, Japan) and acid content was titrated with 0.1 N NaOH using phenolphthalein as indicator and expressed as g of anhydrous citric acid in 100 mL of juice. The maturity index was calculated by dividing the °Brix of the extracted juice by its acid content. Peel color was analyzed by using a Minolta CR-300 Chromameter (Konica Minolta Inc, USA) with a measuring area of 8 mm at four locations around