Postharvest Biology and Technology 127 (2017) 1–13

Contents lists available at ScienceDirect

Postharvest Biology and Technology

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

Dynamic controlled atmosphere storage suppresses metabolism and enhances volatile concentrations of 'Galaxy' apple harvested at three maturity stages

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ARTICLE INFO

Article history:
Received 2 August 2016
Received in revised form 3 January 2017
Accepted 4 January 2017
Available online xxx

Keywords:
Malus domestica
Respiratory quotient
Aroma
Anaerobic metabolism
Ethylene
Respiration rate

ABSTRACT

The objective of this study was to assess the interaction between four storage conditions and three maturity stages of 'Galaxy' apple at harvest (unripe, ripe and overripe, based on starch pattern index) on its metabolism and volatile concentrations after harvest and 9 months of storage plus 7 days of shelf life at 20 °C. Storage conditions were: [1] Controlled atmosphere (CA) (1.2 kPa O_2 + 2.0 kPa CO_2); [2] CA + 1-methylcyclopropene (1-MCP) (0.625 μL L^{-1}); [3] Dynamic controlled atmosphere based on respiratory quotient 1.3 (DCA-RQ 1.3) + 1.2 kPa CO_2 and [4] DCA-RQ 1.5 + 1.2 kPa CO_2. Fruit stored under DCA-RQ 1.5 had higher concentrations of total esters and characteristic aroma volatile compounds in extracted juice. Highest ester concentrations occurred in overripe fruit stored at DCA-RQ 1.3 and DCA-RQ 1.5. 1-MCP application suppressed volatile compound production, not allowing its increment with advanced of the maturity stage and reduced the main esters after storage. DCA-RQ suppressed internal ethylene concentration, ethylene production, and respiration rate, but the low metabolism in fruit stored under DCA-RQ 1.5 did not result in lower volatile compound production.

1. Introduction

Volatile compounds are critically important for apple acceptance by consumers (López et al., 2007). These compounds change in response to fruit maturity and ripening and to storage conditions (Bangerth et al., 2012). Among the volatile compounds produced by apples, esters have a major impact on aroma. Apples of the 'Gala' group, such as 'Galaxy', have high butyl acetate, 2-methylbutyl acetate and hexyl acetate concentrations, which are the most important esters of this cultivar group (Salazar and Orozco, 2011; Both et al., 2014). These compounds increase with advancing maturity, but are suppressed by storage, showing the importance to develop a technology that allows to harvest the fruit in different maturity stages without volatile compound reduction.

Controlled atmosphere (CA), is a worldwide used storage technique for apple fruit. Nevertheless, CA storage, strongly reduces ester formation (Brackmann et al., 1993; Fellman et al., 2003; López et al., 2007; Raffo et al., 2009; Lumpkin et al., 2014, 2015). Low O_2 partial pressure (pO_2) employed during CA storage suppresses ethylene biosynthesis and action, which are important for activity of ester forming enzymes (Burg and Burg, 1965; Yang et al., 2016). Low pO_2 may also suppress volatile compound precursor production via β-oxidation and lipoxygenase (LOX) pathway (Brackmann et al., 1993; Song and Bangerth, 2003), because both pathways require O_2 (Echeverría et al., 2004).

1-MCP application is often used in several countries for extending the storage period of apples, due to its ethylene action blocking (Watkins, 2006; Lee et al., 2012) and delaying fruit ripening. 1-MCP treated apples maintain higher flesh firmness (Fawbush et al., 2009; Moggia et al., 2010; Brackmann et al., 2013), titratable acidity, soluble solids (Watkins, 2006) and have lower

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http://dx.doi.org/10.1016/j.postharvbio.2017.01.002
0925-5214/

incidences of superficial scald (Lurie and Watkins, 2012) and mealiness (Brackmann et al., 2014), among the benefits on the fruit quality. However, 1-MCP can negatively impacts volatile biosynthesis, especially alcohols and esters, that contributes to the characteristic apple aroma (Lurie et al., 2002; Ferenczi et al., 2006; Lee et al., 2012; Thewes et al., 2015; Yang et al., 2016). Additionally, the 1-MCP application presents additional costs for the storage operators and is not allowed for the storage of organic apples. Therefore, there is a need to develop a storage technology that can reduce the fruit metabolism similarly to the 1-MCP application, without significant volatile compounds loss maintaining the apple characteristic aroma.

The apple storage based on the lowest oxygen limit (LOL) tolerated by the fruit has become available for commercial use. Three methodologies to detect the LOL during storage in dynamic controlled atmosphere (DCA) are available: ethanol production by fruit (Storex®, Swinglos®, ILOS-Plus® and Fruit control®) (Veltman et al., 2003), fruit chlorophyll fluorescence emission (HarvestWatch™ and Fruit Observer®)(Prange et al., 2007; Wright et al., 2010, 2012) and respiratory quotient (Advanced control respiration®, Storage Control Systems Safe Pods® and RQ store fresh®) (Gasser et al., 2008; Wright et al., 2012; Brackmann, 2015; Weber et al., 2015). According to Raffo et al. (2009), the storage of 'Pinova' apple under DCA based on chlorophyll fluorescence (DCA-CF), resulted in reduced main esters in comparison with CA storage (1.5 kPa O_2 + 1.3 kPa CO_2), but higher ester concentrations when compared with CA + 1-MCP. Nevertheless, there is no literature evaluating the effect of DCA based on respiratory quotient (DCA-RQ) on the volatile profile of apples. This technology induces a small level of fermentation by the fruit (Brackmann et al., 2015; Weber et al., 2015), which supply ethanol to the volatile compounds biosynthesis, resulting in increased volatile compound biosynthesis in comparison to CA and CA + 1-MCP. In oriental sweet melons, the ethanol application increased the volatile compound production, like ethyl esters, butyl acetate and hexyl acetate (Liu et al., 2012).

The objective of this study was to assess the interaction between four storage conditions and three maturity stages of the fruit (unripe, ripe and overripe) on the metabolism and volatile compound concentration of 'Galaxy' apples after harvest and 9 months of storage plus 7 days of shelf life at 20 °C.

2. Material and methods

2.1. Plant material, orchard location, harvest maturity and sample preparation

'Galaxy' apples (*Malus domestica*), a 'Royal Gala' sport (Okie, 1999), were harvested in a commercial orchard located in the town of Vacaria, RS, Brazil. The 'Galaxy' apples were grafted on M9 rootstocks at a density of 3,575 plants ha^{-1}. The following fertilization was carried out during the growing season: 80 kg ha^{-1} of nitrogen and 120 kg ha^{-1} of potassium.

Fruit were harvested at three maturity stage. The starch pattern index (SPI) (Streif, 1984) was used to differentiate maturity stages in the laboratory: Unripe apples <3.5, ripe fruit index between 3.5–7.0, and overripe >7.0. In each harvest, SPI were determined using 3 replicates of 20 fruit, resulting in averages of 3.4, 6.5 and 9.7 units, for unripe, ripe and overripe, respectively.

Fruit were transported to the Postharvest Research Center of the Federal University of Santa Maria, RS, Brazil, on the day of harvest, where fruit with any damage were eliminated and fruit randomized. Three replication of 25 fruit were used for each treatment.

2.2. Storage conditions

Fruit replications were placed into 233-L galvanized iron CA chambers in which the following treatments were applied: [1] controlled atmosphere – CA (1.2 kPa O_2 + 2.0 kPa CO_2); [2] CA + 1-MCP (0.625 μL L^{-1}); [3] DCA-RQ 1.3 + 1.2 kPa CO_2 and [4] DCA-RQ 1.5 + 1.2 kPa CO_2.

The storage temperature was 1.5 ± 0.1 °C and it was monitored daily during the 9 months of storage with the aid of mercury thermometers inserted inside the fruit flesh to determine the pulp temperature. Inside the storage chamber, the relative humidity (RH), was monitored manually with psycrometers and controlled by the addition of calcium chloride, which absorbed the excess of humidity inside the chamber to maintain an average relative humidity at 94 ± 2%.

2.3. 1-MCP treatment

1-MCP treatment of fruit was carried out in the chamber with 0.625 μL L^{-1} 1-MCP (EthylBloc®, 0.14% of active ingredient). The fruit were treated during 24 h. The air inside the chamber was circulated with a fan. This process was carried out at the storage temperature of 1.5 ± 0.1 °C.

2.4. CA and DCA − RQ setup and maintenance

During the first day of storage, the temperature was reduced down to 5.0 °C and thereafter gradually down to 1.5 °C, a process that took 5 days. CA and DCA-RQ conditions were applied on day 5, when fruit temperatures had reached 1.5 °C. The experimental chambers were hermetically closed and the CA and DCA-RQ conditions were applied.

The chambers were flushed with nitrogen until the pO_2 reached 1.2 kPa for CA, and to achieve the DCA-RQ conditions, O_2 was reduced to 0.5 kPa. This process was carried out over 5 days. The pCO_2 was obtained by its accumulation in the storage chamber due to fruit respiration. Therefore, the CA and DCA-RQ conditions were installed from days 5 to 10, to simulate the commercial CA and DCA storage establishment.

The pO_2 was changed according to the fruit metabolism throughout the DCA storage period, while it was maintained constant during CA storage. The RQ was measured two times a week, to measure the LOL during the storage period, according to the method proposed by Brackmann (2015). The RQ was set at 1.3 and 1.5, and pO_2 changed accordingly to maintain this RQ level (Supplementary Fig. S1). A standard deviation of 0.09 was obtained for DCA – RQ 1.3 and of 0.07 for DCA – RQ 1.5. The RQ was calculated once the chamber had been closed for 13 h between the first and second reading. The RQ was calculated by the ratio between CO_2 production and O_2 uptake. CA conditions were maintained according to the method of Brackmann et al. (2014).

2.5. Internal ethylene concentration (IEC)

The IEC was determined according to Mannapperuma et al. (1991) on 10 fruit per replicate. The internal gas of fruit was withdrawn, and two samples (1 mL) were injected into a gas chromatograph (Varian®, model Star 3400CX) equipped with a flame ionization detector (FID) and a Porapak N80/100 column. The temperatures of the column, the injector and the detector were 90, 140 and 200 °C, respectively. Results were expressed in ug L^{-1}.

2.6. Ethylene production and respiration rate

To determine the ethylene and respiration rate, 10 to 12 fruit were stowed inside a 5-L flask and hermetically closed for about