



## Effect of storage under extremely low oxygen on the volatile composition of 'Royal Gala' apples



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### ABSTRACT

The aim of this work was to assess the profile of volatile compounds in 'Royal Gala' apples stored under controlled atmosphere (CA), with O<sub>2</sub> levels ranging from 1.0 kPa to as low as 0.5 kPa during 8 months (0.5 °C), followed by 7 days of shelf-life at 20 °C. Volatile compounds were collected via solid-phase microextraction (HS-SPME) and analysed by gas chromatography. Straight and branched-chain esters exhibited a distinct pattern. The emission of straight-chain esters decreased under extremely low O<sub>2</sub> (0.5 kPa), while branched-chain esters were not significantly affected in such condition. 2-Methyl-butyl acetate, a significant contributor to the 'Royal Gala' aroma, was higher in intermediate O<sub>2</sub> concentration, suggesting that lowering the O<sub>2</sub> levels down to 0.7 kPa does not negatively affect the volatile composition of 'Royal Gala' apples, as compared to the standard CA (1.0 kPa O<sub>2</sub>). The remaining volatile compounds were not strongly affected by storing fruits under extremely low O<sub>2</sub>.

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### 1. Introduction

Apples are one of the most produced and consumed fruits in the world. In this production, Brazil occupied the 9th position with 1.3 million tonnes of apples produced per year (Agriannual, 2012; Fao., 2011). In Brazil, apple production remains to two cultivars, 'Gala' and 'Fuji' and its respective strains. One of the most important 'Gala' strains is the 'Royal Gala' with almost 25% of total Brazilian apple production (Agapomi., 2013).

The storage of apples in a controlled atmosphere (CA), is widely used by fruit storage companies because of the beneficial effect of CA in maintaining fruit quality and reducing the incidence of physiological disorders, both during and after storage. However, despite the positive effect of CA on the postharvest quality of apples, exposing these fruits to long-term storage under low O<sub>2</sub>, can have a negative impact on the release of volatile compounds. In fact, CA-stored apples produce significantly less volatile compounds during shelf-life than those stored under low temperature with natural air (López et al., 2007; Plotto, Mc Daniel, & Mattheis, 1999). Thus, although CA storage negatively affects fruit aroma, the fact that it efficiently maintains other quality parameters after long-term storage results in good acceptance of CA-stored fruits by consumers (López et al., 2007).

In Brazil, it is common practice to extend the apple storage under CA up to 8 or 9 months (Brackmann et al., 2013; Weber, Brackmann, Anese, Both, & Pavanello, 2013). This long storage life is due the fact that Brazil does not commonly import apples during the off season. So, this long-term storage can impact the apple volatile emission. Furthermore, it is becoming a common practice in packing houses to lower the partial pressures of O<sub>2</sub> below those conventionally used. In fact, O<sub>2</sub> levels as low as 0.5 kPa or even lower are nowadays commonly used. Lowering the partial pressure of O<sub>2</sub> to such levels has only been possible by concomitantly monitoring the minimum O<sub>2</sub> levels tolerated by fruits during the storage, as for example under dynamic CA (DCA) storage (Gasser, Eppler, Naunheim, Gabioud, & Hoehn, 2008; Prange, DeLong, Harrison, Leyte, & McLean, 2003; Watkins, 2008; Wright, DeLong, Harrison, Gunawardena, & Prange, 2010; Zanella et al., 2005). It is noteworthy, however, that in DCA, the O<sub>2</sub> levels are kept at levels that do not cause harm to fruits. In the case of apples, the storage under DCA also reduces significantly the synthesis of ethylene and, as a consequence, maintains quality parameters such as pulp firmness and peel colour, and reduces storage scald (Watkins, 2008).

Besides fruit respiration, also the presence of ethylene in an active form can affect the emission of volatile compounds. The effect of ethylene on the metabolism of fruits has been assessed by inhibiting biochemical steps of its synthesis, such as preventing the activity of the 1-aminocyclopropane-1-carboxylic acid (ACC) synthase by the application of aminoethoxyvinylglycine (AVG). It has

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been previously shown that the pre-harvest application of AVG on apples had a similar effect as CA storage on the emission of volatiles, albeit AVG-treated fruits were stored only under cold air (Bangerth, Streif, Song, & Brackmann, 1998). Thus, the study of Bangerth et al. (1998) suggests that low O<sub>2</sub> levels are not the only factor inhibiting the release of volatile compounds by fruits. In this regard, it has been reported that whereas the application of 1-methylcyclopropene (1-MCP), a potent inhibitor of ethylene action, significantly delays ripening and senescence in apples (Blankenship & Dole, 2003), it has the disadvantage of reducing the production of volatiles (Fan, Mattheis, & Buchanan, 1998; Kondo, Setha, Rudell, Buchanan, & Mattheis, 2005).

The aroma of apples results from a complex interaction between several organic compounds. Although esters are of major importance, other compounds such as alcohols, aldehydes, ketones, terpenes, among others, also significantly contribute to the characteristic aroma of apples (Mehinagic, Royer, Symoneaux, Jourjon, & Prost, 2006). Moreover, the perception of aroma is strongly influenced not only by the relative quantities, but also by the interaction of the volatile compounds present in a specific fruit (Aprea et al., 2012). In the case of cv. Royal Gala apples grown in New Zealand, it has been found that the ester 2-methyl-butyl acetate contributes strongly to the sensory properties of these fruits (Young, Gilbert, Murray, & Ball, 1996). An increase in 2-methyl-butyl acetate is associated with a “sweet” aroma, a property also influenced by the presence of hexyl acetate and butanol (Young et al., 1996). According to Echeverría, Fuentes, Graell, Lara, and López (2004), the storage of cv. Fuji apples under CA with either 1.0 or 3.0 kPa O<sub>2</sub> results in reduced concentrations of hexyl acetate. Such results suggest that at least part of the negative effect of CA on aroma may be related to a decrease in the accumulation of this volatile compound. Thus, low partial pressures of O<sub>2</sub> seem to negatively affect the emission of volatile compounds. This effect may either be directly associated with the low O<sub>2</sub> levels or indirectly because of the effect of low O<sub>2</sub> on ethylene synthesis. However, up to date there are no reports indicating the consequence of ultra-low O<sub>2</sub> levels, as for example those commonly used in DCA systems, on the composition of volatile compounds in apples stored during long term.

Against this background, the main goal of the present work was to assess the influence of ultra-low O<sub>2</sub> during CA storage on the emission or synthesis of volatile compounds in ‘Royal Gala’ apples.

## 2. Material and methods

### 2.1. Fruit harvest and storage conditions

Apples (cv. Royal Gala) were harvested on the 19th February 2011 from a commercial orchard in Vacaria, RS, Brazil. At harvest, fruits had an iodide-starch index of 6.25 (scale 1–10, where 1 = green and 10 = ripe), 0.39 μl C<sub>2</sub>H<sub>4</sub> kg<sup>-1</sup> h<sup>-1</sup> of ethylene synthesis, 5.89 ml CO<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup> of respiration rate, 4.35 mEq 100 ml<sup>-1</sup> of NaOH (0.1 N) for titratable acidity and 11.2 °Brix of soluble solids. Apples were stored in experimental CA chambers (230 l volume each) placed inside a cold chamber, where the temperature was maintained at 0.5 °C (±0.1 °C) throughout the whole experiment. The levels of O<sub>2</sub>, CO<sub>2</sub> and relative humidity in each of these chambers were monitored and controlled daily.

Fruits were stored under four different O<sub>2</sub> conditions (with three replicates each): (1) 1.0 kPa O<sub>2</sub>; 0.8 kPa O<sub>2</sub>; 0.7 kPa O<sub>2</sub> or 0.5 kPa O<sub>2</sub>. In the first day of storage, the partial pressure of O<sub>2</sub> was lowered down to 5.0 kPa by flushing the storage chamber with N<sub>2</sub>. From this day onwards, O<sub>2</sub> was consumed by fruit respiration and decreased to 1.5 kPa at the end of the first week of storage. Then, during one week, fruits were conditioned to low O<sub>2</sub> levels

by gradually decreasing the partial pressure of O<sub>2</sub> down to the level stipulated for each treatment. The atmosphere composition of each storage chamber was monitored daily with the help of a gas analyzer Ultramat 23 (Siemens, Germany). These conditions were then maintained until the end of the experiment by injecting natural air inside the store chambers, whenever the O<sub>2</sub> levels dropped below the desirable levels. The partial pressure of CO<sub>2</sub> was maintained at 1.2 kPa, and the excess was removed by circulating the atmosphere of the chamber through calcium hydroxide.

### 2.2. Sample preparation

Apples were stored during 8 months under the conditions mentioned above. At the end of the storage period, fruits were kept at 20 °C for seven days to simulate shelf-life. The production of volatile compounds was assessed during the shelf-life. A transversal slice from the equatorial part was removed from fruits previously cooled down to 0 °C (pulp temperature) and the endocarp and seeds were discharged. The whole procedure was undertaken under low temperature (approximately 5 °C), to prevent the chemical and enzymatic oxidation of samples. Then, samples were ground and centrifuged and the juices were placed inside amber glass flasks and immediately frozen to –30 °C.

### 2.3. Analysis of volatile compounds

Samples were stored during three weeks and then thawed for 24 h in a refrigerator (5 °C). An aliquot of 10 ml was taken, mixed with 3 g NaCl and 10 μl of a 3-octanol standard solution (82.2 μg ml<sup>-1</sup>) and placed inside a 20 ml vial and sealed with PTFE-coated silicone lid seals.

In order to extract volatile compounds from the headspace, samples were subjected to solid phase microextraction (HS-SPME). A Divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibre (Supelco, 50/30 μm × 20 mm) was preconditioned following the manufacturer's protocol. The vials containing the samples were submerged in a water bath at 35 °C for 5 min. Afterwards the fibre was exposed to the headspace of each sample for 60 min under constant stirring.

The volatile compounds were quantified by a Varian Star 3400CX (CA, USA) gas chromatograph equipped with a flame ionisation detector (GC-FID). The fibre was thermally desorbed into the injection port at a temperature of 250 °C for 10 min, in a splitless mode for 2 min. The compounds were separated in a polar fused silica capillary column CP-WAX 52 CB (Chrompack, USA; 60 m × 0.25 mm × 0.25 μm). Hydrogen was used as carrier gas at constant pressure (15 psi) and flow rate (1.2 ml min<sup>-1</sup>). The initial column temperature was set to 35 °C and held for 3 min. Then, a temperature gradient was started off at 2 °C min<sup>-1</sup> until 80 °C, followed by a 5 °C min<sup>-1</sup> increase until 230 °C, and maintained at isothermal conditions for 5 min. The temperature in the detector was kept at 230 °C. A series of homologous n-alkanes were analysed under the same conditions to calculate the Kovats index (KI). The concentration of volatile compounds was determined by internal standardisation according to Ban, Oyama-Okubo, Honda, Nakayama, and Moriguchi (2010) and Qin et al. (2012) methods.

Qualitative analysis of volatile compounds was carried out by a Shimadzu QP2010 Plus gas chromatography coupled to mass spectrometry (GC/MS; Shimadzu Corporation, Kyoto, Japan). For these analyses, the same chromatographic conditions described above were used and helium was the column carrier gas. The detector was operated in the electron impact ionisation mode with ionisation energy of +70 eV and a scan mass range from 35 to 350 m/z. The analytes were identified based on comparison with mass spectra available in the National Institute of Standards and Technology

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