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Effects of dynamic controlled atmosphere by respiratory quotient on some quality parameters and volatile profile of 'Royal Gala' apple after long-term storage

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ABSTRACT

The effects of dynamic controlled atmosphere (DCA) storage based on chlorophyll fluorescence (DCA-CF) and respiratory quotient (DCA-RQ) on the quality and volatile profile of 'Royal Gala' apple were evaluated. DCA storage reduces ACC (1-aminocyclopropane-1-carboxylate) oxidase activity, ethylene production and respiration rate of apples stored for 9 months at 1.0 °C plus 7 days at 20 °C, resulting in higher flesh firmness, titratable acidity and lesser physiological disorders, and provided a higher proportion of healthy fruit. Storage in a regular controlled atmosphere gave higher levels of key volatiles (butyl acetate, 2-methylbutyl acetate and hexyl acetate), as compared to fruit stored under DCA-CF, but fruit stored under DCA-RQ 1.5 and RQ 2.0 also showed higher amounts of key volatile compounds, with increment in ethanol and ethyl acetate, but far below the odour threshold. Storage in DCA-CF reduces fruit ester production, especially 2-methylbutyl acetate, which is the most important component of 'Royal Gala' apple flavour.

1. Introduction

In many countries, consumers prefer apples that have a red skin colour and there is a tendency to replace traditional apple cultivars by their mutants because of their deeper red skin coloration. Among 'Gala' mutants, 'Royal Gala' shows a significant production area, especially due to its richer red skin colour in comparison to the 'Gala' standard. This 'Gala' mutant ranges from 20 to 25% of the total Brazilian apple production. However, its harvest window is about two weeks, which is short. Therefore, a significant part of the production has to be stored.

Apples are stored under controlled atmosphere (CA) with oxygen partial pressure ranging from 1.0 up to 1.2 kPa and carbon dioxide from 2.0 to 3.0 kPa (Brackmann, Weber, Pinto, Neuwald, & Steffens, 2008). Nevertheless, even under these storage conditions, significant quality losses occur after long-term storage, which are related to the high incidence of physiological disorders (Brackmann et al., 2008), flesh firmness loss (Weber et al., 2015) and a significant reduction of volatile compounds production and emission (Bangerth, Song, & Streif, 2012; Fellmann, Rudell, Mattinson, & Mattheis, 2003; Song & Bangerth, 1996). The storage of apple in lower O_2 conditions, such as 0.3 kPa, may negatively affect the fruit, due to anaerobic metabolism, resulting in higher levels of fermentative volatiles (Lumpkin, Fellman, Rudell, & Mattheis, 2014). Thus, it is necessary to develop a technology that allows fruit storage with low physiological disorders incidence, flesh firmness loss and better volatile compounds maintenance.

During the last few years, a new trend in oxygen partial pressure monitoring has been developed and tested for apple storage under CA. This new technology, called dynamic controlled atmosphere (DCA), is based on the lowest oxygen limit (LOL) tolerated by the fruit in their metabolic stage. When monitoring the LOL, the oxygen partial pressures can be reduced in the storage rooms to the lowest limit tolerated by the fruit and changed according to the LOL throughout the storage. Nowadays, there are three methods to monitor the LOL in real time during apple storage: based on the ethanol production by fruit (Veltman, Verschoor, & Ruijsch van Dugteren, 2003), through chlorophyll fluorescence (Prange et al., 2007; Wright, DeLong, Gunawardena, & Prange, 2012) and by assessing the respiratory quotient of the fruit during storage (Bessemans, Verboven, Verlinden, & Nicolaï, 2016; Weber

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et al., 2015; Wright et al., 2012). The storage of apples under DCA significantly decreases the ACC oxidase enzyme activity, ethylene production and maintains high flesh firmness and lower physiological disorder (Weber et al., 2015). However, there are few reports regarding the DCA effect on volatile profile in comparison to CA. Brackmann, Weber, and Both (2015) found higher flesh firmness and amount of healthy fruit in apples stored under DCA-RQ, compared to those submitted to DCA-CF. Additionally, when evaluating 'Royal Gala' apple, the fruit stored in DCA-RQ maintained a similar quality to the samples submitted to DCA-CF (Weber et al., 2015).

A complex mixture of organic compounds, such as esters, alcohols, aldehydes, among others, composes the volatile profile of apple. These volatile compounds are very dynamic and can change according to the maturity stage, the cultivar, the application of 1-methylcyclopropene (1-MCP), the storage conditions, besides some other factors. Apples that have a red peel generally show a higher total ester concentration, as compared to those with low red skin coloration, which may be related to the anthocyanin content (Young, Chu, Lu, & Zhu, 2004). 'Royal Gala' apple stored under ultralow oxygen partial pressure (0.5 kPa) significantly reduced the straight-chain ester production, but branched-chain esters were not reduced by lowering oxygen (Both, Brackmann, Thewes, Ferreira, & Wagner, 2014). Another study comparing ultralow oxygen (ULO) with DCA-CF (dynamic controlled atmosphere based on chlorophyll fluorescence) found a significant ester reduction in 'Pinova' apple stored in DCA-CF compared to ULO (1.5 kPa O_2 + 1.3 kPa CO_2), but showed lower reduction in relation to ULO + 1-MCP (Raffo, Kelderer, Paoletti, & Zanella, 2009). However, there are no reports in the literature evaluating and comparing CA, DCA-CF and DCA-RQ (dynamic controlled atmosphere based on respiratory quotient) and their effects on quality and volatile profile of apples.

The aim of this paper was to evaluate the effect of DCA, based on chlorophyll fluorescence and respiratory quotient, on the quality and volatile profile of 'Royal Gala' apples after long-term storage, since there are no results in the literature evaluating the effect of DCA-RQ on the volatile profile of apples.

2. Material and methods

2.1. Fruit harvest and selection process

'Royal Gala' apples were randomly harvested in a commercial orchard in the town of Vacaria-RS, Brazil. Thereafter the fruit were transported to the Postharvest Research Center of the Federal University of Santa Maria, where selection was once again carried out, discarding any damaged fruit. Experimental samples, with 50 fruit each, were put into small experimental chambers (233 L) and different storage conditions were used. Three samples of 50 fruit were used in each treatment.

2.2. Controlled atmosphere and dynamic controlled atmosphere conditions

The experiment was composed of 4 different storage conditions: [1] controlled atmosphere with 1.2 kPa O_2 + 2.0 kPa CO_2 (CA); [2] dynamic controlled atmosphere based on chlorophyll fluorescence (DCA-CF) + 1.2 kPa CO_2; [3] dynamic controlled atmosphere based on respiratory quotient (DCA-RQ), with respiratory quotient 1.5 (DCA-RQ 1.5) + 1.2 kPa CO_2; [4] DCA-RQ 2.0 + 1.2 kPa CO_2. The DCA-CF was performed according to methodology proposed by Prange et al. (2007). DCA-RQ was based on the methodology proposed by Weber et al. (2015). Respiratory quotient (RQ) was daily calculated, by the ratio of CO_2 production through O_2 uptake, and chambers remained closed for 24 h for this evaluation. Thus, the RQ was set at 1.5 (DCA-RQ 1.5) and 2.0 (DCA-RQ 2.0) and the O_2 level was changed daily, in order to keep the RQ at the assigned value for each treatment. The treatments were composed of 3 replicates of 50 fruit each, totalling 150 fruit per treatment.

2.3. Atmosphere establishment

In the first week of storage, fruit were stored at 5 °C and then the temperature was decreased to 1 °C in one week. On the day that the temperature reached 1 °C, the oxygen partial pressure was reduced down to 5 kPa with N_2 flushing and, for one week, the oxygen was decreased to the desired condition by fruit respiration. This gradual temperature and oxygen reduction was undertaken in order to simulate the commercial conditions adopted by the CA stores. Oxygen and carbon dioxide partial pressures were monitored and corrected by an automatic CA and DCA-RQ control system (Valis®, Lajeado, RS, Brazil). The equipment compared the oxygen and carbon dioxide partial pressure at a set point. If the oxygen partial pressure was below the set point, O_2 was injected up to the set point. The same method was used for carbon dioxide correction, but generally the CO_2 was above the desired concentration and excess CO_2 in the chamber was automatically absorbed with a lime scrubber. The O_2 and CO_2 levels during storage and the calculated RQ are shown in Fig. 1. The process of oxygen monitoring and correction in DCA-CF was carried out according to Prange et al. (2007) and in DCA-RQ according to Weber et al. (2015).

2.4. Temperature and relative humidity

The storage temperature was 1.0 ± 0.1 °C. Mercury thermometers, with 0.1 °C resolution, were inserted in the apple flesh (3 apples), in order to monitor the temperature. The apple with the thermometer was allocated inside the refrigeration chamber.

Relative humidity was set at 94 ± 1% throughout the storage and monitored weekly with a psychrometer. In order to absorb the humidity, calcium chloride was placed in the experimental chamber (7.5 g kg^{-1} of fruit).

2.5. Biochemical analyses

After 9 months of storage plus 7 days of shelf life (20 ± 1 °C and relative humidity 80 ± 5%), aiming to simulate commercial practice, the fruit were submitted to quality analyses.

2.5.1. ACC oxidase enzyme activity

ACC oxidase enzyme activity was evaluated according to methodology developed by Bufler (1986). Thus, 3 g skin samples extracted from fruit equatorial region were immediately dipped into a solution containing 0.1 mol L^{-1} ACC and 10 mmol L^{-1} MES (2-(*N*-morpholino)ethanesulfonic acid) buffer at pH 6.0; after 30 min, samples were transferred to hermetic 50-mL syringes, to which 1 mL CO_2 was added. The ethylene concentration in the syringes was measured by gas chromatography (as described for ethylene in the next section) after 30 min, and the results were expressed in ng C_2H_4 kg^{-1} s^{-1}.

2.5.2. Ethylene production and respiration rate

Ethylene production was evaluated by gas chromatography. About 1.5 kg of fruit were put into a 5-L glass container and hermetically closed for 2 h. Thereafter, 2 aliquots of 1 mL were taken from the container and injected in a Varian® gas chromatograph model Star 3400CX (Varian, Palo Alto, CA) equipped with a flame ionisation detector (FID) and a Porapak N80/100 column (2 m length × ⅛″ diameter). The temperatures of the injector, column and detector were 140, 90 and 200 °C respectively. The ethylene