



# The different impacts of dynamic controlled atmosphere and controlled atmosphere storage in the quality attributes of ‘Fuji Suprema’ apples

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## ABSTRACT

The objectives of the present study were to evaluate the interaction between controlled atmosphere (CA), dynamic controlled atmosphere based on chlorophyll fluorescence (DCA – CF), and respiratory quotient (DCA – RQ 1.5 and DCA – RQ 2.0), with either immediate or delayed atmosphere establishment (30 days of delay) in the quality and volatile profile of ‘Fuji Suprema’ apple after long-term storage. Fruit stored under DCA, regardless the method, had lower ethylene production and higher flesh firmness, both at immediate and delayed atmosphere establishment. DCA – RQ resulted in lower decay incidence when the atmosphere was established immediately. Fruit stored under CA had the highest butyl acetate, 2-methylbutyl acetate and hexyl acetate, both at immediate and delayed atmosphere establishment, and its production was closely related to the ethylene and respiration rate. However, the ‘Fuji Suprema’ apple stored in DCA – RQ 2.0 had the highest total ester concentration and the highest volatile compounds that are characteristic to Fuji apples, such as ethyl 2-methyl butanoate, ethyl butanoate and ethyl hexanoate. The storage under DCA – CF resulted in the lowest production of volatile compounds by ‘Fuji Suprema’ apple.

## 1. Introduction

‘Fuji’ is one of the most important apple cultivar produced worldwide. However, during the last few years, new orchards are being installed with mutants of ‘Fuji’, like ‘Fuji Suprema’, due to its better red skin coloration (Silveira et al., 2007). Apples have a seasonal production, which makes necessary to store the fruit throughout the off-season. Nowadays, ‘Fuji Suprema’ apples are generally stored under controlled atmosphere (CA) with oxygen partial pressures ( $pO_2$ ) far above the lower oxygen limit (LOL), which is the lowest  $pO_2$  that fruit can be stored without the occurrence of undesirable quality changes.

The storage of ‘Fuji Suprema’ apples under CA results in fruit with low quality after long-term storage (8 up to 9 months), due to physiological disorders (Corrêa et al., 2010), flesh firmness reduction (Echeverría et al., 2003, 2004), acidity loss (Echeverría et al., 2004; Corrêa et al., 2010), and drastic reduction in volatile compounds (Echeverría et al., 2003, 2004; Argenta et al., 2004; Lara et al., 2006). The  $pO_2$  employed during CA storage is much higher than the LOL, which results in quality reduction. Nevertheless, in order to

decrease the  $pO_2$  until extremely low levels ( $< 0.4$  kPa) is necessary to monitor the LOL periodically throughout the entire storage period. This technology was originally proposed by Wolfe et al. (1993). Currently, there are three methodologies to monitor the fruit metabolism during the storage: 1) based on the ethanol production (Veltman et al., 2003), 2) chlorophyll fluorescence (Prange et al., 2007), and 3) respiratory quotient (Brackmann, 2015; Van Schaik et al., 2015; Weber et al., 2015).

Between these dynamic controlled atmosphere (DCA) technologies, the one based on chlorophyll fluorescence (DCA – CF) is the most widely studied around the world (Wright et al., 2010, 2012; Aubert et al., 2015; Eren et al., 2015; Thewes et al., 2015; Tran et al., 2015; Zanella and Stürz, 2015). Apple stored under DCA – CF had higher flesh firmness, greener skin coloration and higher acidity when compared to the ones stored under CA (Aubert et al., 2015; Thewes et al., 2015; Tran et al., 2015). Nevertheless, the storage under DCA – CF significantly reduced the volatile compounds production in ‘Pink Lady’ (Aubert et al., 2015), ‘Pinova’ (Raffo et al., 2009) and ‘Royal Gala’ apples (Both et al., 2017) in comparison to CA stored apples. During the last few

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years has been developed a new DCA method based on the respiratory quotient (DCA – RQ) (Brackmann et al., 2015; Weber et al., 2015; Bessemans et al., 2016), and there are still very few findings regarding this technology and the effect on quality maintenance of ‘Fuji Suprema’ apples.

The LOL monitoring by DCA – RQ is based on the ratio between CO<sub>2</sub> production and O<sub>2</sub> uptake (Brackmann, 2015; Van Schaik et al., 2015; Weber et al., 2015; Both et al., 2017; Thewes et al., 2017a,b). Thus, this method detects the LOL directly and not an event triggered by the low pO<sub>2</sub>, as in DCA – CF. The studies evaluating this technology have shown high-quality maintenance after long-term storage (Brackmann et al., 2015; Weber et al., 2015; Bessemans et al., 2016; Thewes et al., 2017a). In ‘Royal Gala’ apple, the storage under DCA – RQ resulted in fruit with higher volatile compounds in comparison to the ones stored under DCA – CF (Both et al., 2017). However, there are no studies evaluating the effect of DCA – RQ on the volatile compounds profile of ‘Fuji Suprema’ apple and its interaction with delayed atmosphere establishment.

Delayed CA establishment has been reported to reduce the physiological disorders related to CO<sub>2</sub>, especially in CO<sub>2</sub>-sensitive apple cultivars, as such ‘Fuji’ (Argenta et al., 2000; Brackmann et al., 2009), ‘Pink Lady’ (Castro et al., 2007, 2008), and ‘Empire’ apples (DeEll and Ehsani-Moghaddam, 2012). Nevertheless, the delayed CA establishment results in significant fruit softening (Argenta et al., 2000; Brackmann et al., 2009; DeEll and Ehsani-Moghaddam, 2012). Thus, it is important to evaluate the interaction between the delayed atmosphere establishment and DCA storage technologies, with lower pO<sub>2</sub>, on the apples’ overall quality maintenance and volatile compounds production after long-term storage.

In this context, the present study aimed to evaluate the interaction between CA, DCA – CF, DCA – RQ 1.5 and DCA – RQ 2.0 with either immediate or delayed atmosphere establishment (30 days of delay) on the quality and volatile profile of ‘Fuji Suprema’ apple stored during 9 months plus 7 days of shelf life at 20 °C.

## 2. Material and methods

### 2.1. Plant material

Apples of the cultivar ‘Fuji Suprema’ were harvested at a commercial orchard located in the town of São Joaquim, state of Santa Catarina, Brazil. The fruit were harvested at the commercial harvest peak for this cultivar. Immediately after, the apples were transported to the post-harvest research center, at Federal University of Santa Maria (UFMS).

The apples were sorted at the postharvest research center, where fruit with any type of mechanical lesion, due to the transportation, were eliminated. Thereafter, 3 samples of 22 fruit each were submitted to an initial analysis to estimate fruit maturity (Table 1). Afterward, the apples were randomly sampled, 24 samples of 22 fruit, with each treatment composed by 3 replications of 22 fruit. The treatments evaluated were: [1] CA with 1.0 kPa O<sub>2</sub> + < 0.5 kPa CO<sub>2</sub>; [2] DCA – CF + 0.8 kPa CO<sub>2</sub>; [3] DCA – RQ 1.5 + 0.8 kPa CO<sub>2</sub> and [4] DCA – RQ 2.0 + 0.8 kPa CO<sub>2</sub>. The fruit from all four treatments were either stored in immediate atmosphere establishment or with 30 days of delayed atmosphere establishment, totaling 8 treatments.

### 2.2. Delayed atmosphere establishment, storage temperature and relative humidity

The delay in atmosphere establishment was carried out at the temperature of –0.5 °C and 94 ± 1% in a regular atmosphere (20.9 kPa O<sub>2</sub> + 0.04 kPa CO<sub>2</sub>) during a period of 30 days before the atmosphere setup. The temperature throughout the 9 months of storage was –0.5 ± 0.1 °C and a relative humidity 94 ± 1%. A thermometer inserted in pulp and psychrometer were used to monitor the temperature and relative humidity, respectively.

### 2.3. Dynamic controlled atmosphere monitored by chlorophyll fluorescence (DCA – CF)

In order to monitor the LOL by chlorophyll fluorescence (CF), two samples of 6 fruit were put into two baskets with a CF sensor on its upper side. These baskets were allocated inside the DCA – CF chamber to monitor the LOL in real time throughout the storage. During the storage, the CF was monitored every hour and the oxygen partial pressure changed according to the method proposed by Prange et al. (2007).

### 2.4. Dynamic controlled atmosphere monitored by respiratory quotient (DCA – RQ)

The RQ is the ratio between the CO<sub>2</sub> production by O<sub>2</sub> uptake, and it is near 1.0 in aerobic conditions. Nevertheless, if the pO<sub>2</sub> is lowered below the anaerobic compensation point the RQ level rises above 1.0 (Brackmann, 2015; Van Schaik et al., 2015; Bessemans et al., 2016). In the present study, two RQ levels were evaluated: DCA – RQ 1.5 and DCA – RQ 2.0. The variation of oxygen according to the two RQ levels are showed in Supplementary Fig. S1. The LOL was monitored by the respiratory quotient (RQ) every day by an automatic DCA control system (Valis®, Lajeado, RS, Brazil).

### 2.5. CA and DCA setup and maintenance

The CA and DCA storage conditions were obtained by flushing the chamber with nitrogen until the O<sub>2</sub> pressure was decreased to 5.0 kPa and thereafter gradually reduced to the set point by fruit respiration, which took approximately 5 days after chamber flushing with nitrogen. The target carbon dioxide partial pressure was obtained by fruit respiration. During the 9 months of storage the pO<sub>2</sub> and pCO<sub>2</sub> were determined and corrected 4 times every day with an automatic DCA control system (Valis®, Lajeado, RS, Brazil). Thereby, the automatic DCA control system compared the pO<sub>2</sub> and pCO<sub>2</sub> of the chamber to a set point. If the pO<sub>2</sub> was lower than the set point, cold air was injected until the desired concentration and the excess of CO<sub>2</sub> was absorbed with a lime scrubber.

### 2.6. ACC oxidase enzyme activity, ethylene production and respiration rate

The ACC oxidase enzyme activity was indirectly determined by the ethylene production of a sample of fruit peel, according to the methodology proposed by Bufler (1986), one day after harvest and after 9 months of storage plus 7 days of shelf life at 20 °C. The ethylene production rate was determined at chamber opening (ethylene 0) and after 7 days of shelf life at 20 °C (ethylene 7) by stowing of 1.5 kg sample in a 5-L container, which was hermetically closed for about one hour. Thereafter, 2 samples of 1 mL of the headspace were taken from the container and injected into a gas chromatograph Star CX 3400 (Varian, Palo Alto, CA, USA), equipped with a flame ionization detector to determine the ethylene concentration in the container headspace. The temperature of the injector, column and detector were: 140, 90 and 200 °C, respectively. The ethylene concentration was positively quantified by a standard gas injection in the same chromatographic condition described above. The respiration rate was evaluated at chamber opening (respiration 0) and after 7 days of shelf life at 20 °C (respiration 7) by the CO<sub>2</sub> accumulated in the same container used to assess the ethylene production, with an electronic gas analyzer (Isolcell®, Italy).

### 2.7. Decay incidence, flesh breakdown, healthy fruit and flesh firmness

These variables were evaluated after 9 months of storage plus 7 days of shelf life at 20 °C. Decay incidence and flesh breakdown were determined by counting the fruit that presented these disorders in relation to the total amount of fruit from each sample. The healthy fruit

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