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The influence of temperature and 1-MCP on quality attributes of 'Galaxy' apples stored in controlled atmosphere and dynamic controlled atmosphere



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ABSTRACT

Controlled atmosphere (CA) associated with 1-methylcyclopropene (1-MCP) or dynamic controlled atmosphere (DCA) with lower oxygen partial pressures (pO_2), are strategies to long-term storage of apples (*Malus x domestica* Borkh). However, the effect of 1-MCP application in DCA stored fruit and the storage temperature adopted in this condition still need to be determined. The storage temperature increase is desired, aiming to reduce energy consumption. 'Galaxy' apples were stored for 9 months at 1.0, 1.5 and 2.0 °C, in either CA or DCA monitored by respiratory quotient of 1.5 (DCA-RQ). Another set of fruit that received 1-MCP application was also stored in each condition. DCA-RQ drastically reduces the metabolism, allowing the storage at higher temperatures, even without 1-MCP application, with lower physiological disorders, higher healthy fruit amount and retention of flesh firmness and acidity. The quality maintenance after long-term apple storage in CA is only feasible associated to 1-MCP treatment.

1. Introduction

'Galaxy' apple is a natural red-fruit mutation of 'Royal Gala' and is well appreciated by consumers for their intense red skin color, covering the entire fruit epidermis. In southern states Brazil, which is the main production region, the harvest window comprises few weeks in February. In order to supply this apple cultivar and other 'Gala' mutants to the market throughout the year, it is necessary to use an efficient storage method to keep the quality for long-term.

Controlled atmosphere (CA), which comprise in reducing the pO_2 and increasing the CO_2 in the storage room, is a technique worldwide adopted for apple storage. However, for 6–8 months of storage, it is linked to 1-methylcyclopropene (1-MCP) application (Thewes, Both, Brackmann, Ferreira, & Wagner, 2015a; Watkins, 2006). 'Gala' mutants can be stored in CA with pO_2 ranging from 0.8 up to 1.0 kPa and 2.5 kPa CO_2 , with temperature of 0.5 to 1.0 °C (Brackmann, Weber, Pinto, Neuwald, & Steffens, 2008).

1-Methylcyclopropene (1-MCP) application suppresses ethylene action and reduces quality losses in CA storage (Thewes et al., 2015a; Watkins & Nock, 2012). However, it has the weakness to decreases the ester production, responsible for apple aroma (Thewes et al., 2015a) and have restrictions in organic apple production (Rebeaud & Gasser, 2015). 1-MCP application combined with CA is a strategy aiming

energy saving, with elevated storage temperatures. The storage in ultralow oxygen (ULO) with $1.2 \text{ kPa O}_2 + 2.5 \text{ kPa CO}_2$ at 5 °C + 1-MCP, compared to ULO at 1 °C reduced the energy consumption by 70%, without negative influence on apple quality (Kittemann, McCormick, & Neuwald, 2015). According to East, Smile, and Trujillo (2013), shifting the refrigeration of chambers to off-peak periods resulted in less than 0.5 °C in average fruit temperature oscillation and saved around 40% of energy in CA storage. Dynamic controlled atmosphere based on chlorophyll fluorescence (DCA-CF) is a storage method that allow the pO₂ stepwise decrease until a stress is detected by a sensor inserted in the chamber. The sensor capture the fluorescence emitted by chlorophyll of the fruit skin, in response to a stress caused by low oxygen. Then, the pO_2 is increased in 0.2 kPa or, at a minimum, to 0.4 kPa and is monitored for the entire storage period. DCA-CF combined or not with 1-MCP, could be used with elevated storage temperatures (2°C to 3,5–10 °C), without quality losses (Köpcke, 2015). Nevertheless, there is no information regarding the use of higher storage temperatures in DCA-RQ storage yet.

The concept of DCA storage is built on the possibility to lowering the pO_2 in the storage rooms at the lower oxygen limit (LOL). It is only possible if the onset of anaerobic metabolism may be detected during oxygen reduction. Ethanol production was used to detect and establishes the lowest possible O_2 concentration in 'Elstar' apple (Veltman,

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Verschoor, & Ruijsch van Dugteren, 2003). Also, DCA-CF is worldwide used to monitor LOL and establish the pO₂ according to fruit response (Köpcke, 2015; Thewes, Both, Brackmann, Weber, & Anese, 2015b). Currently, there are some reports of DCA-CF on 'Greenstar' (Tran, Verlinden, Hertog, & Nicolai, 2015), 'Elstar', 'Jonagold' and 'Gloster' apples storage at higher temperatures (Köpcke, 2015). On the other hand, DCA-CF decreased volatile production in 'Royal Gala' (Both et al., 2017) and showed the same quality maintenance as ultralow oxygen (ULO) storage in 'Gala' mutants (Thewes et al., 2015b). However, DCA may also be monitored by respiratory quotient (DCA-RQ), which is the ratio between CO₂ production and O₂ uptake, measured during a determined period (12-24h) of storage. DCA-RO reduced superficial scald, maintained flesh firmness and skin color in 'Granny Smith' apple in the same manner as CA + 1-MCP (Bessemans, Verboven, Verlinden, & Nicolaï, 2016), retained quality (Weber et al., 2015) and volatile production (Both et al., 2017) in 'Royal Gala' apples. In 'Galaxy' apple, DCA-RQ maintains higher quality than DCA-CF or CA (Brackmann, Weber, & Both, 2015) and similar as CA + 1-MCP, in apple harvested at three maturity stages (Thewes et al., 2017). Recent studies have found that the best RQ level for 'Gala' mutants ranged from 1.3, 1.5 and not above 2.0 (Both et al., 2017; Brackmann et al., 2015; Thewes et al., 2017; Weber et al., 2015). Nevertheless, these studies with Gala mutants were performed in temperatures recommended for CA storage and there are no studies with higher storage temperature in such storage conditions.

The possibility of monitoring the LOL tolerated by the fruit during storage in DCA may be a reliable tool to increase the storage temperature while still maintaining low metabolic activities of the fruit. In DCA monitored by chlorophyll fluorescence, Köpcke (2015) found that the pO_2 need to be of 0.6 kPa at 2.0 °C, 0.7 at 3.5 °C and 0.8 kPa at 8.5 °C, because the fruit metabolism increases, as storage temperatures increase. Moreover, in this study was adopted 0.2 kPa O_2 above the anaerobic compensation point (as a response of chlorophyll fluorescence), in order to avoid anaerobic metabolism stress. Experimental research also reported a decrease of heat production of the fruit by the lower respiration in the storage monitored by respiratory quotient, resulting in lower cooling energy requirement (Van Schaik, Van de Geijn, Verschoor, & Veltman, 2015).

In this context, the main goal of the present study was to evaluate the possibility to adopt more elevated storage temperature combined with dynamic controlled atmosphere monitored by respiratory quotient, on quality maintenance of 'Galaxy' apple after long-term storage. We also aim to evaluate the need of 1-MCP application in different storage methods and temperatures.

2. Material and methods

2.1. Fruit harvest, selection and sample preparation

'Galaxy' apples were harvested from a commercial orchard located in Vacaria – RS, a traditional apple production area in the south of Brazil. Fruit were picked on the same day that growers harvested it to commercial storage and, at this time, fruit showed a 6.95 starch index (1: unripe and 10: overripe), 78.6 Newton (N) flesh firmness, $5.22 \text{ meq } 100 \text{ mL}^{-1}$ titratable acidity, respiration rate of $9.77 \,\mu g \, kg^{-1} \, s^{-1}$ and ethylene production of $0.35 \, ng \, kg^{-1} \, s^{-1}$. The storage and quality analyses were performed at the Postharvest Research Center of the Federal University of Santa Maria – RS, Brazil.

2.2. Treatments evaluated

Apple samples (four replications of 40 fruit per treatment) were put into hermetically closed experimental chambers with the volume of 0.23 m^3 (1 m × 0.58 m × 0.40 m), made of galvanized iron, in which it was applied the storage conditions. These chambers were put inside different storage rooms (40 m³), each with a given temperature

according the following treatments: [1] Controlled atmosphere (CA) with 1.2 kPa $O_2 + 2.0$ kPa CO_2 ; [2] Dynamic controlled atmosphere monitored by respiratory quotient of 1.5 (DCA-RQ), in which the pO_2 was changed according to the fruit metabolism, and 1.2 kPa CO_2 . For each of these storage conditions, three temperatures were evaluated: 1.0 °C; 1.5 °C and 2.0 °C (\pm 0.1 °C). Also for each storage condition and temperature, four replicates received 1-methylcyclopropene (1-MCP) treatment and other four were stored without 1-MCP. Temperature was controlled by thermostats and monitored daily by Hg bulb thermometers (with a 0.1 °C resolution), inserted in the apple flesh and allocated inside each of the three rooms.

2.3. Atmosphere establishment and monitoring

During five days, fruit remained at 5.0 °C in air and, thereafter, the O₂ was reduced to 5.0 kPa in each experimental chamber, by N₂ flushing. The temperatures were set according to the treatments, in three different storage rooms (40 m³). During additional five days, the pO2 was reduced stepwise to 1.2 kPa for CA storage, and to 0.5 kPa for DCA, at the time which the respiratory quotient (RQ) determination started. The target level of CO₂ was obtained by fruit respiration. Oxygen and carbon dioxide partial pressures were daily monitored with a gas analyzer (Siemens[®], model Ultramat, Germany) and corrected by an automatic CA and DCA-RQ control system (Valis Automação TI[©], Lajeado, RS, Brazil). Each experimental chamber was connected to the device by two hoses and the air was circulated using a pump. The equipment compared the O2 and CO2 partial pressure to a set point. If the pO_2 was below the set point, O_2 was injected up to the set point and when the CO₂ was above the desired concentration, the excess CO₂ in the chamber was automatically absorbed with a lime scrubber.

CA establishment and DCA-RQ setup was performed in each experimental chamber (0.23 m³). The relative humidity was set at 94 \pm 1% inside these chambers and monitored with the aid of a psychrometer. To avoid excessive relative humidity, calcium chloride (8–10 g kg⁻¹ fruit) was allocated in trays inside the chambers, in which the air of the experimental chamber circulated by a fan allocated inside, at least 4 times per day (4–8 min).

2.4. Respiratory quotient

The respiratory quotient (RQ) of 1.5 was chosen based on previous studies (Both et al., 2017; Brackmann et al., 2015; Thewes et al., 2017; Weber et al., 2015). Every two days, during the entire storage period, the CO₂ scrubbing and O₂ injection of experimental chambers was disabled in the control system (aiming to prevent CO₂ scrubbing and O₂ injection) during 14 h. During this time-lapse, the O₂ and CO₂ partial pressures were recorded and, from the ratio of CO₂ production by O₂ uptake in this period, the RQ was calculated. The set point of the O₂ remained the same if the RQ resulted in 1.5 ± 0.1, however with RQ below or above 1.5, the O₂ set point was slightly decreased and increased, respectively, aiming to obtain the desired RQ level in the next determination. This procedure was performed with experimental chamber in the three temperatures evaluated.

In this manner, the pO_2 was changed during the entire storage period allowing keeping the RQ at 1.5, lowering the fruit metabolism and without risks of disorders caused by excessive anaerobic respiration. During 9 months of storage, the mean of O_2 in each experimental chamber was 0.21, 0.19 and 0.24 kPa in 1.0, 1.5 and 2.0 °C, respectively, and O_2 and CO_2 oscillation and RQ levels are shown in supplementary Fig. 1.

2.5. 1-Methylcyclopropene treatment

1-MCP treatment was performed at the beginning of storage, at 5.0 °C. Fruit samples were inserted in experimental chambers with the volume of 0.23 m^3 and 1-MCP (SmartFresh^{*}, 0.14% of active

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