

'Royal Gala' apple quality stored under ultralow oxygen concentration and low temperature conditions

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Abstract – The objective of this work was to evaluate the interaction of ultralow oxygen concentrations (ULO) with storage temperatures and carbon dioxide partial pressures and its influence on fruit quality preservation and on the occurrence of physiological disorders in 'Royal Gala' apples. The experiment was carried out in a completely randomized design, with four replicates 25-fruit. ULO conditions (1.0 kPa O₂ + 2.0 kPa CO₂; 0.8 kPa O₂ + 1.5 kPa CO₂; 0.8 kPa O₂ + 1.0 kPa CO₂; 0.6 kPa O₂ + 1.5 kPa CO₂; and 0.6 kPa O₂ + 1.0 kPa CO₂) were tested at 0, 0.5 and 1.0°C, in a 5x3 factorial arrangement. Fruit quality and ripening analyses were performed after eight-month storage plus seven days of shelf-life at 20°C. Oxygen partial pressures below 0.8 kPa increased the occurrence of internal breakdown and mealiness. The best ULO condition was 1.0 kPa O₂ + plus 2.0 kPa CO₂ at 1.0°C. The interaction of ULO conditions and storage temperatures shows the need of increasing O₂ partial pressure at higher storage temperatures.

Index terms: *Malus domestica*, carbon dioxide, controlled atmosphere, decay, physiological disorders.

Qualidade de maçã 'Royal Gala' armazenada em condições de concentração ultrabaixa de oxigênio e baixa temperatura

Resumo – O objetivo deste trabalho foi avaliar a interação de concentrações ultrabaixas de oxigênio (ULO) com diferentes temperaturas e pressões parciais de gás carbônico sobre a conservação da qualidade e a ocorrência de distúrbios fisiológicos em maçãs 'Royal Gala'. O experimento foi realizado em delineamento inteiramente casualizado, com quatro repetições de 25 frutos. As concentrações ULO (1,0 kPa O₂ + 2,0 kPa CO₂, 0,8 kPa O₂ + 1,5 kPa CO₂, 0,8 kPa O₂ + 1,0 kPa CO₂, 0,6 kPa O₂ + 1,5 kPa CO₂ e 0,6 kPa O₂ + 1,0 kPa CO₂) foram avaliadas a 0,0, 0,5 e 1,0°C, em arranjo fatorial de 5x3. As análises da qualidade e amadurecimento dos frutos foram realizadas após oito meses de armazenamento e mais sete dias de exposição a 20°C. As pressões parciais de O₂ abaixo de 0,8 kPa aumentaram a ocorrência de degenerescência de polpa e polpa farinácea. A melhor condição de ULO foi a de 1,0 kPa O₂ + 2,0 kPa CO₂ a 1,0°C. A interação entre condições de ULO e temperaturas de armazenamento evidencia necessidade de aumento das pressões parciais de O₂ quando a temperatura de armazenamento é mais elevada.

Termos para indexação: *Malus domestica*, gás carbônico, atmosfera controlada, podridões, distúrbios fisiológicos.

Introduction

Controlled-atmosphere storage extend the postharvest life of apple fruit. The system combines low temperatures with low partial pressures of O₂ and high partial pressure of CO₂, which reduce respiration rates, ethylene (C₂H₄) biosynthesis and action (Yang & Hoffman, 1984), thereby maintaining the physical and chemical properties of fruit. However, there are still significant postharvest losses of fruit due to pathogens and physiological disorders, which may reach up to 60% depending on the year (Brackmann

et al., 2002). Therefore, it has become necessary to improve the currently used storage techniques to reduce fruit loss.

Partial pressures of O₂ and CO₂ recommended for the storage of 'Royal Gala' apples in Brazil are 1.0 and 2.5 kPa, respectively (Brackmann et al., 2008). It is well documented that low levels of O₂ in controlled-atmosphere storage reduce fruit respiration and ethylene production and action. Generally, the lower the metabolic rates, the longer the postharvest life and quality of apples. Therefore, studies have been conducted to define the use of ultralow oxygen

partial pressures (Zanella, 2003), to suppress fruit metabolism, and to obtain higher fruit quality by reducing losses.

Under controlled-atmosphere storage, the oxygen partial pressure cannot fall below the minimum limit needed for aerobic energy production. Below this minimum limit, also known as the anaerobic compensation point, the anaerobic respiration becomes the major energy production pathway (Saquet & Streif, 2008). This change in metabolism may cause the accumulation of volatile substances, like ethanol and acetaldehyde, and may ultimately result in the development of physiological disorders such as flesh breakdown (Pedreschi et al., 2009). The lowest oxygen limit may vary among fruit of the same species (Prange et al., 2003), depending on the region, the year of production (Delong et al., 2004), and on the storage temperature (Wright et al., 2010). Therefore, ultralow oxygen must be studied before it is applied commercially for 'Royal Gala' apples.

The lower oxygen limits recommended for the 'Delicious', 'Law Rome' and 'McIntosh' apples are 0.7 kPa, 0.9 kPa and 1.9 kPa, respectively (Gran & Beaudry, 1993). 'Granny Smith' apples stored under O_2 partial pressures of 0.7 kPa, from four to six months, showed a ground color identical to the one they had at harvest time, according to Zanella (2003), who also reported a lower occurrence of flesh breakdown, better retention of flesh firmness and total absence of scald in apples stored under ultralow oxygen conditions. For apples cultivated in Brazil, Brackmann et al. (2000) reported that the optimum storage conditions for 'Gala' apples were 0.75 to 1.0 kPa O_2 , at 1.0°C. However, Ceretta et al. (2010) reported, for the same cultivar stored at 0°C and ≤ 0.8 kPa O_2 conditions, an increase of skin cracking occurrences, internal breakdown and decay compared to fruit stored under 1.0 kPa O_2 . Discrepancy between these reports is probably due to the distinct temperatures employed in each study, which shows that there might be a relationship between storage temperature and O_2 levels in the prolonged maintenance of apple quality.

The objective of this work was to evaluate the interaction of ultralow oxygen partial pressure with different temperatures and carbon dioxide partial pressures and its influence on fruit quality preservation and on the occurrence of physiological disorders in 'Royal Gala' apples.

Materials and Methods

The experiment was carried out at the Núcleo de Pesquisa em Pós-colheita, Departamento de Fitotecnia, Universidade Federal de Santa Maria, Santa Maria, RS, Brazil, in 2008. Fruit samples were harvested in a commercial orchard, in Vacaria county, RS, Brazil. At harvest, fruit showed 6.70 starch index (scale 0, minimum; 10, maximum starch hydrolysis), 92.8 N flesh firmness, and 21.8 $\eta L g^{-1} h^{-1}$ C_2H_4 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase activity, and produced 1.33 $\mu L kg^{-1} h^{-1}$ C_2H_4 and 6.86 $mL kg^{-1} h^{-1}$ CO_2 (respiration rate).

The experiment was conducted in a completely randomized design, with four replicates of 25 fruit. Five ultralow oxygen conditions were tested at three different temperatures, in a factorial arrangement (5x3). The controlled-atmosphere storage conditions were: 1.0 kPa O_2 + 2.0 kPa CO_2 ; 0.8 kPa O_2 + 1.5 kPa CO_2 ; 0.8 kPa O_2 + 1.0 kPa CO_2 ; 0.6 kPa O_2 + 1.5 kPa CO_2 ; and 0.6 kPa O_2 + 1.0 kPa CO_2 . All these conditions were tested at 0, 0.5 and 1.0°C.

The fruit were stored in hermetically sealed experimental chambers with 0.232 m^3 , which were placed inside a cold storage room with 48 m^3 each, at 0, 0.5 and 1.0°C (oscillation of $\pm 0.1^\circ C$). The relative humidity inside the chambers was maintained at $96 \pm 1\%$. Temperature was controlled by thermostats and checked daily by bulb mercury thermometers with a 0.1°C resolution inserted in the fruit flesh. The dilution of O_2 in the storage chamber was done with injections of N_2 obtained from a pressure swing adsorption (PSA) nitrogen generator (Janus & Pergher, Porto Alegre, RS, Brazil). Partial pressures of CO_2 were obtained by gas injection from a high pressure cylinder. The desired partial pressures were maintained by automatic gas control equipment (Kronenberger/Climasul, Caxias do Sul, RS, Brazil). The O_2 consumed by respiration was replaced by injections of atmospheric air into the chambers, and excessive CO_2 produced by respiration was absorbed by a 40% potassium hydroxide solution.

Fruit quality and ripening parameters were evaluated after eight-month storage, plus seven days of shelf-life at 20°C, except for respiration, which was assessed at the removal from the storage chambers and after the six days of air-exposure at 20°C. The parameters decay occurrence, flesh firmness, mealiness, internal breakdown, ACC oxidase activity, ethylene production

rate and respiration rate were assessed and detailed as follows.

Decay occurrence was evaluated by counting fruit showing typical fungal lesions larger than 5 mm in diameter.

Flesh firmness was determined with a penetrometer model FT 327 (Effegi Systems, Milan, Italy), equipped with a 11 mm probe and measured in both sides of fruit equatorial region, from which the skin had been previously removed.

Mealiness was determined by visual quantification of fruit exhibiting symptoms related to this physiological disorder (dry and non-juicy flesh).

Internal breakdown was measured by visual evaluation of flesh browning, after transversal cuts across fruit.

ACC oxidase activity was determined in 3 g skin samples extracted from fruit equatorial region, from each experimental unit; these samples were immediately dipped into a solution containing 0.1 mol L⁻¹ ACC and 10 mmol L⁻¹ MES buffer [2-(N-morpholino) ethanesulfonic acid] at pH 6.0; after 30 min, samples were transferred to hermetic 50 mL syringes, to which 1 mL CO₂ was added. The ethylene concentration in the syringes was measured by gas chromatography after 30 min, and the results were expressed as $\eta\text{L g}^{-1} \text{h}^{-1} \text{C}_2\text{H}_4$ (Bufler, 1986). To analyze ethylene concentration, two gas samples of 1 mL extracted from the headspace of each syringes were injected in a gas chromatograph, Varian Gas Chromatograph Star CX 3400 model, (Varian, Palo Alto, CA, USA), equipped with a flame ionization detector (FID) and a Porapak N80/100 steel column. The column, injector and detector temperatures were 90, 140 and 200°C, respectively.

Ethylene production rate was determined by the use of approximately 1,200 g fruit which were placed into containers with 5,000 mL volume. These containers were hermetically sealed for approximately two hours. Ethylene synthesis, expressed as $\mu\text{L kg}^{-1} \text{h}^{-1} \text{C}_2\text{H}_4$, measured by gas chromatography, was calculated by taking into account the ethylene concentration, the mass of fruit, the free space inside the container, and time.

Respiration rate was determined by the volume of CO₂ production. The air from the same container, used to determine the production of ethylene, was circulated through an electronic CO₂ analyzer, with the infrared gas analyzer (IRGA) system (Agri-datalog, Lana, BZ,

Italy). Based on CO₂ concentration, free space inside the container, fruit weight and closure time, the respiration rate was calculated and expressed as mL kg⁻¹ h⁻¹ CO₂.

Before the analysis of variance, the results were tested for normality and homogeneity of errors by the Lilliefors and Bartlett tests, respectively. The parameters that fell outside the normality of errors were transformed by the formula arc sen square root $(x + 0.5)/100$, while the parameters with heteroscedasticity errors were transformed by the formula $\log_{10}(x + 1.0)$ to be returned to the group and then submitted to ANOVA tests. Average values were compared by Tukey's test, at 5% probability.

Results and Discussion

Fruit stored under 0.8 kPa O₂ + 1.5 kPa CO₂ conditions and exposed for seven days at 20°C showed the lowest occurrence of decay, independently of the storage temperature (Table 1). Low O₂ partial pressures during the storage reduced the occurrence of decay during the post-storage period, possibly due to the fungistatic effect of low O₂ concentration (Neuwald, 2004). Cellular energy production was likely induced by anaerobic respiration under 0.6 kPa O₂. As a result, cells were damaged, probably due to accumulation of fermentation products, and became sensitive to infections (Kader, 2002). Ceretta et al. (2010) also reported a higher decay occurrence in 'Gala' apples stored at O₂ partial pressures below 0.8 kPa. This partial pressure of O₂ (0.8 kPa) also increased decay susceptibility in 'Fuji' apples (Brackmann et al., 2001).

Flesh firmness was not significantly affected by the different ultralow oxygen and CO₂ conditions (Table 1). Storage temperatures of 0.5 and 1.0°C kept higher flesh firmness, in comparison to 0°C. Brackmann et al. (2008) reported that 'Royal Gala' and 'Galaxy' apples showed greater flesh firmness at 0.5°C in comparison to -0.5°C. The loss of flesh firmness is usually linked to rates of respiration and ethylene production (Hiwasa, et al., 2003). In the present study, it was observed that after a six-day exposure to air at 20°C, fruit stored at 0°C showed higher rates of respiration and ethylene production than fruit stored at 0.5 and 1.0°C (Table 2). It has been reported that increased ethylene synthesis induces the expression and the activity of cell-wall degrading enzymes (Prasanna et al., 2007) resulting in loss of firmness.

The occurrence of mealiness had no interaction with ultralow oxygen conditions and temperatures (Table 1). There was a lower occurrence of this disorder at 1.0°C storage temperature. However, the storage of apples at 0.8 kPa O₂ with 2.0 or 1.5 kPa CO₂ resulted in a higher occurrence of mealiness. This physiological disorder is associated with ripening, when protopectin degradation is enhanced (Prasanna et al., 2007), reducing the cell-to-cell adhesion and, ultimately, leading to the development of a mealy aspect. The activity of cell-wall degrading enzymes is highly correlated with ethylene biosynthesis and respiration rate (Wei et al., 2010).

There was a significant interaction between storage temperatures and ultralow oxygen conditions for flesh breakdown (Table 1). The temperature increase from 0 to 1.0°C decreased the occurrence of this disorder, especially in higher O₂ partial pressure. According to Streif et al. (2003), many postharvest factors affect the occurrence of flesh breakdown in apples, such as harvest date, time between harvest and cooling, CO₂ and O₂ partial pressures, and storage interval and storage temperature. Flesh breakdown occurrence was lower at 1.0°C with the highest O₂ levels (0.8 and

1.0 kPa O₂ associated with 2.0 kPa CO₂). However, no statistic difference in the treatment 0.8 kPa O₂ + 1.5 kPa CO₂ was observed. At 0°C there was a lower occurrence of the disorder in conditions with a lower O₂ level of (0.6 kPa O₂ + 1.0 kPa CO₂), although not differing from the conditions with 0.6 and 0.8 kPa O₂ associated to 1.5 kPa CO₂. The interaction between temperature and oxygen partial pressure resulted in the need to increasing O₂ partial pressures as temperature was increased from 0 to 1.0°C. A possible explanation to this result would be that the solubility of O₂ inside the apple flesh decreases as the temperature rises (Yearsley et al., 1997). Consequently, if a lower solubility limits the O₂ availability, it is expected that the minimum oxygen limit increases as a function of storage temperature rise. Similar results were reported by Wright et al. (2010) for 'Honeycrisp' apple. Thus, a higher occurrence of this disorder occurs probably due to a higher anaerobic respiration observed in fruit submitted to lower O₂ partial pressure. The final products of this reaction, namely ethanol and acetaldehyde, and the imbalance between oxidative and reductive processes cause an energetic collapse

Table 1. Occurrence of decay, flesh firmness, mealiness and flesh breakdown in 'Royal Gala' apples, after eight months of storage at different conditions of controlled-atmosphere and temperature and an added exposition for seven days at 20°C⁽¹⁾.

O ₂ + CO ₂ (kPa)	Decay (%)			Mean	Flesh firmness (N)			Mean	Mealiness (%)			Mean	Flesh breakdown (%)			Mean
	0°C	0.5°C	1.0°C		0°C	0.5°C	1.0°C		0°C	0.5°C	1.0°C		0°C	0.5°C	1.0°C	
Initial analysis ⁽²⁾	0.0				92.8				0.0				0.0			
1.0 + 2.0	14.0	8.8	7.5	10.1ab	76.6aA	75.4aA	76.4aA	76.2	26.7	30.3	16.3	24.4b	32.9aA	17.7abB	5.0cC	18.5
0.8 + 2.0	10.0	12.7	7.6	10.1ab	70.5bB	77.5aA	77.5aA	75.2	42.5	38.0	17.8	32.8a	36.3aA	17.8abB	8.9cC	21.0
0.8 + 1.5	7.6	8.9	6.3	7.6b	73.2abB	79.3aA	75.8aAB	76.1	34.1	29.1	20.3	27.9ab	29.0abA	13.9bB	10.1bcB	17.7
0.6 + 1.5	10.5	14.3	16.4	13.7a	75.1aA	77.7aA	77.3aA	76.7	28.6	29.4	11.4	23.2b	27.2abA	14.0bAB	19.1abB	20.1
0.6 + 1.0	8.7	12.5	11.4	10.9ab	73.5abA	76.3aA	76.8aA	75.5	27.5	32.5	17.8	25.9b	25.0bA	27.5aA	22.8aA	25.1
Mean	10.2A	11.4A	9.8A	-	73.8	77.2	76.8	-	31.9A	31.9A	16.7B	-	30.1	18.2	13.2	-

⁽¹⁾Means followed by equal letters, lowercase in the columns and uppercase in the lines, do not differ by Tukey's test, at 5% probability. ⁽²⁾Initial analysis took place before fruit storage.

Table 2. ACC oxidase activity ($\eta\text{L g}^{-1} \text{h}^{-1} \text{C}_2\text{H}_4$), ethylene production ($\mu\text{L kg}^{-1} \text{h}^{-1} \text{C}_2\text{H}_4$) and respiration rate ($\text{mL kg}^{-1} \text{h}^{-1} \text{CO}_2$) of 'Royal Gala' apples, after eight months at different conditions of controlled-atmosphere and temperature⁽¹⁾.

O ₂ + CO ₂ (kPa)	ACC oxidase activity			Mean	Ethylene production rate			Mean	Respiration rate chamber opening			Mean	Respiration rate six days at 20°C			Mean
	0°C	0.5°C	1.0°C		0°C	0.5°C	1.0°C		0°C	0.5°C	1.0°C		0°C	0.5°C	1.0°C	
Initial analysis ⁽²⁾	21.8				1.33				6.86				6.86			
1.0 + 2.0	43.0aA	44.8aA	23.3bB	37.0	0.26aA	0.16abB	0.18aB	0.20	5.36bAB	5.54aA	4.68aB	5.19	8.89	5.71	5.78	6.80a
0.8 + 2.0	42.5aA	29.7bB	19.3bC	30.5	0.25aA	0.19abB	0.20aB	0.21	5.91abA	5.44aA	4.51aB	5.28	8.09	6.04	6.31	6.82a
0.8 + 1.5	37.3aA	33.8abAB	25.4abB	32.2	0.19bA	0.15bA	0.19aA	0.18	5.25bA	5.24aA	4.55aA	5.01	7.40	5.19	5.86	6.15a
0.6 + 1.5	22.1bA	25.0bA	20.9bA	22.7	0.16bAB	0.14bB	0.20aA	0.17	5.47bA	4.05bB	5.11aA	4.88	7.26	5.26	6.28	6.27a
0.6 + 1.0	33.3abA	29.5bA	35.1aA	32.7	0.19bA	0.21aA	0.17aA	0.19	6.60aA	5.77aB	4.88aC	5.75	7.60	7.08	6.03	6.90a
Mean	35.7	32.6	24.8	-	0.21	0.17	0.19	-	5.72	5.21	4.74	-	7.85A	5.86B	6.05B	-

⁽¹⁾Means followed by equal letters, lowercase in the columns and uppercase in the lines, do not differ by Tukey's test, at 5% probability. ⁽²⁾Initial analysis took place before fruit storage.

and loss of membrane integrity, resulting in flesh breakdown (Franck et al., 2007).

As to ACC oxidase activity, partial pressures of O₂ and CO₂ showed contrasting effects at different storage temperatures (Table 2). At 0°C, the storage of fruit at 0.6 kPa O₂ + 1.5 kPa CO₂ resulted in a reduction in ACC activity, although not differing from the conditions with 0.6 kPa O₂ + 1.0 kPa CO₂. At 0.5°C, however, the controlled-atmosphere storage with 0.6 kPa O₂ + 1.5 kPa CO₂ caused an increasing effect by inhibiting the ACC oxidase activity, but a difference with 0.8 kPa O₂ + 1.5 or 2.0 kPa CO₂ condition was not observed. Thus, when storage temperature was 0.5°C, a higher O₂ partial pressure (0.8 kPa) was required to supply the enzyme activity, due to the lower O₂ solubility at higher temperatures (Yearsley et al., 1997). Comparing the temperatures, under conditions where O₂ partial pressure were maintained at 0.8 or 1.0 kPa, associated to 2.0 kPa CO₂, the lowest ACC oxidase activity was measured in fruit stored at 1.0°C. The ACC oxidase, ethylene production rate and flesh breakdown results, showed that, when the storage temperature is increased from 0 to 1.0°C, the O₂ partial pressures must also be raised.

Fruit exposed to 0.6 kPa O₂, with the two partial pressures of CO₂ (1.0 and 1.5 kPa) and 0.8 kPa O₂ + 1.5 kPa CO₂ at 0°C, showed the lowest ethylene production (Table 2). Comparing the tested temperatures to 0°C, there was a lower ethylene production at 0.5 and 1.0°C, for conditions of 0.8 and 1.0 kPa O₂ combined with 2.0 kPa CO₂. Lower ethylene production was a result of the lower ACC oxidase activity visible in fruit at high temperature. These results may appear incompatible, but excessive reduction of temperature can cause cell damage and a concomitant increase in ethylene production (Knee et al., 1983) resulting in low fruit quality. In agreement with that, the lower occurrence of physiological disorder was observed in fruit stored at 1.0°C (Table 1).

By the analysis performed soon after removing fruit from the storage chamber, respiration rate was lower in fruit stored under conditions of higher CO₂ partial pressure (1.5 and 2.0 kPa) at 0°C (Table 2). However, at 0.5°C, only fruit stored at 0.6 kPa O₂ + 1.5 kPa CO₂ showed lower respiration rate. After a six-day exposure to air at 20°C, there was no interaction between temperature and controlled-atmosphere storage conditions. Fruit stored at 0.5 and 1.0°C showed lower respiration rate than those kept at 0°C. It

has been reported that 'Royal Gala' and 'Galaxy' apples stored in controlled-atmosphere storage exhibited lower respiration at 0.5°C in comparison to -0.5°C (Brackmann et al., 2008), thus showing an inverted relationship between temperature (in the range of 0 to 1.0°C) and respiration rate.

Conclusions

1. The storage of 'Royal Gala' apples under ultralow oxygen (below 0.8 kPa of O₂) conditions reduces the post-storage quality and increases the occurrence of physiological disorders in fruit.
2. The 1°C storage temperature can keep the quality of 'Royal Gala' apples after eight months of controlled-atmosphere storage.
3. The best ultralow oxygen condition for the storage of 'Royal Gala' apples is 1.0 kPa O₂ combined with 2.0 kPa of CO₂ at 1.0°C.
4. The O₂ partial pressures must be increased when storage temperature is higher.

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