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Relative humidity and its interaction with the storage temperature of 'Gala' apples and mutants

Umidade relativa e sua interação com temperatura de armazenamento de maçãs 'Gala' e mutantes

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ABSTRACT

The aim of this study was to evaluate the effect of relative humidity at two different temperatures on the postharvest quality of 'Gala', 'Royal Gala', 'Maxi Gala' and 'Brookfield' apples during eight months under controlled atmosphere storage. The experiment was conducted in a completely randomized design with split-plot scheme, where the main plots were composed of temperatures, 0.5 and 1.0°C, the subplots were the low (LRH) and high (HRH) relative humidity and the sub subplots were the four cultivars, resulting in a tri-factorial experiment: ((2x2) x4) with four repetitions of 25 fruits each. The results showed that there is a high respiratory rate in 'Maxi Gala' and 'Brookfield' apples in all storage conditions. The 'Brookfield' apples presented high pulp cracks when stored under a HRH environment. The temperature of 1.0°C decreases the ACC oxidase enzyme activity and ethylene production and maintains higher flesh firmness. The LRH maintains higher flesh firmness, total soluble solids and decreases the flesh breakdown. The fruits of the 'Brookfield' cultivar presented a lower storage potential in relation to the other cultivars.

Key words: *controlled atmosphere, Malus domestica, physiological disorders.*

RESUMO

O objetivo do presente estudo foi avaliar o efeito da umidade relativa em duas temperaturas sobre a qualidade pós-colheita de maçãs 'Gala', 'Royal Gala', 'Maxi Gala' e 'Brookfield' durante oito meses de armazenamento em atmosfera controlada. O experimento foi conduzido no delineamento inteiramente casualizado com parcela subdividida, sendo as temperaturas de 0,5 e 1,0°C as parcelas principais, a baixa umidade relativa do ar (BUR) e alta

umidade relativa do ar (AUR) as subparcelas e as quatro cultivares as subsubparcelas, resultando num experimento trifatorial: ((2x2) x4) com quatro repetições de 25 frutos em cada. Pelos resultados, observa-se uma elevada taxa respiratória nos frutos das cultivares Maxi Gala e Brookfield em todas as condições testadas. Os frutos da cultivar Brookfield apresentaram maior rachadura de polpa quando estes foram armazenados em ambiente com AUR. A temperatura de 1,0°C diminui a atividade da enzima ACC oxidase, a produção de etileno e mantém maior firmeza de polpa. A BUR mantém maior firmeza de polpa, sólidos solúveis totais e diminui a degenerescência de polpa. Os frutos da cultivar Brookfield apresentam menor potencial de armazenagem em relação às demais cultivares.

Palavras-chave: *atmosfera controlada, Malus domestica, distúrbios fisiológicos.*

INTRODUCTION

The 'Gala' apple is one of the most produced cultivars in the states of Rio Grande do Sul and Santa Catarina (Brazil), although it has been replaced in recent years by others derived from its mutation among which the 'Royal Gala', 'Galaxy' and 'Brookfield' stand out (BRACKMANN et al., 2009). The increase of these mutants culture is stimulated mainly due to its intense red coloration of the fruit skin which increases the consumer acceptance. Considering that apples have a short period of harvest, it is necessary to store part of

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the production in order to increase and regulate the offer period.

Temperature is the most relevant factor in the quality maintenance during the storage period (BRACKMANN et al., 2010). The use of low temperature during storage retards the physiological and biochemical ripening processes of fruits and vegetables (KADER, 1986). This temperature reduction has a strong influence on the decrease of the 'Gala' and 'Fuji' apples respiratory rate (FONSECA et al., 2002, STEFFENS et al., 2007). With the temperature reduction there is a drastic decline of fruit metabolism. However, this temperature reduction has a critical level which varies according to each cultivar. Thus, the exposure of fruits to temperature below the ideal may lead to an unbalanced metabolism, causing a high incidence of physiological disorders. WEBER et al. (2011) found a higher occurrence of flesh breakdown and mealiness in 'Royal Gala' apples stored between temperatures of 0.0°C and 0.5°C in relation to those stored at a temperature of 1.0°C.

The relative humidity (RH) control is another factor of extreme importance on the quality maintenance of apples during storage. According to KADER (1986), the RH during storage should not remain very low, because aside causing weight loss, it also damages the fruit appearance by wilting and leads to nutritional losses. Although water vapor has a fundamental importance in storage, its level within the storage chamber has not been established yet. This is mainly due to its difficulty of measurement and control during storage at low temperatures (PRANGE et al., 2005). As reported by LIDSTER (1990), flesh breakdown is higher under RH from 96 to 100%, for the 'McIntosh' apples. This high RH causes the emergence of cracks on the epidermis (EBERT, 1984). However, there is still a lack of studies regarding the best level of RH during storage for the various apple cultivars.

This research aimed to evaluate the RH effect at two different temperatures on the quality maintenance of 'Gala', 'Royal Gala', 'Maxi Gala' and 'Brookfield' apples, after eight months under controlled atmosphere storage.

MATERIAL AND METHODS

The experiment was developed at the Postharvest Research Center of the Department of Plant Sciences at the Federal University of Santa Maria (UFSM). The experimental material consisted of 'Gala', 'Royal Gala', 'Maxi Gala' and 'Brookfield' apples, obtained from commercial orchards in the city of Vacaria, state of Rio Grande do Sul (Brazil).

In the selection of fruits, the ones that presented any kind of injury or defect were eliminated. Thereafter, the experimental samples were homogenized, treatment application in experimental CA chambers with a volume of 233 liters was carried out and then finally, the samples were placed in two refrigerated chambers, at temperatures of 0.5°C(±0,1) and 1.0°C(±0,1).

The experiment was conducted in a completely randomized design with split-plot scheme, where the main plots were the temperatures of 0.5 and 1.0°C, the subplots were the low RH (LRH) (94±1%) and the high RH (HRH) (97±1%) and the sub subplots were the four cultivars, resulting in a tri-factorial experiment: ((2x2)x4), with four repetitions of 25 fruits per treatment. The CA condition for all the treatments, was 1,2kPa of O₂ + 2,5kPa of CO₂ being installed immediately after the placing of the fruits within the chambers. The O₂ partial pressure reduction was obtained through the dilution of O₂ with N₂ in the chamber until it reached the pre-established concentration. The CO₂ partial pressure was acquired by the injection of this gas until the desired concentration. Psychrometers with mercury thermometers were used to monitor the RH, whereas for the LRH absorption and maintenance calcium chloride was used. The HRH condition was obtained by the fruits transpiration within the chambers that remained hermetically sealed. Temperatures was controlled by thermostats and checked daily by bulb mercury thermometers with a 0.1°C resolution inserted in the fruits flesh, that were stowed in the storage chamber.

The quality parameters were evaluated after eight months of storage plus seven days of shelf life at 20°C. The parameters were: a) ACC oxidase enzyme activity: determined according to the methodology proposed by BUFLER (1986); b) Ethylene synthesis: determined through the stowage of approximately 1.5kg of fruits in a container with a volume of 5 liters, that was hermetically sealed for an approximate time of one hour after which two aliquots of each container were drawn and injected into a Varian gas chromatograph Star CX 3400 model, (Varian, Palo Alto, CA, USA), equipped with a flame ionization detector (FID) and a Poropak N80/100 column, being the data expressed in $\mu\text{L C}_2\text{H}_4 \text{ kg}^{-1} \text{ h}^{-1}$; c) Respiratory rate: determined through the air circulation, from the same ethylene synthesis analysis container, using an Agri-Datalog® gas analyzer, with infrared gas analyzer (IRGA) system (Agri - datalog, Lana, BZ, Italy), that analyzed the CO₂ concentration within the container. The data was expressed in $\text{mL CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$; d) Flesh firmness:

determined with the aid of a penetrometer model FT 327 (Effegi Systems, Milan, Italy) equipped with a 11mm probe and expressed in Newton; e) Flesh breakdown; f) Decay incidence; g) Pulp cracking: determined by the counting of fruits that demonstrated such disorder presented as a percentage; h) Titratable acidity: determined by the titration with 0.1N NaOH of a solution with 10mL of juice diluted in 100mL distilled water, until pH 8.1 was reached, data was expressed in meq 100mL⁻¹; and i) Total soluble solids: determined by refractometry and expressed in °Brix.

An analysis of variance (ANOVA) was performed for all the parameters evaluated. The parameters averages where the ANOVA was significant were compared through the Tukey test at a 5% probability of error. The data expressed in percentage was transformed by the formula $\arcsin \sqrt{x/100}$, before the analysis of variance.

RESULTS AND DISCUSSION

After eight months under CA storage, no statistical differences were observed in the incidence of decays, which was extremely low, presenting an average of 3.42% (± 2.05) rotten fruits (data not presented).

Temperature did not influenced ethylene synthesis when the fruits were stored under a higher relative humidity (HRH), however when stored under a low relative humidity (LRH), the temperature of 1.0°C propitiated a lower ethylene synthesis. This result is related to a lower ACC oxidase enzyme activity at this temperature (Table 1), since according to KONDO et al. (2009), low ethylene production might be related to a lower activity of this enzyme. At first this result is somewhat contradictory, once temperature reduction decreases the respiratory metabolism and the ethylene production. Nevertheless, this may be justified by the storage temperature decrease limit, since the excessive temperature reduction may cause damage to the cells and therefore increase the ethylene synthesis (KNEE et al., 1983). Analyzing the different RH within each temperature, it is possible to observe that the fruits stored under LRH at 0.5°C had higher ethylene synthesis in comparison to those stored at HRH. However, this was not noticed at a temperature of 1.0°C, when actually the inverse occurred to the 'Gala' and 'Maxi Gala' cultivars. In the literature is also possible to observe contradictory results. NAKANO et al. (2003) claims that the water loss through LRH storage increases the ethylene synthesis, yet BRACKMANN et al. (2007) noticed a decrease on it with the RH reduction. The 'Gala' and 'Brookfield' cultivars showed

a high ethylene synthesis when stored under a LRH at a temperature of 0.5°C, but no difference was found between the cultivars at 1.0°C under the same RH. In the HRH storage, the 'Maxi Gala' presented the highest ethylene synthesis (Table 1), on both 0.5°C and 1.0°C temperatures.

At the temperature of 0.5°C, the HRH storage provided a lower ACC oxidase enzyme activity in the Gala cultivar, however, no difference was observed between the HRH and the LRH at this temperature (Table 1) in the other cultivars. At the temperature of 1.0°C the LRH kept the enzyme activity low in the 'Royal Gala' and 'Brookfield' cultivars, but no difference occurred between the HRH and LRH. Generally, the temperature of 1.0°C suppressed the ACC oxidase enzyme activity when the fruits were stored in both HRH and LRH (Table 1). These results correspond to ASIF et al. (2009) observations about how storage temperature reduction to 1.0°C decreases the activity of several enzymes, including the ACC oxidase. The 'Maxi Gala' cultivar had higher enzyme activity differing from the others cultivars at both 0.5°C and 1.0°C, with the exception of 'Brookfield' at 1.0°C temperature. BRACKMANN et al. (2009) observed a low ACC oxidase enzyme activity in 'Brookfield' apples, an intermediate activity in the 'Galaxy' and a high activity in 'Royal Gala' apples.

The 'Brookfield' apples stored under HRH showed higher incidence of flesh breakdown, regardless of the storage temperature (Table 1). This is probably related to the difficulty of gas diffusion in the fruits stored under HRH causing physiological disorders, such as flesh breakdown during long periods of storage (PEREIRA et al., 2009) essentially, due to the difficulty of CO₂ diffusion to the fruit outside (CASTRO et al., 2008). At the temperature of 0.5°C and under a HRH, this disorder occurrence was significantly lower in the 'Royal Gala' cultivar, meanwhile, the opposite effect occurred to the 'Gala' cultivar (Table 1). When the fruits were stored at 0.5°C under LRH, the 'Gala' and the 'Royal Gala' had a lower flesh breakdown incidence, whereas at 1.0°C under LHR the 'Royal Gala' showed lower incidence of this disorder, significantly differing from the 'Brookfield' cultivar. Comparing the two RH levels, with the exception of 'Royal Gala' at the temperature of 0.5°C, all others presented lower occurrence of flesh breakdown under LRH or no significant difference for both temperatures. BRACKAMANN et al. (2007) noticed in 'Royal Gala' apples and ALVES et al. (2010) in 'Laetitia' plums that the use of LRH decreased the flesh breakdown significantly. Comparing the different temperatures, there was no much variation between this disorder occurrence for the four cultivars and the two RH levels.

Table 1 - Ethylene production rate, ACC (1-aminocyclopropane-1-carboxylate) oxidase activity, flesh breakdown and titratable acidity of 'Gala' apples and mutants after eight months stored at different conditions of relative humidity and temperature plus seven days shelf life at 20°C.

Treatments		-----Ethylene production rate ($\mu\text{L C}_2\text{H}_4 \text{ kg}^{-1} \text{ h}^{-1}$)-----				-----ACC oxidase activity ($\eta\text{L C}_2\text{H}_4 \text{ g}^{-1} \text{ h}^{-1}$)-----			
		Gala	Royal Gala	Maxi Gala	Brookfield	Gala	Royal Gala	Maxi Gala	Brookfield
0.5°C	I.A.**	0.48	0.52	0.47	1.37	19.6	17.4	22.3	17.4
	HRH***	0.15bB*	0.10bB	1.15bA	0.22bB	42.7bC	40.7aC	112.4aA	69.7aB
	LRH***	4.28aA	2.58aBC	2.23aC	3.31aAB	82.9aB	46.9aC	132.0aA	55.8aC
1.0°C	HRH	0.30aB	0.13aB	1.43aA	0.25aB	33.6aB	36.0aB	50.0aA	53.2aA
	LRH	0.10bA	0.06aA	0.18bA	0.20aA	29.7aB	15.4bC	43.5aA	35.9bAB
HRH	0.5°C	0.15a	0.10a	1.15a	0.22a	42.7a	40.7a	112.4a	69.7a
	1.0°C	0.30a	0.13a	1.43a	0.25a	33.6b	36.0a	50.0b	53.2b
LRH	0.5°C	4.28a	2.58a	2.23a	3.31a	82.9a	46.9a	132.0a	55.8a
	1.0°C	0.10b	0.06b	0.18b	0.20b	29.7b	15.4b	43.5b	35.9b
CV temperature (%)		27.6				2.08			
CV RH (%)		28.2				4.20			
CV cultivar (%)		19.4				3.99			
		-----Flesh breakdown (%)-----				-----Titratable acidity (meq 100mL ⁻¹)-----			
		Gala	Royal Gala	Maxi Gala	Brookfield	Gala	Royal Gala	Maxi Gala	Brookfield
0.5°C	I.A.	0.0	0.0	0.0	0.0	5.0	4.9	5.0	4.9
	HRH	13.1aB	1.00bC	14.2aB	29.2aA	4.33aA	4.25aA	4.30aA	3.73bB
	LRH	5.96bB	3.05aB	15.1aA	11.1bA	4.63aA	4.33aA	4.30aA	4.25aA
1.0°C	HRH	10.1aB	10.9aB	9.72aB	25.2aA	4.13aAB	4.20aA	4.23aA	3.93aB
	LRH	7.14aAB	3.00bB	5.56aAB	13.0bA	4.25aA	3.98bB	4.18aAB	3.63bC
HRH	0.5°C	13.1a	1.0b	14.2a	29.2a	4.33a	4.25a	4.30a	3.73a
	1.0°C	10.1a	10.9a	9.72a	25.2a	4.13a	4.20a	4.23a	3.93 a
LRH	0.5°C	5.96a	3.05a	15.1a	11.1a	4.63a	4.33a	4.30a	4.25a
	1.0°C	7.14a	3.00a	5.56b	13.0a	4.25b	3.98b	4.18a	3.63b
CV temperature (%)		39.0				1.83			
CV RH (%)		32.6				2.89			
CV cultivar (%)		21.9				4.05			

*Means followed by equal letters, lowercase in the columns and uppercase in the lines, do not differ by Tukey's test, at 5% probability.

**Initial analysis took place before fruit storage.

*** HRH: high relative humidity; LRH: low relative humidity.

Temperature did not influenced the titratable acidity when the fruits were stored under HRH, nonetheless when stored under LRH, the 0.5°C temperature kept higher concentrations of organic acids with the exception of the 'Maxi Gala' cultivar (Table 1). Titratable acidity did not differ for the four cultivars when stored at 0.5°C under LRH, but when stored at 1.0°C the 'Brookfield' showed the lowest titratable

acidity (Table 1). At the temperature of 0.5°C, the LRH kept the titratable acidity high on the 'Brookfield' cultivar, while at the temperature of 1.0°C an opposite behavior was observed for this same cultivar and for the 'Royal Gala', with the highest acidity in the fruits stored under HRH. These results are discordant to BRACKMANN et al. (2009), who observed high

titratable acidity in the fruits stored in an environment with low relative humidity.

High respiratory rate was noticed in 'Maxi Gala' and 'Brookfield' cultivars fruits (Table 2). For this variable, there was only interaction between temperature and RH (Table 2). There was no difference (<0.05) between HRH and LRH at the temperature of 0.5°C , while at the temperature of 1.0°C the fruits stored under LRH presented lower respiratory rate in relation to HRH (Table 2). PRANGE et al. (2001), also observed respiration reduction and postharvest quality maintenance when the apples were storage with RH

reduction. Comparing the different temperatures for the same RH rate, it is possible to observe that the temperature did not influence the respiratory rate under a LRH, meanwhile when the fruits were stored under HRH the respiratory rate was lower at the temperature of 0.5°C (Table 2). This result is congruent with STEFFENS et al. (2007), who affirmed that temperature reduction decreases the temperate climate fruits respiration.

The storage temperature did not influence the content of total soluble solids. This result is in agreement with KE et al. (1991), who affirmed that the

Table 2 - Respiration rate, total soluble solids, flesh firmness and pulp crack of 'Gala' apples and mutants after eight months stored at different conditions of relative humidity and temperature plus seven days shelf life at 20°C .

Treatments	-----Respiration rate (mL CO ₂ kg ⁻¹ h ⁻¹) -----					-----Total soluble solids (°Brix) -----				
	Gala	Royal Gala	Maxi Gala	Brook-field	Mean	Gala	Royal Gala	Maxi Gala	Brook-field	Mean
I.A. **	8.81	8.22	10.54	10.66		11.4	11.7	11.5	12.0	
0.5°C	4.53	4.10	5.48	5.15	4.82 b	13.0	13.3	13.4	13.2	13.2 a
1.0°C	4.93	4.61	6.01	6.08	5.41 a	13.0	13.3	13.6	13.2	13.3 a
Mean	4.73 B*	4.35 B	5.75 A	5.62 A		13.0 C	13.3 B	13.5 A	13.2 BC	
HRH***	4.92	4.50	6.00	5.78	5.30 a	12.8	13.2	13.4	13.0	13.1 b
LRH***	4.54	4.21	5.49	5.46	4.92 a	13.3	13.5	13.7	13.4	13.5 a
Mean	4.73 B	4.35 B	5.75 A	5.62 A		13.0 C	13.3 B	13.5 A	13.2 BC	
	HRH		LRH							
0.5°C	4.58bA		5.06aA		4.82					
1.0°C	6.02aA		4.79aB		5.41					
Mean	5.30		4.92							
CV temperature (%)					14.8	1.68				
CV RH(%)					19.4	1.76				
CV cultivar (%)					15.0	1.45				
	----- Flesh firmness (N) -----					----- Pulp crack (%) -----				
	Gala	Royal Gala	Maxi Gala	Brook-field	Mean	Gala	Royal Gala	Maxi Gala	Brook-field	Mean
I.A.	96.8	99.3	103.2	101.2		0.0	0.0	0.0	0.0	
0.5°C	76.3	78.2	77.6	73.3	76.4 b	7.71	6.17	14.7	17.4	11.5 a
1.0°C	77.2	78.3	80.2	77.1	78.2 a	6.60	6.02	9.72	17.5	9.96 a
Mean	76.8 AB	78.2 A	78.9 A	75.2 B		7.16	6.10	12.2	17.5	
HRH	74.5	77.0	76.6	72.0	75.0 b	7.79aB	6.52aB	12.7aB	23.4aA	11.5
LRH	79.0	79.5	81.2	78.4	79.5 a	6.52aA	5.67aA	11.7aA	11.5bA	9.96
Mean	76.8 AB	78.2 A	78.9 A	75.2 B		7.16	3.10	12.2	17.5	
CV temperature (%)					3.60	39.4				
CV RH(%)					3.56	44.4				
CV cultivar (%)					3.22	28.3				

*Means followed by equal letters, lowercase in the columns and uppercase in the lines, do not differ by Tukey's test, at 5% probability.

**Initial analysis took place before fruit storage.

*** HRH: high relative humidity; LRH: low relative humidity.

content of soluble solids does not vary according to the storage temperature. The 'Maxi Gala' apples demonstrated the highest contents of total soluble solids (Table 2), that might be related to the degree of ripeness, indicated by the high flesh firmness (Table 2). WATKINS et al. (2000), observed that the content of soluble solids varies according to the type of storage and to the cultivar. The LRH kept the content of total soluble solids high in relation to the HRH (Table 2), this is due to the water loss of the fruits through transpiration and concentrating the sugar within the cells. WEBER (2010) also noticed a higher content of total soluble solids when the fruits were stored in low relative humidity.

Flesh firmness is one of the attributes that best indicates a fruits postharvest quality, like that of apple. In the present study however, interaction between the factors evaluated to flesh firmness did not occur (Table 2). It is observed between the two temperatures that the storage at 1.0°C maintains higher flesh firmness (Table 2), a fact that may be related to a lower ethylene production, considering that flesh firmness is often related to the respiratory rate and to the ethylene production (HIWASA et al., 2003). The LRH storage also kept the fruits with greater flesh firmness in relation to HRH. This result is possibly related to greater cell wall integrity, taking into account that earlier studies indicate that flesh firmness maintenance has to do with the cell wall constituents such as pectin, which has the function of maintaining the cells union, once it is degraded; a decrease in flesh firmness occurs (PEREIRA et al., 2009). Other studies also witnessed greater flesh firmness in 'Gala' apples when the fruits were submitted to a mass loss through storage in a low RH environment (BRACKMANN et al., 2007). The 'Brookfield' cultivar presented lower flesh firmness compared to the 'Royal Gala' and 'Maxi Gala', although not differing from 'Gala' apples (Table 2). This may be related to the high flesh breakdown in the 'Brookfield' apples (Table 1). These results contradict BRACKMANN et al. (2009), who observed higher flesh firmness in the 'Brookfield' cultivar in relation to the 'Royal Gala', stored at the same atmospheric condition.

The incidence of pulp cracking demonstrated a two-way significant interaction to the cultivars and the RH within the chamber (Table 2), while the storage temperature did not influence this disorder occurrence. No difference was noticed between the cultivars when the fruits were stored under a LRH, however, under HRH the 'Brookfield' cultivar showed a higher occurrence of pulp cracking (Table 2). The higher occurrence of cracks may be related to a higher pectin degradation of the cell wall (STEFFENS et al.,

2005). This fact may explain the lower flesh firmness and higher cracking found in the 'Brookfield' cultivar, which was triggered by the high ethylene production of these fruits (Table 1). Increase pulp cracking incidence was observed only on the fruits of the 'Brookfield' cultivar under HRH, whilst on the other cultivars no difference between storage under LRH and HRH was seen. Studies have demonstrated that the RH storage reduction decreases expressively the pulp cracking incidence (BRACKMANN et al., 2005).

CONCLUSION

The best humidity condition for all cultivars investigated is LRH (94±1%) because maintains higher flesh firmness and total soluble solids and decreases the flesh breakdown. Also the temperature of 1.0°C decreases the ACC oxidase enzyme activity and the ethylene production and keeps higher flesh firmness, therefore being the most indicate to the storage of all cultivars. The 'Brookfield' cultivar shows a lower storage potential in relation to the other cultivars, revealed by the low values of flesh firmness and higher occurrence of disorders.

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