Original Article

‘GALA’, ‘ROYAL GALA’ AND ‘BROOKFIELD’ APPLES STORED UNDER ULTRA-LOW OXYGEN AND TWO TEMPERATURES

MAÇÃS ‘GALA’, ‘ROYAL GALA’ E ‘BROOKFIELD’ ARMAZENADAS EM ULTRA BAIXO OXIGÊNIO E DUAS TEMPERATURAS

Anderson WEBER[1]; Fabio Rodrigo THEWES[2]; Márcio Renan Weber SCHORR[1]; Adriano Roque de GASPERIN[1]; Thiago Liberalesso VENTURINI[3]; Auri BRACKMANN[4]

1. Programa de Pós-Graduação em Agronomia, Departamento de Fitotecnia, Universidade Federal de Santa Maria - UFSM, Santa Maria, RS, Brasil, anweba@yahoo.com.br, 2. Curso de Agronomia – UFSM, Santa Maria, RS, Brasil; 3. Engenheiro agrônomo da Empresa Agropecuária Schio Ldta, Vacaria, RS. 4. Professor, Doutor, departamento de fitotecnia - UFSM, Santa Maria, RS, Brasil.

ABSTRACT: Most apples produced in Brazil have to be stored for regular the supply to the consumer market throughout the year. Thus, the present study aimed to check the differential response of ‘Gala’, ‘Royal Gala’ and ‘Brookfield’ apples stored under different ULO and temperature conditions. The experiment had a completely randomized design with 25 fruits per experimental unit and a three-factor design (2x5x3) with split-plots. After eight months of storage plus seven days of simulated shelf life, there was a significant triple interaction among the factors temperature/CA/cultivar for enzyme 1-aminocyclopropane-1-carboxilate (ACC) oxidase activity, ethylene production, respiration rate and flesh breakdown. For flesh firmness, there was no interaction among the factors tested in the present study. However, the observation of the main effects of each factor shows that flesh firmness can be preserved more effectively at 1.0° than at 0.5°C. Temperature of 1.0°C promotes better storage condition due the high flesh firmness and low ACC oxidase enzyme activity of fruit after storage. The best ULO condition stay between 0.8 up to 1.0 kPa of O_2 and 1.5 up to 2.0 kPa of CO_2 that reduces the ACC oxidase enzyme and ethylene production. ‘Brookfield’ apples have lower storage potential because the high respiration rate and flesh breakdown.

KEYWORDS: *Malus domestica*. Postharvest. Quality. Physiological disorders.

INTRODUCTION

Most apples produced in Brazil - more than a million tonnes (IBGE, 2012) – have to be stored so that a regular supply can be offered to the consumer market throughout the year. Apples have a high storage potential; hence they can be stored under controlled atmosphere (CA) for up to nine months (BRACKMANN et al., 2005). Under CA, low O_2 partial pressures and high CO_2 partial pressures cause a reduction in respiration rate (BRACKMANN et al., 2008) and ethylene production (LAU, 1985), thus preserving the physical and chemical characteristics of the fruits (KE et al., 1991).

The new technologies developed by researchers are being quickly adopted by fruit storage companies, whose storage costs are high because of items such as electricity consumption. For this reason, researchers have been testing higher storage temperatures which do not result in fruit quality loss. Recent studies showed that temperatures such as 1ºC are more suitable than 0ºC or 0.5ºC to preserve the quality of 'Royal Gala' apples after eight months of storage under CA (WEBER et al., 2011; WEBER et al., 2012). According to WEBER et al. (2011), O_2 level has to be increased as storage temperature is increased (1ºC).

Nowadays, Ultra Low Oxygen (ULO) has been shown to be an alternative to conventional CA. This technique promotes minimum O_2 pressure, right above the anaerobic compensation point, where aerobic respiration is low and also occurs minimal CO_2 production (BOERSIG et al., 1988). As a result, the loss of acids and sugars during respiration and the rate of ethylene biosynthesis are lower, and no losses are caused by the incidence of physiological disorders (GRAN; BEAUDRY, 1993; YEARSLEY et al., 1996). According to ORTIZ et al. (2011), ULO delays overall ripening because of a decrease in ethylene production. However, tolerance to ULO and high CO_2 levels depends on cultivar and storage temperature (WEBER et al., 2011).

Approximately 60% of the apples produced in Rio Grande do Sul belong to the cultivar ‘Gala’ and its mutants ‘Royal Gala’, ‘Brookfield’ and ‘Galaxy’ (AGRIANUAL, 2009). As the production of ‘Galaxy’ and the other mutants has been higher than that of ‘Gala’ standard, recent studies seek to identify the best storage conditions for each mutant, because storage companies generally apply the same CA conditions and temperature used for ‘Gala’. Brackmann et al. (2008) found that the best condition for the 'Galaxy' apples is 0.8 to 1.0kPa O_2 + 2.5kPa CO_2 and 1.0kPa O_2 + 2.5kPa CO_2 for 'Royal Gala', with 'Galaxy' having a higher storage

Received: 21/03/13
Accepted: 03/09/13

potential than 'Royal Gala'. The present study aimed to check the differential response of 'Gala', 'Royal Gala' and 'Brookfield' apples stored under different ULO and temperature conditions.

MATERIAL AND METHODS

The experiment was carried out at Department of Plant Science from the Federal University of Santa Maria (UFSM). The fruits were harvested in a commercial orchard located in Vacaria, RS, and selected previous to the application of the treatments: injured and/or damaged fruits were discarded, and the experimental units were then homogenized.

The experiment had a completely randomized design with 25 fruits per experimental unit. A three-factor design (2x5x3) with split-plots was used. The main plot was composed of two temperatures (0.5 and 1.0°C); the subplot, of five CA conditions [1] 1.2kPa O_2 and 2.5kPa CO_2; [2] 1.2kPa O_2 and 2.0kPa CO_2, [3] 1.0kPa O_2 and 2.0kPa CO_2, [4] 0.8kPa O_2 and 2.0kPa CO_2 and [5] 0.8kPa O_2 and 1.5kPa CO_2; and the subsubplot was composed of three cultivars ('Gala', 'Royal Gala' and 'Brookfield').

The fruits were stored for eight months in hermetically small experimental chambers (volume = 0.232m^3) which were connected to a control unit with gas analyzers. The mini-chambers were placed inside 48m^3 cold storage room. Relative humidity inside the mini-chambers was maintained constant at 96±1%.

The desired CA conditions were created by injecting nitrogen (N_2) from a generator that uses the Pressure Swing Adsorption (PSA) technology. By means of the N_2 dilution method, O_2 partial pressure was reduced to the level previously established for each treatment. The desired CO_2 conditions were obtained by injecting it from high pressure cylinders into the experimental chambers. During the storage period, the partial pressure of the gases (O_2 and CO_2) was monitored and corrected daily by Shelle® gas controller, which was calibrated weekly with standard gases.

Fruit quality analyses were performed after eight months of storage plus seven days at 20±1°C (UR 84±5%) to simulate the shelf life period. The assessed variables were: a) *ACC oxidase activity*, according to the methodology proposed by BUFLER (1986); b) *Ethylene production*: detected by a Varian® Star 3400CX gas chromatograph; a 2.0m Porapak® N column was used as the stationary phase, and nitrogen was used as the mobile phase. The temperatures respectively used for column, injector and detector were 90ºC, 140ºC and 200ºC. About 1.5kg of fruit mass was placed in airtight 5L glass containers for a specified period of time. A 1mL sample was then removed from the headspace of each container and injected into the gas chromatograph. Average values were obtained in ppm and later converted and expressed in µL C_2H_4 $kg^{-1}h^{-1}$; c) *Respiration rate*: obtained by circulation the gas from the fruits of the same containers used to determine ethylene through an *Agri-datalog*® gas analyzer and expressed in mL CO_2 $kg^{-1}h^{-1}$ as a function of fruit mass, free volume in the container, closing time and the CO_2 concentration found in the containers; d) *Flesh breakdown*: determined by the number of fruits which had darkened patches on the flesh when cut into several longitudinal slices; e) *Soluble solids* (SS): determined with a manual refractometer; the reading was adjusted as a function of the effect of temperature and expressed in °Brix; f) *Mealiness*: determined subjectively by analysis fruits that showed mealy flesh, i.e., with low juiciness, and expressed in percentage; g) *Flesh firmness*: determined with a penetrometer (11mm plunger), pushed into the two opposite sides of the equatorial axis area of the fruit whose epidermis had been previously removed, expressed in Newtons (N); h) *Titatrable acidity*: 10mL of juice was diluted in 100mL distilled water and titrated with a solution of NaOH 0.1N up to pH8.1, expressed in meq100mL^{-1};

Prior to analysis of variance (ANOVA), the data were tested for normality and homogeneity of errors by the Lilliefors and Bartlett's tests, respectively. The parameters without normality of errors were transformed by the formula arc.sine$((x+0,5)/100),^{1/2}$ and the parameters with heteroscedastic errors were transformed by the formula $\log_{10}(x + 1.0)$ for subsequent analysis of variance together with the other parameters. The means were compared by the Tukey's test at 5% level of probability.

RESULTS AND DISCUSSION

After eight months' storage plus seven days of simulated shelf life, there was a significant triple interaction among the factors temperature/CA/cultivar for enzyme 1-aminocyclopropane-1-carboxilate (ACC) oxidase activity, ethylene production (Table 1), respiration rate and flesh breakdown (Table 2).

Table 1. ACC (1-aminocyclopropane-1-carboxilate) oxidase activity and ethylene production rate of 'Gala', 'Royal Gala' and 'Brookfield' apples after eight months stored at different CA conditions and temperature plus seven days shelf life at 20°C.

Treatments		ACC Oxidase activity ($\eta L\ C_2H_4\ g^{-1}\ h^{-1}$)			Ethylene production rate ($\mu L\ C_2H_4\ kg^{-1}\ h^{-1}$)		
		Gala	Royal Gala	Brookfield	Gala	Royal Gala	Brookfield
Initial analysis		19.6	17.4	17.4	0.48	0.52	1.37
0.5°C	1.2 + 2.5	37.1 aB*	40.7 bB	62.9 aA	0.15 bAB	0.10 bB	0.22 aA
	1.2 + 2.0	42.8 aB	60.4 aA	58.8 aA	0.52 aA	0.27 aB	0.24 aB
	1.0 + 2.0	38.0 aB	31.8 cC	55.7 aA	0.54 aA	0.11 bB	0.17 aB
	0.8 + 2.0	30.8 bA	22.2 dB	33.1 bA	0.24 bA	0.09 bB	0.18 aAB
	0.8 + 1.5	18.8 cB	16.6 eB	35.7 bA	0.22 bA	0.08 bB	0.17 aAB
1.0°C	1.2 + 2.5	33.6 bB	36.0 bB	53.2 aA	0.20 aAB	0.13 bB	0.25 aA
	1.2 + 2.0	49.2 aB	50.0 aB	64.1 aA	0.17 abB	1.13 aA	0.19 abB
	1.0 + 2.0	12.7 cC	33.6 bA	26.0 cB	0.09 bcA	0.07 bA	0.12 bA
	0.8 + 2.0	11.2 cB	6.6 dC	33.8 bA	0.07 cA	0.07 bA	0.13 bA
	0.8 + 1.5	10.9 cC	13.6 cB	23.9 cA	0.07 cA	0.06 bA	0.12 bA
1.2 + 2.5	0.5°C	37.1 a	40.7 a	62.9 a	0.15 a	0.10 a	0.22 a
	1.0°C	33.6 a	36.0 a	53.2 b	0.20 a	0.13 a	0.25 a
1.2 + 2.0	0.5°C	42.8 a	60.4 a	58.8 a	0.52 a	0.27 b	0.24 a
	1.0°C	49.2 a	50.0 b	64.1 a	0.17 b	1.13 a	0.19 a
1.0 + 2.0	0.5°C	38.0 a	31.8 a	55.7 a	0.54 a	0.11 a	0.17 a
	1.0°C	12.7 b	33.6 a	26.0 b	0.09 b	0.07 a	0.12 a
0.8 + 2.0	0.5°C	30.8 a	22.2 a	33.1 a	0.24 a	0.09 a	0.18 a
	1.0°C	11.2 b	6.6 b	33.8 a	0.07 b	0.07 a	0.13 a
0.8 + 1.5	0.5°C	18.8 a	16.6 a	35.7 a	0.22 a	0.08 a	0.17 a
	1.0°C	10.9 b	13.6 b	23.9 b	0.07 b	0.06 a	0.12 a

*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.

Table 2. Respiration rate and flesh breakdown of 'Gala', 'Royal Gala' and 'Brookfield' apples after eight months stored at different CA conditions and temperature plus seven days shelf life at 20°C.

Treatments		Respiration rate ($mL\ CO_2\ kg^{-1}\ h^{-1}$)			Flesh breakdown (%)		
		Gala	Royal Gala	Brookfield	Gala	Royal Gala	Brookfield
Initial analysis		8.81	8.22	10.66	0.0	0.0	0.0
0.5°C	1.2 + 2.5	4.29 abAB*	3.81 aB	5.13 abA	13.10 aB	1.00 aC	29.25 aA
	1.2 + 2.0	3.63 bB	4.08 aB	5.02 abA	4.60 aB	7.00 aB	21.97 aA
	1.0 + 2.0	5.04 aA	4.69 aA	4.40 bA	5.95 aAB	1.00 aB	14.78 abA
	0.8 + 2.0	4.13 abB	4.29 aB	5.51 aA	4.82 aB	6.04 aAB	15.94 abA
	0.8 + 1.5	3.75 bB	4.25 aAB	5.04 abA	7.14 aA	2.00 aA	7.71 bA
1.0°C	1.2 + 2.5	5.55 aAB	5.11 aB	6.42 aA	10.08 aB	10.88 aB	25.25 aA
	1.2 + 2.0	5.59 aA	5.44 aA	5.71 abA	6.08 aAB	2.17 bB	11.96 bA
	1.0 + 2.0	4.66 abB	5.07 aAB	5.87 abA	7.21 aAB	5.13 abB	15.96 abA

	0.8 + 2.0	4.18 bB	4.99 aB	6.52 aA	9.52 aAB	3.92 abB	18.48 abA
	0.8 + 1.5	3.94 bA	4.50 aA	4.86 bA	4.76 aB	2.97 abB	26.09 aA
1.2 + 2.5	0.5°C	4.29 b	3.81 b	5.13 b	13.1 a	1.0 b	29.2 a
	1.0°C	5.55 a	5.11 a	6.42 a	10.1 a	10.9 a	25.2 a
1.2 + 2.0	0.5°C	3.63 b	4.08 b	5.02 a	4.6 a	7.0 a	22.0 a
	1.0°C	5.59 a	5.44 a	5.71 a	6.1 a	2.2 a	12.0 a
1.0 + 2.0	0.5°C	5.04 a	4.69 a	4.40 b	6.0 a	1.0 a	14.8 a
	1.0°C	4.66 a	5.07 a	5.87 a	7.2 a	5.1 a	16.0 a
0.8 + 2.0	0.5°C	4.13 a	4.29 a	5.51 b	4.8 a	6.0 a	15.9 a
	1.0°C	4.18 a	4.99 a	6.52 a	9.5 a	3.9 a	18.5 a
0.8 + 1.5	0.5°C	3.75 a	4.25 a	5.04 a	7.1 a	2.0 a	7.7 b
	1.0°C	3.94 a	4.50 a	4.86 a	4.8 a	3.0 a	26.1 a

*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.

Higher ACC oxidase activity was observed for 'Brookfield' apples compared to 'Royal Gala' and 'Gala', for nearly all the combinations of temperature and AC (Table 1). This is a different result from the one found by BRACKMANN et al. (2009), who observed higher activity in 'Royal Gala' compared to 'Brookfield'. As for CA, 'Gala' and 'Brookfield' apples stored at 0.8 kPa O_2 at 0.5°C showed lower ACC oxidase activity than those stored at higher oxygen partial pressures. At 1.0°C, lower activity was observed at 0.8 and 1.0 kPa O_2. This result was also found by WEBER et al. (2011) with 'Royal Gala' apples, where an increase in temperature from 0.0°C to 1.0°C required an increase in O_2 partial pressure from 0.6 to 1.0kPa, respectively. For 'Royal Gala', higher activity was observed in the condition 1.2 + 2.0 at both temperatures, with gradual reduction in activity as O_2 pressure decreased. A comparison of the storage temperatures in most CA conditions showed lower activity of the enzyme at 1.0°C, particularly in lower O_2 conditions. According to YEARSLEY et al. (1997), this happens because as temperature increases, there is an increase in the lowest oxygen limit (LOL) and, as O_2 is needed for ACC oxidase to be active (YANG; HOFFMANN, 1984), its activity is reduced.

Ethylene production was lower for 'Royal Gala' at 0.5°C, when compared to 'Gala' (Table 1). There were no differences among the cultivars at 1.0°C and O_2 below 1.0kPa, but differences were observed when the fruits were stored at 1.2kPa O_2. As for storage conditions, O_2 partial pressures of 0.8 and 1.0 kPa resulted in lower ethylene production at 1.0°C for the three cultivars. Low O_2 not only reduces ACC oxidase activity but also helps reduce ethylene synthesis because it is a substrate for such synthesis (YANG; HOFFMAN, 1984). Concerning storage temperature, 'Gala' had higher ethylene synthesis with storage at 0.5°C in four of the five CA conditions tested, unlike 'Royal Gala' and 'Brookfield', which showed hardly any differences, even though there was a little difference in ACC oxidase activity.

The comparison among the cultivars showed that 'Brookfield' had higher respiration rate than the other cultivars both at 0.5 and at 1.0°C (Table 2). The respiration rate for 'Royal Gala' and 'Brookfield' had little or no variation among the CA conditions at both temperatures. For 'Gala' and 'Brookfield' at 1.0°C, the fruits that were submitted to the lowest O_2 conditions (0.8 and 1.0 kPa) had lower respiration rate. On 'Gala' and 'Royal Gala' apples the temperature only showed significant differences when storage O_2 was 1.2 kPa (CO_2 = 2.0 and 2.5 kPa), where 1.0°C resulted in a higher respiration rate; however, there was statistical difference when O_2 was below 1.0kPa. For 'Brookfield', the conditions 1.2 + 2.5, 1.0 + 2.0 and 0.8 + 2.0 also resulted in a higher respiration rate at 1.0°C. Temperature has a direct effect on the rate of chemical reactions; thus, higher temperature results in a higher respiration rate (WRIGHT et al., 2010). However, there is no oxygen for the respiratory process under low O_2 conditions (0.8 and 1.0 kPa, for 'Gala' and 'Royal Gala'), even at a higher temperature.

Flesh breakdown, just like ACC oxidase activity and respiration rate, was higher for 'Brookfield' than for the other two cultivars for all

the combinations of temperature and CA (Table 2), as opposed, once again, to the result obtained by BRACKMANN et al. (2009), who found higher flesh breakdown for 'Royal Gala' than for 'Brookfield'. The analysis of the CA conditions shows that there were no statistical differences for 'Gala' at 0.5 and 1.0°C, and for 'Royal Gala' at 0.5°C. For 'Brookfield' at 0.5°C there was lower incidence of this disorder at 0.8+1.5 kPa (O_2+CO_2), without a distinction between 0.8+2.0 and 1.0+2.0. 'Royal Gala' and 'Brookfield' at 1.0°C had lower incidence of flesh breakdown under higher O_2 (1.2+2.0). Again, these results are indicative that O_2 partial pressure has to be increased when the temperature is increased, which agree with the results found by WEBER et al. (2011) in 'Royal Gala' apples. According to CERETTA et al. (2010), very low O_2 conditions increase susceptibility to damage by high CO_2.

For flesh firmness, there was no interaction among the factors tested in the present study. Therefore, the treatment of a factor behaved the same way within another factor (Table 3). The observation of the main effects of each factor shows that flesh firmness can be preserved more effectively at 1.0° than at 0.5°C. The higher loss of flesh firmness of the fruits of this treatment may be associated with high ethylene production of the fruits stored under this condition (HIWASA et al., 2003), mainly, the fruits of the cultivar Gala. When different cultivars are compared, 'Brookfield' apples loss more firmness compared to the other cultivars, although they had shown higher flesh firmness at the beginning of storage. The lower flesh firmness in this cultivar is probably due to the fact that the fruits were extremely mature after eight months of storage, as evidenced by the higher incidence of mealiness and flesh breakdown (Table 2 and 3). Such physiological disorders reduce the integrity of the cell wall and adhesion among cells (PRASANA et al., 2007; PAYASI et al., 2009; PEREIRA et al., 2009) and, therefore, they also reduce flesh firmness. The rate of ethylene biosynthesis was also lower in this cultivar (Table 1), but this is likely occurred because the fruits were already at an early stage of senescence, and thus, less able to synthesize ethylene. Among the different CA conditions, lower flesh firmness was observed in the fruits stored at 1.2 kPa O_2 + 2.5 kPa CO_2 compared to the other conditions.

Table 3. Flesh firmness and mealiness of 'Gala', 'Royal Gala' and 'Brookfield' apples after eight months stored at different CA conditions and temperature plus seven days shelf life at 20°C.

Treatments	Flesh firmness (N)				Mealiness (%)			
	Gala	Royal Gala	Brook-Field	Mean	Gala	Royal Gala	Brook-field	Mean
I. A.	96.8	99.3	101.2		0.0	0.0	0.0	
0.5°C	78.6	78.7	75.6	77.6 b	10.0	7.5	20.6	12.7 a
1.0°C	79.7	80.6	76.7	79.0 a	7.7	6.7	19.5	11.3 a
	79.1 A	79.6 A	76.2 B		8.8	7.1	20.1	
1.2 + 2.5	74.5	77.0	72.0	74.5 b	7.8 aB	6.5 abB	23.4 aA	12.6
1.2 + 2.0	79.4	78.2	77.1	78.2 a	9.5 aB	8.1 abB	17.5 aA	11.7
1.0 + 2.0	80.9	81.2	76.9	79.7 a	9.0 aB	5.6 abB	20.4 aA	11.6
0.8 + 2.0	81.0	80.7	77.5	79.7 a	10.8 aA	11.1 aA	17.8 aA	13.2
0.8 + 1.5	79.8	81.1	77.4	79.4 a	7.1 aB	4.0 bB	21.3 aA	10.8
	79.1 A	79.6 A	76.2 B		8.8	7.1	20.1	

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

Regarding the incidence of mealiness, interaction was observed across the CA conditions and the cultivars (Table 3). In all the CA conditions tested, except for 0.8 kPa O_2 + 2.0 kPa CO_2, where there was no statistical differences across the cultivars, higher incidence of mealiness in the fruits of the cultivar Brookfield. This disorder is associated with higher activity of enzymes polygalacturonase and pectinametilesterase, which transform protopectin molecules into soluble pectin (PRASANA et al., 2007; PAYASI et al., 2009), thus reducing adhesion among cells and turning the flesh mealy. When the different CA conditions are compared in each cultivar, statistical differences occur only in the cultivar Royal Gala, where 0.8 kPa O_2 combined with 2.0kPa CO_2 resulted in a higher

incidence of this disorder. For cultivars non-sensitive to high CO_2, WEBER et al. (2012) recommends that CO_2 partial pressure should be approximately twice as high as O_2 partial pressure. Another study conducted with 'Royal Gala' also found a high incidence of mealiness in the apples submitted to the same partial pressure of O_2 and CO_2 (WEBER et al., 2011). Between the two temperatures tested, there was no significant difference for this variable.

Storage temperature did not have a significant effect on the concentration of total SS (Table 4). However, the observation of the different cultivars shows that total soluble solids content was high for 'Royal Gala', intermediate for 'Brookfield' and low for 'Gala'. These results differ from those found by BRACKMANN et al. (2009), who observed a higher total soluble solids content in 'Brookfield' compared to 'Royal Gala' and 'Galaxy'. Although, the SS level is related with the ripening at harvest and season that fruit growing. When the different CA conditions were observed, it could be seen that storage at 1.0 kPa O_2 + 2.0 kPa CO_2 maintained a high content of total soluble solids compared to the 1.2 kPa O_2 + 2.5 kPa CO_2 condition.

For titratable acidity, there was significant interaction between storage temperature and CA conditions (Table 4). At 1.2 kPa O_2, there was no difference between the temperatures, while in the other conditions the temperature of 0.5°C maintained higher content of organic acids. A comparison of the different CA conditions in each temperature shows that at the lowest temperature was higher consumption of organic acids at the highest O_2 partial pressures, while at 1°C there was higher consumption of organic acids at the lowest O_2 partial pressures. This is indicative that when the temperature is increased, O_2 partial pressures have to be increased as well, a similar result was found in 'Royal Gala' apples (WEBER et al., 2011). Among the cultivars, Gala had the highest acidity, 'Royal Gala' had an intermediate value, and 'Brookfield' had the lowest acidity. The lower titratable acidity of 'Brookfield' was due to the high respiration rate of the fruits of this cultivar (Table 2). Other studies associate the consumption of organic acids with respiration (BRACKMANN et al., 2009; SWEETMAN et al., 2009).

Table 4. Soluble solids and titratable acidity of 'Gala', 'Royal Gala' and 'Brookfield' apples after eight months stored at different CA conditions and temperature plus seven days shelf life at 20°C.

Treatments	Soluble solids (°Brix)				Titratable acidity (mEq 100mL^{-1})			
	Gala	Royal Gala	Brook-field	Mean	Gala	Royal Gala	Brook-field	Mean
I. A.	11.4	11.7	12.0		5.0	4.9	4.9	
0.5°C	12.9	13.3	13.0	13.1 a	4.46	4.23	3.91	4.20
1.0°C	12.9	13.3	13.1	13.1 a	4.30	4.10	3.80	4.06
	12.9 C	13.3 A	13.0 B		4.38 A	4.16 B	3.85 C	
1.2 + 2.5	12.8	13.2	13.0	13.0 b		0.5°C	1.0°C	Mean
1.2 + 2.0	12.9	13.3	13.1	13.1 ab	1.2 + 2.5	4.10 bA	4.08 abA	4.09
1.0 + 2.0	13.0	13.4	13.1	13.2 a	1.2 + 2.0	4.13 bA	4.22 aA	4.17
0.8 + 2.0	12.9	13.4	13.0	13.1 ab	1.0 + 2.0	4.37 aA	4.10 abB	4.23
0.8 + 1.5	13.0	13.3	12.9	13.1 ab	0.8 + 2.0	4.20 abA	4.00 abB	4.10
	12.9 C	13.3 A	13.0 B		0.8 + 1.5	4.20 abA	3.91 bB	4.05
						4.20	4.06	

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

Therefore, storage at 1.0°C resulted in lower ACC oxidase activity and lower ethylene production, but did not increase the respiration rate and, as a consequence, provided better maintenance of flesh firmness. This result was also found by WEBER et al. (2012). Thus, increasing storage temperature and, for that matter, the possibility of using slightly higher oxygen partial pressures can be a good alternative for cost reduction under cold storage and controlled atmosphere.

CONCLUSIONS

Temperature of 1.0°C promotes better storage condition due the high flesh firmness and

low ACC oxidase enzyme activity of fruit after storage.

The best ULO condition stay between 0.8 up to 1.0 kPa of O_2 and 1.5 up to 2.0 kPa of CO_2 that reduces the ACC oxidase enzyme and ethylene production.

'Brookfield' apples have lower storage potential because the high respiration rate and flesh breakdown.

ACKNOWLEDGEMENTS

To Conselho Nacional de Desenvolvimento Científico e Tecnológico, Coordenação de aperfeiçoamento de Pessoal e de Nível Superior and Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul, for financial support.

RESUMO: A maioria das maçãs produzidas no Brasil deve ser armazenada para um fornecimento regular durante todo ano ao mercado consumidor. Assim, o presente estudo tem por objetivo avaliar a resposta de maçãs 'Gala', 'Royal Gala' e 'Brookfield' armazenadas em diferentes condições de ULO e temperaturas. O experimento foi conduzido em um delineamento inteiramente casualizado com 25 frutos por unidade experimental e foi usado um esquema trifatorial (2x5x3) com parcelas subdivididas. Após oito meses de armazenamento mais sete dias de vida de prateleira, houve uma significativa interação entre os fatores temperatura/CA/cultivar para atividade da enzima ACC oxidase, produção de etileno, taxa respiratória e degenerescência de polpa. Para firmeza de polpa, não houve interação entre os fatores testados no presente estudo. Entretanto, a observação dos efeitos principais de cada fator verifica-se que a firmeza de polpa e mantida mais elevada quando os frutos foram armazenados na temperatura de 1.0°C do que 0.5°C. A temperatura de 1.0°C promove melhores condições de armazenamento pela maior firmeza de polpa e menor atividade da enzima ACC oxidase dos frutos após o armazenamento. A melhor condição de ULO esta entre 0.8 até 1.0 kPa de O_2 e 1.5 até 2.0 kPa de CO_2 que reduz a atividade da enzima ACC oxidase e a produção de etileno. Maçãs 'Brookfield' tem menor potencial de armazenamento pela elevada taxa respiratória e degenerescência de polpa.

PALAVRAS-CHAVE: *Malus domestica*. Pós-colheita. Qualidade. Desordens fisiológicas.

REFERENCES

AGRIANUAL. **Anuário da Agricultura Brasileira**. São Paulo: FNP, 2009. 497 p.

BOERSIG, M. R.; KADER, A. A.; ROMANI, R. J. Aerobic-anaerobic respiratory transition in pear fruit and cultured pear fruit cells. **Journal of American Society of Horticultural Science,** Alexandria, v. 113, p. 869-873, Nov. 1988.

BRACKMANN, A.; WEBER, A.; PINTO, J. A. V.; NEUWALD, D. A.; STEFFENS, C. A. Manutenção da qualidade pós-colheita de maçãs 'Royal Gala' e 'Galaxy' sob armazenamento em atmosfera controlada. **Ciência Rural,** Santa Maria, v. 38, p. 2478-2484, Dec. 2008.

BRACKMANN, A.; SESTARI, I.; GUARIENTI, A. J. W.; PINTO, J. A. V.; STEFFENS, C. A. Qualidade da maçã 'Gala' armazenada em diferentes pressões parciais de O_2 e CO_2. **Revista da FZVA - Faculdade de Zootecnia, Veterinária e Agronomia,** Uruguaiana, v. 12, p. 98-105, Jan./dec. 2005.

BRACKMANN, A.; WEBER, A.; PAVANELLO, E. P.; BOTH, V.; SESTARI, I. Armazenamento em atmosfera controlada de maçãs mutantes da cultivar Gala. **Revista Brasileira de Armazenamento,** Viçosa, v. 34, n. 2, p. 50-57, Jan./Jun. 2009.

BUFLER, G. Ethylene-promoted conversion of 1- aminocyclopropene-1-carboxylic acid to ethylene in peel of apple at various stages of fruit development. **Plant Physiology,** Rockville, v. 80, p. 539-543, Feb.1986.

CERETTA, M.; BRACKMANN, A.; PINTO, J. A. V.; LUCIO, A. C.; ANESE, R. O. Tolerância de maçãs 'Gala' a pressões parciais extremas de O_2 e CO_2 durante o armazenamento. **Revista Brasileira de Armazenamento,** Viçosa, v. 35, n. 1, p. 60-69, Jul./Dec. 2010.

FRANCK, C.; LAMMERTYN, J.; HO, Q. T.; VERBOVEN, P.; VERLINDEN, B.; NICOLAI, B. M. Browning disorders in pear fruit. **Postharvest Biology and Technology,** Amsterdam, v. 43, p. 1-13, Jan. 2007.

GRAN, C. D.; BEAUDRY, R. M. Determination of the low oxygen limit for several commercial apple cultivars by respiratory quotient breakpoint. **Postharvest Biology and Technology,** Amsterdam, v. 3, p. 259-267, Oct. 1993.

HIWASA. K.; KINUGASA, Y.; AMANO, S.; HASHIMOTO, A.; NAKANO, R.; INABA, A.; KUBO, Y. Ethylene is required for both the initiation and progression of softening in pear (*Pyrus communis* L.) fruit. **Journal of Experimental Botany**, Lancaster, v. 54, p. 771‑779, Feb. 2003. Disponível em: <http://jxb.oxfordjournals.org/content/54/383/771.short>. Acesso em: 14 mar. 2013.

INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA (IBGE). **Banco de dados agregados:** Disponível em:<http://www.sidra.ibge.gov.br/bda/agric/default.asp?t=2&z=t&o=11&u1=1&u2=1&u3=1&u4=1&u5=1&u6=1>. Acesso em: 17 Mar. 2013.

KE, D.; RODRIGUES-SINOBAS, L.; KADER, A. A. Physiology and prediction of fruit tolerance to low-oxygen atmosphere. **Journal of the American Society for Horticultural Science,** Alexandria, v. 116, n. 2, p. 253-260, Mar. 1991. Disponível em: < http://ucce.ucdavis.edu/files/datastore/234-292.pdf>. Acesso em: 14 mar. 2013.

LAU, O. L. Storage procedures, low oxygen and low carbon dioxide atmospheres on storage quality of 'Golden Delicious' apples. **Journal of American Society of Horticultural Science,** Alexandria. v. 110, p. 541-547, Feb. 1985.

ORTIZ, A.; GRAELL, J.; LARA, I. Cell wall-modifying enzymes and firmness loss in ripening 'Golden Reinders' apples: A comparison between calcium dips and ULO storage. **Food Chemistry,** Chicago, v. 128, p. 1072-1079, Oct. 2011.

PAYASI, A.; MISHRA, N. N.; CHAVES, A. L. S.; SINGH, R. Biochemistry of fruit softening: an overview. **Physiology and Molecular Biology of Plants**, Lucknow, v. 15, n. 2, p. 103-113, Apr. 2009. Available from: < http://link.springer.com/article/10.1007%2Fs12298-009-0012-z?LI=true> Accessed: Mar, 02. 2013.

PEREIRA, T.; ALMEIDA, P. S. G.; AZEVEDO, I. G.; CUNHA, M.; OLIVEIRA, J. G.; SILVA, M. G.; VARGAS, H. Gas diffusion in 'Golden' papaya fruit at different maturity stages. **Postharvest Biology and Technology,** Amsterdam, v. 54, p. 123-130, Dec. 2009.

PRASANNA, V.; PRABHA, T. N.; THARANATHAN, R. N. Fruit ripening phenomena ‑ an overview. **Critical Reviews in Food Science and Nutrition**, Boca Raton, v. 47, p. 1‑19, Oct. 2007.

SWEETMAN, C.; DELUC, L. G.; CRAMER, G. R.; FORD, C. M.; SOOLE, K. L. Regulation of malate metabolism in grape berry and other developing fruits. **Phytochemistry,** Oxford, v. 70, p. 1329-1344, Jul./Aug. 2009.

WEBER, A.; BRACKMANN, A.; ANESE, R. O.; BOTH, V.; PAVANELLO, E. P. 'Royal Gala' apple quality stored under ultralow oxygen concentration and low temperature conditions. **Pesquisa Agropecuária Brasileira,** Brasília, v. 46, n. 12, p. 1597-1602, Dec. 2011.

WEBER, A.; BRACKMANN, A.; THEWES, F. R.; BOTH, V.; ANESE, R. O.; SCHORR, M. R. W. Relative humidity and its interaction with the storage temperature of 'Gala' apples and mutants. **Ciência Rural**, v. 42, n. 12, p. 2159-2165, 2012. Available from: < http://www.scielo.br/pdf/cr/2012nahead/a35312cr2012-0007.pdf> Accessed: Mar, 03. 2013.

WRIGHT, H.; DELONG, J.; HARRISON, P. A.; GUNAWARDENA, A. H. L. A. N.; PRANGE, R. The effect of temperature and other factors on chlorophyll *a* fluorescence and the lower oxygen limit in apples (*Malus domestica*). **Postharvest Biology and Technology**, Amsterdam, v. 55, p. 21‑28, Jan. 2010.

YANG, S. F.; HOFFMAN, N. E. Ethylene biosynthesis and its regulation in higher plants. **Annual Review of Plant Physiology,** Palo Alto, v. 35, p. 155-189, Jun. 1984.

YEARSLEY, C. W.; BANKS, N. H.; GANESH, S. Temperature effects on the internal lower oxygen limits of apple fruit. **Postharvest Biology and Technology**, Amsterdam, v. 11, p. 73-83, Jun.1997

YEARSLEY, C. W.; BANKS, H. K.; GANESH, S.; CLELAND, D. J. Determination of lower oxygen limits for apple fruit. **Postharvest Biology and Technology,** Amsterdam, v. 8, p. 95-109, May. 1996.