

Storability of 'Tupy' and 'Guarani' blackberries in controlled atmosphere

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ABSTRACT: The aim of this work was to compare the storage potential of 'Guarani' and 'Tupy' blackberries in controlled atmosphere (CA) and thereafter to determinate the best CA storage conditions for 'Tupy' blackberries. Two experiments were carried out. In the first one, 'Guarani' and 'Tupy' blackberries were stored in CA condition (10 kPa O₂ + 15 kPa CO₂). We found that 'Tupy' blackberry has the best storage potential. Therefore, in the second experiment, several CA conditions were evaluated for 'Tupy' blackberries storage, as follows: [1] cold storage (CS): 20.9 kPa O₂ + 0.04 kPa CO₂; [2] CA with 10 kPa O₂ + 15 kPa CO₂; [3] CA with 5 kPa O₂ + 15 kPa CO₂; [4] CA with 10 kPa O₂ + 10 kPa CO₂. All treatments were stored at 1 °C and

98% (± 1) of relative humidity. The 'Tupy' blackberry, despite showing a significant decrease in ascorbic acid content, presented a higher storage potential than 'Guarani' blackberry, if stored at recommended CA condition for other cultivars. 'Tupy' blackberry stored at 5 kPa O₂ + 15 kPa CO₂ showed higher ascorbic acid content, soluble solids and titratable acidity as compared to the other storage conditions. In CA with 10 kPa O₂ + 15 kPa CO₂, the juice was lighter (high L), but differed significantly only from those fruit of CS. The best CA condition for 'Tupy' blackberries storage is 5 kPa O₂ with 15 kPa CO₂.

Key words: *Rubus* spp., oxygen, carbon dioxide, conservation, small fruit.

INTRODUCTION

Among the small fruits, blackberries (*Rubus* spp.) show significant production in Brazil (Antunes et al. 2014), which has important nutraceutical properties, providing ascorbic acid, anthocyanins and phenolic compounds, including ellagic acid for the consumers. These components contribute to human health due to their high antioxidant activity (Krüger et al. 2011). In Brazil, the 'Tupy' and 'Guarani' cultivars are produced for fresh consumption; they have a black color and average weight from 5 up to 6 g and show a significant yield (Antunes 2002). Additionally, 'Tupy' is the most important cultivar produced in Brazil (Antunes et al. 2014).

Fragile structure and high respiration rate lead to a short postharvest life, limiting the commercialization of fresh blackberry in the fruit market (Cia et al. 2007). Cold storage (CS) did not maintain efficiently bioactive compounds, and a significant antioxidant activity reduction is verified after storage, requiring other storage techniques together with CS

to improve postharvest life and keep quality of blackberry after storage (Antunes et al. 2014). Cold storage associated with modified atmosphere packaging (MAP) or controlled atmosphere (CA) storage may result in additional quality maintenance (Schaker and Antonioli 2009). Antunes et al. (2003) reported that 'Brazos' and 'Comanche' blackberries were stored during nine days at 2 °C in MAP, where cv. Comanche presented higher ascorbic acid than cv. Brazos after storage, but there are little reports in literature about the storage of blackberry in CA. Because of the difference among cultivars, it is important to evaluate the storage potential of the distinct blackberries cultivars. Actually, there are only general recommendations for the blackberries storage conditions, which lead to a short storage period and significant quality losses after storage and shelf life.

CA storage is a complementary technique to cold storage, where low O₂ and high CO₂ partial pressures are performed around the fruit, aiming to reduce its metabolism and the proliferation of pathogenic microorganism (Brackmann et al. 2012). Kader (2002) recommends O₂ partial pressure from 5 up →

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to 10 kPa and CO₂ from 15 up to 20 kPa for blackberries storage, considering that high CO₂ partial pressure (> 20 kPa) reduces the ascorbic acid content (Agar et al. 1997). Low O₂ and high CO₂ partial pressures are crucial to lower respiration rate. Low O₂ inhibits cytochrome-C oxidase in electron transport chain (Gupta et al. 2009) and high CO₂ reduces isocitrate dehydrogenase enzyme in tricarboxylic acid cycle (Liu et al. 2004), leading to a dramatically metabolism reduction during storage.

Although each cultivar can differ in their optimal requirements for O₂ and CO₂ partial pressures, it is necessary to establish the best CA conditions for blackberry cultivars produced in Brazil. Albeit the CA storage is widely used in pomaceous fruits storage around the world, in Brazil it is not used yet for blackberry. However, it is very important to evaluate its efficiency in blackberry quality maintenance to recommend its adoption by fruit growers. This will provide a great economic impact, by reducing postharvest losses, and offer high quality products to the consumers.

Therefore, the objective of this work was to compare the storage potential of 'Guarani' and 'Tupy' blackberries in CA. Additionally, we determined the best CA storage conditions for 'Tupy' blackberries.

MATERIALS AND METHODS

Fruit harvest and sample selection

The work was carried out with 'Tupy' and 'Guarani' blackberries, harvested in an experimental orchard. Thereafter, the samples were submitted to a selection process aiming to eliminate fruit with mechanical injury due to the transportation. Two experiments were conducted in a completely randomized design, with six replicates of 0.2 kg per treatment.

Experiment 1: comparison of the storage potential of 'Tupy' and 'Guarani' blackberries in CA condition

In the first experiment it was carried out a comparison between the two cultivars stored in the same CA condition (10 kPa O₂ + 15 kPa CO₂) at 1 °C and relative humidity (RH) of 98% (± 1). This CA condition was chosen according to Perkins-Veazie and Collins (2002). The harvest stage for the

two cultivars was set when fruit showed 100% of black-color skin, which is the commercial harvest stage.

Experiment 2: controlled atmosphere conditions for 'Tupy' blackberries

In the second experiment, different controlled atmosphere conditions were evaluated for cultivar Tupy: [1] CS: 20.9 kPa O₂ + 0.04 kPa CO₂; [2] CA with 10 kPa O₂ + 15 kPa CO₂; [3] CA with 5 kPa O₂ + 15 kPa CO₂; [4] CA with 10 kPa O₂ + 10 kPa CO₂. All treatments were stored at 1 °C and 98% (± 1) of RH.

Experimental chambers, CA achieving and maintenance

The fruit were stored in hermetically sealed 5-L experimental chambers, which were placed inside a cold storage room, at 1 °C (± 0.1). The temperature was controlled by thermostats and checked daily with bulb mercury thermometers with a 0.1 °C resolution inserted in the blackberry flesh. The dilution of O₂ in the storage chamber was done with injection of N₂ obtained from a pressure swing adsorption (PSA) nitrogen generator (Janus & Pergher, Porto Alegre, RS, Brazil). The desired CO₂ partial pressure was obtained by gas injection from a high-pressure cylinder until the pre-established concentration. The CA conditions were maintained by a gas control equipment (Kronenberger/ Climasul, Caxias do Sul, RS, Brazil). The O₂ consumed by respiration was replaced by atmospheric air injection into the chambers, and excessive CO₂ produced by respiration was absorbed by a 40% potassium hydroxide solution.

Quality evaluation after storage

Fruit quality and ripening were evaluated after 12 days of storage, plus two days of shelf life at 20 °C. The parameters assessed were: a) mass loss: obtained by the difference between the total mass before and after the storage, data expressed in percentage of total mass; b) healthy fruit: determined by the total fruit minus the fruit that showed any symptom of decay incidence, damage, wilting and juice leakage, results expressed in percentage of total fruit; c) soluble solids (SS): obtained by refractometry, results expressed in °Brix; d) titratable acidity: determined by titration of a solution that →

contained 10 mL of juice diluted in 100 mL of distilled water, with a solution of NaOH 0.1 N, results expressed in percentage of citric acid; e) ascorbic acid: determined by titration, with potassium iodate 0.01 N, of a solution with 12 g of blackberry juice, 20 mL of sulfuric acid at 20%, 1 mL of potassium iodide at 10% and 1 mL of starch solution at 1%. The following formula was used: ascorbic acid = $(100 \times V \times F) / P$, where: V = potassium iodate consumption (mL); F = potassium iodate equivalence factor according to normality (0.8806) and P = samples weight (g), results were expressed in mg of ascorbic acid per 100 g of fruit pulp; f) phenolic compounds: assessed using the Folin-Ciocalteu phenol reagent method (Singleton and Rossi Junior 1965); 5 mL of bidistilled water and 500 μ L of Folin-Ciocalteu reagent were added to 200 μ L of the samples. After 30 s to 8 min, 1.5 mL of sodium carbonate was added. The extract was left to rest for 30 min at 40 °C. A calibration curve in several dilutions was made based on the absorbance measured at 765 nm (with a spectrophotometer FEMTO, model 600S). The total phenolic content was expressed as gallic acid equivalents (GAE) in $\text{mg}\cdot\text{L}^{-1}$; g) respiration rate: determined at chamber opening and after 2 days of shelf life by CO_2 production. The fruits were placed into containers, with 0.8 L of volume, hermetically sealed for approximately one hour. The air of the container was circulated through an electronic CO_2 analyzer, with an infrared gas analyzer (IRGA) system (Agri-datalog, Lana, BZ, Italy). Based on CO_2 concentration, free room inside the container, fruit weight and closure time, the respiration rate was calculated and expressed in $\text{mL CO}_2\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$; h) juice color: determined with a Minolta colorimeter, model CR 310, with tridimensional color system (CIE L a b). The results were expressed by L, which represents color lightness (0 = black and 100 = white), by the a^* -value, which is ranged from green (-a) to red (+a), by the b^* -value axis, which is ranged from blue (-b) to yellow (+b), and hue angle.

Statistical analysis

All data were subjected to a multivariate analysis via principal component analysis (PCA) using the Genes software (Viçosa, UFV). Before multivariate analysis, the data matrix was auto scaled for each variable in order to assume the same weight during analysis. In addition, an analysis of variance (ANOVA) was performed for all the parameters evaluated. Significant parameters averages were compared through Tukey's test ($p < 0.05$). The data expressed in percentage

were transformed by the formula $\arcsin \sqrt{(x + 0.5)/100}$, before the ANOVA.

RESULTS AND DISCUSSION

Comparison of the storage potential of 'Tupy' and 'Guarani' blackberries in CA storage (experiment 1)

Comparing 'Tupy' and 'Guarani' blackberries stored in CA, the 'Tupy' blackberries showed a higher healthy fruit percentage and phenolic compounds content as compared to cv. Guarani (Table 1). Another important result is that 'Tupy' had lower SS and titratable acidity degradation than 'Guarani' blackberries in relation to initial analysis. Besides, 'Tupy' blackberry increased phenolic compounds during the storage. High phenolic compounds in 'Tupy' blackberry lead to more benefits to human health concerning this cultivar after storage in relation to 'Guarani', once these compounds have high antioxidant activity (Krüger et al. 2011).

Furthermore, the characteristics associated with the color of 'Tupy' blackberries seem to be more suitable due to lower L and b^* -values, indicating a dark violet color. However, the ascorbic acid was lower in this cultivar, indicating higher ascorbic acid degradation during 'Tupy' blackberries storage as compared to that of 'Guarani'. This result shows a distinct behavior between the blackberries during storage, with lower ascorbic acid degradation in 'Guarani'. Evaluating other cultivars, Antunes et al. (2003) found higher ascorbic acid content in 'Comanche' cultivar as compared to 'Brazos'.

Thereby, despite showing a significant reduction in ascorbic acid content, if stored in recommended CA condition for other cultivars, 'Tupy' blackberry has a better storage potential in relation to 'Guarani'. This result shows a practical application for fruit growers, once they can choose the cultivar with better storability, especially if they are distant of the market, handling and consumers. Taking into account that 'Tupy' blackberries have potential to storage, we evaluated CA conditions for this cultivar in experiment 2, aiming to maintain the highest quality after storage and shelf life.

Controlled atmosphere conditions for 'Tupy' blackberry storage (experiment 2)

In order to better visualize the effect of the controlled atmosphere conditions on quality parameters, we performed an →

exploratory multivariate analysis — the (PCA). Principal components I and II (PC I and PC II) explained the 49.94 and 38.59% of the overall variance, respectively (Figure 1). These analyses allowed a separation according to storage condition, where, along PC I, cold storage and 5 + 15 (kPa O₂ + CO₂) condition were located in opposite sides and CA

with 10 kPa O₂ (10 + 10 and 10 + 15) condition in the center of the PC I plot (Figure 1a). The left-hand side of PC I, which is related to 5 + 15, exhibited high titratable acidity, ascorbic acid and SS (Figure 1b). On the other hand, the parameters related with cold storage were mass loss and respiration rate at 2 days of shelf life (Figure 1c). CA condition

Table 1. Physic and chemical quality of 'Guarani' and 'Tupy' blackberry fruits after 12 days of controlled atmosphere (10 kPa O₂ + 15 kPa CO₂) storage at the temperature of 1 °C plus two days of shelf life at 20 °C.

Parameter	Cultivars						CV (%)
	Initial analysis		After storage		Change during storage		
	Guarani	Tupy	Guarani	Tupy	Guarani	Tupy	
Mass loss (%)	0.0 ± 0.00	0.0 ± 0.00	1.27 ± 0.70 ^{ns}	1.70 ± 1.40	-1.27	-1.70	74.2
Healthy fruits (%)	100 ± 0.00	100 ± 0.00	71.8 ± 3.67*	78.7 ± 3.49	-28.2	-21.3	4.76
Soluble solids (°Brix)	7.75 ± 0.07	7.0 ± 0.00	7.1 ± 0.08 ^{ns}	7.0 ± 0.14	-0.65	0.00	1.65
Titratable acidity (% citric acid)	1.16 ± 0.17	1.0 ± 0.04	0.80 ± 0.03 ^{ns}	0.84 ± 0.04	-0.36	-0.16	4.78
Ascorbic acid (mg·100 g ⁻¹)	63.4 ± 1.24	70.9 ± 0.62	31.5 ± 0.73*	13.9 ± 2.81	-31.9	-57.0	9.05
Respiration rate ^I (mL CO ₂ ·kg ⁻¹ ·h ⁻¹)	ND	ND	36.7 ± 3.51 ^{ns}	40.2 ± 2.92	-	-	8.40
Respiration rate ^{II} (mL CO ₂ ·kg ⁻¹ ·h ⁻¹)	ND	ND	54.6 ± 5.74 ^{ns}	56.4 ± 7.17	-	-	11.7
Phenolic compounds (mg·L ⁻¹)	10.2 ± 0.15	9.92 ± 0.07	9.5 ± 0.13*	10.9 ± 0.25	-0.70	+0.98	1.97
Luminosity (L)	26.2 ± 0.50	29.8 ± 1.0	37.6 ± 2.73*	31.3 ± 3.35	+11.4	+1.50	8.87
a*-values	10.3 ± 1.53	18.2 ± 0.37	41.4 ± 0.82*	29.9 ± 7.50	+31.1	+23.2	15.0
b*-values	3.19 ± 0.87	6.67 ± 0.22	24.3 ± 1.64*	11.5 ± 5.69	+21.1	+4.83	23.4

*Significant difference between the two cultivars by F test at 5% of probability of error; ^{ns}No significant difference between the two cultivars by F test at 5% of probability of error. Numbers after ± mean standard deviation. ^IRespiration rate at the opening of chambers; ^{II}Respiration rate after two days of shelf life; -Reduction during storage; +Increase during storage. ND = not determined.

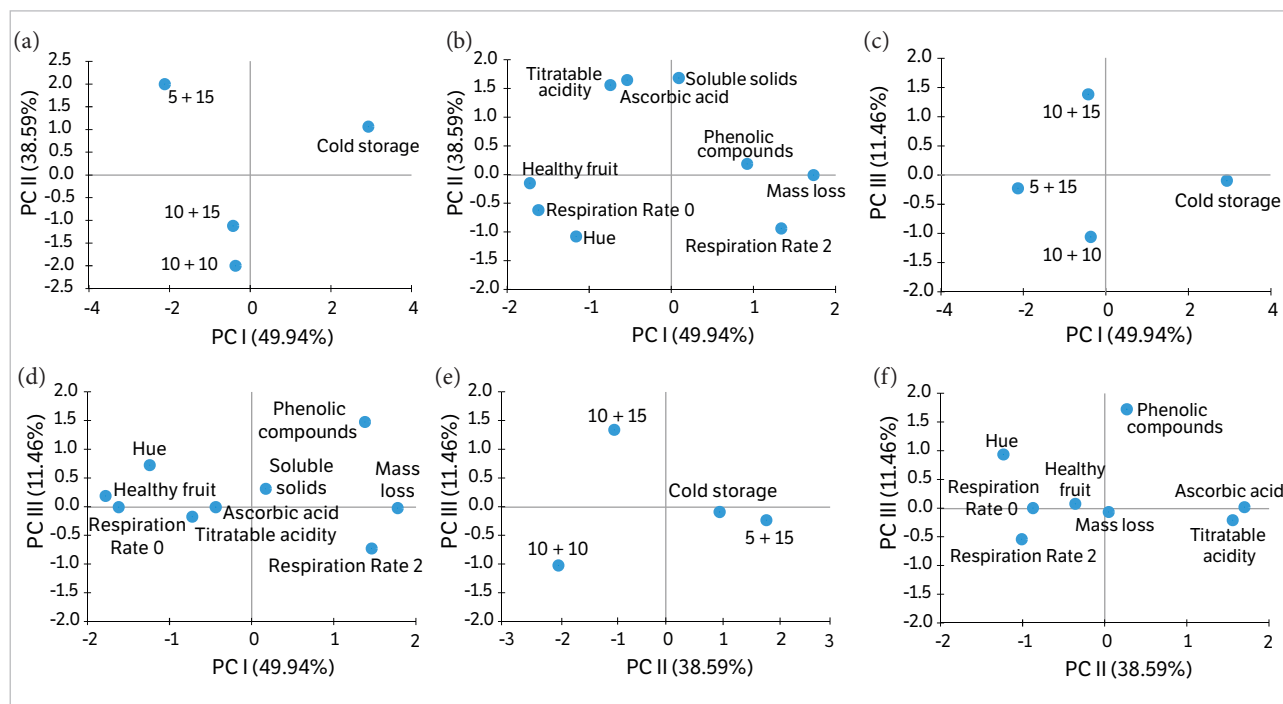


Figure 1. (a), (c) and (e) – Scores of treatments; (b), (d) and (f) – Loadings of variables showing the three major principal components from the analysis of 'Tupy' blackberry fruit after 12 days of controlled atmosphere storage at the temperature of 1 °C plus two days of shelf life at 20 °C.

of 5 + 15 resulted in high healthy fruits, titratable acidity, ascorbic acid and hue (Figure 1d). Therefore, low O₂ partial pressure was responsible to reduce degradation metabolism, which results in fruits with high titratable acidity, ascorbic acid and, consequently, more healthy fruits. According to PCII and PCIII, cold storage condition is located at the same position of 5 + 15, but, if PCI and PCIII are observed, these conditions stay in opposite sides (Figures 1c,d). PCII and PCIII strengthen positive relation between low O₂ partial

pressure (5 + 15) and ascorbic acid content (Figure 1e). Ascorbic acid is an important reactive oxygen species scavenging metabolites during fruit ripening (Mondal et al. 2009). Furthermore, this compound has crucial benefits for human health.

Mass loss (ML) was lower in blackberry stored under CA, where the fruit stored under 5 kPa O₂ + 15 kPa CO₂ had 0.9% of ML in relation to 4.45% of ML in fruits that were stored under CS (Figure 2a). ML directly results in →

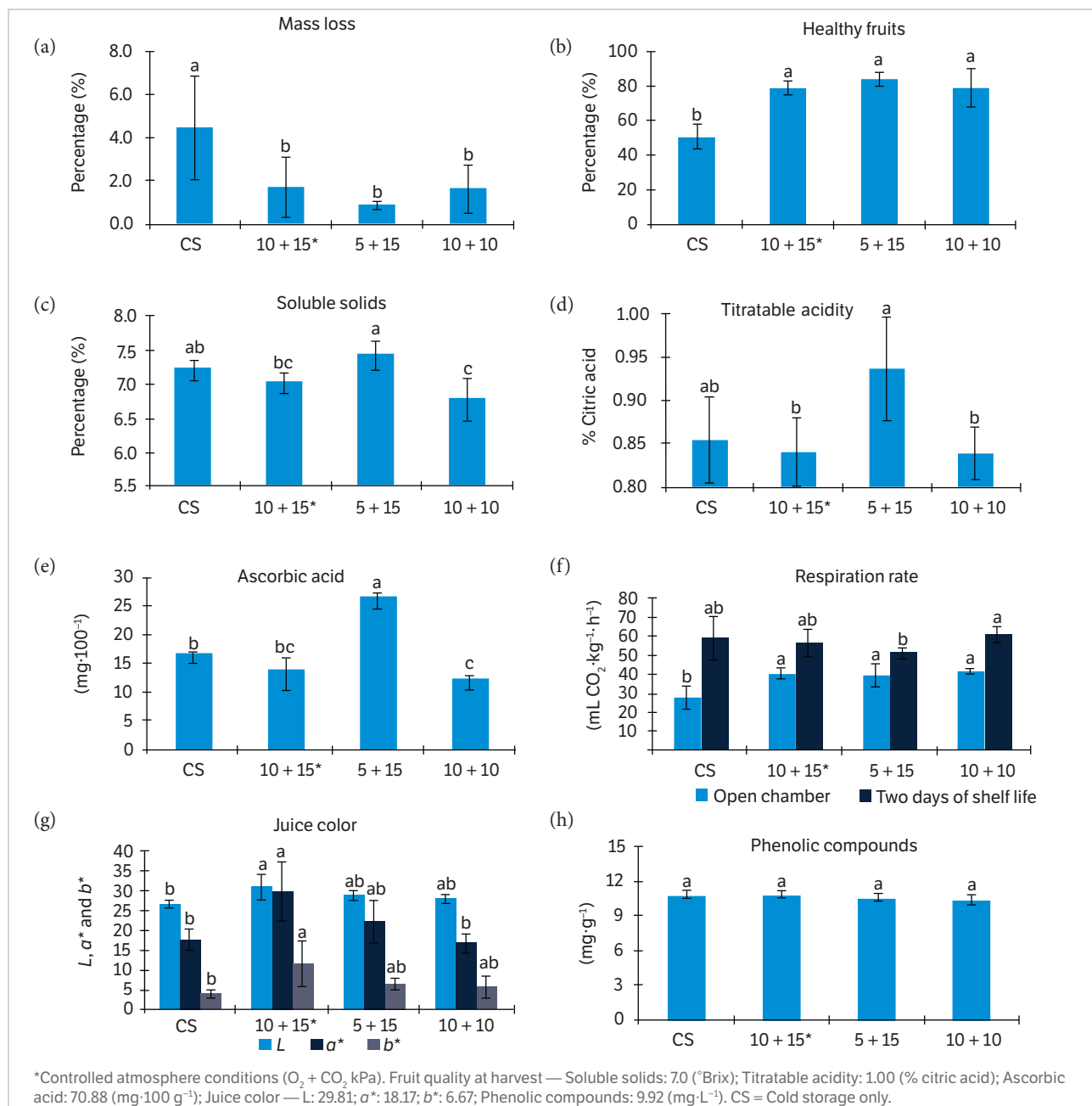


Figure 2. Physic and chemical qualities of 'Tupy' blackberry fruit after 12 days of controlled atmosphere storage at the temperature of 1 °C plus two days of shelf life at 20 °C. Bars mean standard deviation.

economic losses, thus the CA reduces this loss, independently of the CA condition adopted. ML occurs mainly due to transpiration and, at a lower intensity, due to respiration; however, in this case, most of the ML is related to respiration, because of the high RH ($98 \pm 1\%$) inside the CA chamber (Maguire et al. 2000). Brackmann et al. (2014) found that transpiration is the main responsible for ML in 'Fuyu' persimmons, stored with 95% of RH, which represents about 76% of all ML, and the remaining 24% are due to carbon losses by respiration rate.

Blackberries submitted to CA also presented a higher percentage of healthy fruits, with lesser decay incidence, damage, wilting, juice leakage, contributing to the marketing quality after two days of shelf life if compared to CS fruits. Usually SS and acidity are reduced after harvest (Tosun et al. 2008); these parameters are closely related to eating quality (Chitarra and Chitarra 2005), so the higher these two parameters the better the storage. Fruit stored under 5 kPa O₂ + 15 kPa CO₂ showed higher SS and titratable acidity after storage (Figures 2c,d). Perhaps this CA condition resulted in a lower metabolism during the storage and especially during the shelf life period, explained by the low respiration rate (Figure 2f). Some previous studies also suggest that the lower the respiratory rate the higher the SS and titratable acidity (Chitarra and Chitarra 2005; Steffens et al. 2007).

Ascorbic acid content of blackberries was also higher at 5 kPa O₂ + 15 kPa CO₂ (Figure 2e). Brackmann et al. (2012) found an increase in ascorbic acid during CA storage in 'Paluma' guava, but, in the present study, the opposite was observed: a reduction in ascorbic acid level after storage, being less significant under 5 kPa O₂ + 15 kPa CO₂. Importantly, if 'Tupy' blackberry was stored in high O₂ partial pressure (10 kPa), the fruit showed 13.9 mg·100 g⁻¹ of ascorbic acid; the fruit kept with low O₂ partial pressure (5 kPa) showed 25 mg·100 g⁻¹ of ascorbic acid, which supports the CA use with low O₂ to supply blackberry to the market with high quality. In this work, high CO₂ partial pressure did not reduce ascorbic acid in 'Tupy' blackberry. This result is not in accordance

with Agar et al. (1997), which found that high CO₂ reduces ascorbic acid during blackberry fruit storage.

The respiration rate, which was evaluated by the CO₂ production, was higher in CA-stored fruit at the opening of the chambers, likely due high CO₂ partial pressure inside the fruit flesh, since it remained with 10 or 15 kPa CO₂ partial pressure during the storage time (Figure 2f). After two days of shelf life at 20 °C, fruit stored with 5 kPa O₂ + 15 kPa CO₂ showed the lowest respiration rate, but did not differ from CS and 10 kPa O₂ + 15 kPa CO₂. Probably the lowest respiration rate by fruit submitted to these two CA conditions has relation with the oxygen level employed in these conditions. In other fruits, some early studies verified a reduction in respiration rate with the O₂ partial pressure reduction (Gupta et al. 2009; Brackmann et al. 2012).

In CA condition with 10 kPa O₂ + 15 kPa CO₂, the juice was lighter (high L), but differed significantly only from those stored in CS (Figure 2g). Concerning *a**-value, fruit stored in CA with 10 kPa O₂ + 15 kPa CO₂ showed redder color (high *a**-value), but did not differ from the CA condition with 5 kPa O₂ + 15 kPa CO₂. According to Tosun et al. (2008), the *a**-values increased at the beginning and decreased at the end of the fruit ripening, when fruit acquired a violet color. The *b**-values were higher in fruit stored in CA with 10 kPa O₂ + 15 kPa CO₂; however, they did not differ as compared to the other CA conditions, which indicates a less blue color. Phenolic compounds were not affected by the CA conditions evaluated in the present experiment (Figure 2h).

CONCLUSION

The 'Tupy' blackberry, despite showing a significant decrease in ascorbic acid content, presents a higher storage potential as compared to 'Guarani' blackberry. The best controlled atmosphere condition for 'Tupy' blackberry storage is 5 kPa O₂ with 15 kPa CO₂. This condition is efficient to maintain high ascorbic acid after storage.

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