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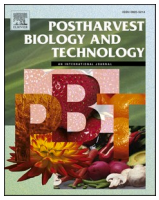

Anaerobic compensation point can effectively extend 'Palmer' mango shelf-life in CA storage

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ABSTRACT

The objective of this study was to identify the anaerobic compensation point (ACP) required to maximally inhibit aerobic respiration and extend postharvest life of 'Palmer' mangos produced on 'Espada' rootstock in the summer and winter growing seasons in Brazil. The study was composed by two experiments. The first was carried out to determine the ACP (minimum pO_2 required to maximally inhibit aerobic respiration) of 'Palmer' mangos during storage at 9 °C. The second was carried out to validate the minimum pO_2 to store the fruit under dynamic controlled atmosphere (DCA). In the first experiment, the fruit were harvested at the commercial maturity in the summer and winter growing seasons and stored for 49 days at 9 °C with 90–95 % relative humidity. Every week, the fruit were hermetically sealed in 20 L containers, where O_2, CO_2, and ethanol concentrations were monitored during 35 h. The minimum pO_2 required to maximally inhibit fruit aerobic respiration was determined at the beginning of ethanol production, which indicates a shift of fruit aerobic to anaerobic respiration (ACP). The minimum pO_2 to efficiently inhibit aerobic respiration of 'Palmer' mangos ranged from 0.3 kPa to 4.7 kPa in summer and 1.75–11.15 kPa in winter growing seasons. Fruit harvested in the following growing season and stored in DCA with the minimum pO_2 showed lower aerobic respiration, mass loss, as well as better maintenance of skin and pulp color, firmness, soluble solids (SS), titratable acidity (TA), and SS/TA ratio, compared to fruit stored only under refrigerated atmosphere at 9 °C for 60 days.

1. Introduction

Mango (*Mangifera indica* L.) is the second most produced tropical fruit in the world, being an important source of nutrients with functional and health benefits (Brecht and Yahia, 2009; Vilvert et al., 2023). Brazil is one of the main producers, exporters and consumers of mango, occupying the 6th position worldwide, with the Northeast region being responsible for most of the fruit production in the country (Brazilian Horti and Yearbook., 2023; Sanches et al., 2024).

Although mangos are produced and consumed all over the world, many producing regions have to transport the fruit to long-distance markets, as is the case with mangos produced in Brazil. In addition, mango has short postharvest life, which is characterized by accelerated metabolism and intense ripening-related changes (Liu et al., 2022), which are important to achieve high consumer quality, but also limit fruit commercialization in distant markets (Bambalele et al., 2021; Vilvert et al., 2023).

Refrigeration is the most important technology to maintain postharvest fruit quality (Pereira et al., 2020). However, in the case of mangos, refrigeration alone is not sufficient to reduce fruit metabolic activity and extend postharvest life, when the fruit are intended for distant markets that require transportation time above 30 days (Nadeem et al., 2022; Vilvert et al., 2023). In that case, other technologies must also be applied to effectively inhibit fruit metabolism and increase postharvest life.

Controlled atmosphere (CA) has been used, together with

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refrigeration, to reduce oxygen partial pressure (pO_2) and increase carbon dioxide partial pressure (pCO_2) in the storage environment to further inhibit the metabolic activity and extend postharvest life of different fruit species (Mditshwa et al., 2018; Thewes et al., 2021). Previous studies have applied static pO_2 levels throughout storage to maintain the quality of mangoes (Teixeira and Durigan, 2011; Hailu, 2016; Ntsoane et al., 2019; Bender et al., 2000; Bender et al., 2021). However, a more recent study accomplished with 'Tommy Atkins' mango has shown that pO_2 needs to be constantly monitored and adjusted according to fruit metabolism to maximally inhibit aerobic respiration and extend postharvest life of the fruit, technology known as dynamic controlled atmosphere (DCA) (Santos et al., 2023).

The DCA optimizes fruit storage conditions, considering that the ideal pO_2 for quality maintenance varies according to several factors, including genotype, fruit maturity, growth regulators, temperature, metabolic stress and others (Wright et al., 2015). In the DCA, the pO_2 is gradually reduced until it reaches the anaerobic compensation point (ACP), which is the lowest pO_2 concentration tolerated by the fruit, below which the respiratory metabolism changes from aerobic to anaerobic (Deuchande et al., 2016).

The definition of ACP is key in the establishment of DCA conditions, considering that a precise monitoring of the critical pO_2 level is essential for a long storability and maintenance of fruit postharvest quality without leading to the development of irreversible damages and off-flavors caused by anaerobic metabolism. The critical pO_2 level can be determined by measuring the production of anaerobic metabolism compounds such as ethanol (Schouten et al., 1998), monitoring chlorophyll fluorescence (Prange et al., 2002), determining the respiratory quotient (CO_2/O_2 ratio) (Gabioud et al., 2009), or measuring the CO_2 production during fruit storage (Thewes et al., 2021). DCA storage, determined based on ethanol production, has been reported to effectively preserve the postharvest quality of different fruit species (Schouten et al., 1998; Veltman et al., 2003), as well as 'Tommy Atkins' mango produced in different growing seasons (Santos et al., 2023). However, there are no reports on the anaerobic compensation point (ACP) and efficiency of DCA to maintain postharvest quality of 'Palmer' mangos intended to distant markets.

The DCA has been widely used to store apples, effectively maintaining quality and reducing postharvest losses of fruit, from harvest to marketing. However, there is limited information on the application of DCA to tropical fruits, such as mango, papaya, avocado, and others, which are highly perishable and generally require storage at relatively high temperatures to avoid chilling injury. This represents a significant knowledge gap, since tropical fruit production and export are rapidly expanding in global markets, and the development of advanced storage strategies is crucial for reducing postharvest losses. Therefore, studies on DCA in mango not only contribute to filling this technological gap, but also provide insights that can be extended to other tropical commodities.

The objective of this study was to identify the anaerobic compensation point (ACP) required to maximally inhibit aerobic respiration and extend postharvest life of 'Palmer' mangos produced in the summer and winter growing seasons.

2. Material and methods

2.1. Fruit and storage conditions

'Palmer' mangos (*Mangifera indica* L.) were produced on 'Espada' rootstock during the summer (December to March) and winter (June to September) growing seasons in the same commercial orchard in the São Francisco Valley (SFV), Petrolina, PE, Brazil (latitude of 9°20'58.8" S, longitude of 40°33'16.7" W, altitude of 376 m). The plants were six years old. The summer growing season was characterized by temperatures ranging from 22.4 °C to 34.1 °C, relative humidity ranging from 31.7 % to 88.2 %, and global solar radiation ranging from 18.0 MJ m^{-2} to 22.1 MJ m^{-2}. The winter growing season was characterized by temperatures ranging from 19.3 °C to 30.5 °C, relative humidity ranging from 34.3 % to 92.2 %, and global solar radiation ranging from 14.4 MJ m^{-2} to 18.5 MJ m^{-2}. Standard crop management practices were applied to all growing seasons, including pruning, irrigation, fertilization, and control of pests and diseases, following technical recommendations. The study was composed by two experiments. The first was carried out to determine the ACP (minimum pO_2 required to maximally inhibit aerobic respiration) of 'Palmer' mango during storage at 9 °C, based on the presence of ethanol gas in the storage environment. The second was carried out to validate the minimum pO_2 under DCA.

In both experiments, medium-sized fruit (400–535 g) were harvested at the physiological maturity, characterized by full shoulders and light green skin color (National Mango Bord, 2010). After harvest, the fruit were transported to the Postharvest Laboratory at the Brazilian Agricultural Research Corporation (EMBRAPA), Petrolina, PE, Brazil, where fruit without any visual defect were non-destructively homogenized based on dry matter content, using a Vis-NIR spectrometer model F-750 (Felix Instruments, Cama, WA, USA), previously calibrated for 'Palmer' mangos (Freitas et al., 2022). In both growing seasons, fruit with dry matter content higher then 130 g kg^{-1} were selected and sanitized with sodium hypochlorite at 200 μL L^{-1} for 15 min, before storage.

2.2. Experiment 1. minimum pO_2 to maximally inhibit 'Palmer' mango aerobic respiration

A total of 728 fruit, harvested in the summer and winter growing seasons, were placed in commercial cardboard boxes and stored at 9 °C (±0.5 °C) with 90–95 % relative humidity for 49 days to simulate long distance transport. Temperature and relative humidity were continuously monitored by an electronic data logger, confirming that the actual values remained within ± 0.5 °C and ± 5 % of the set points throughout the storage period. At harvest and weekly thereafter at 9 °C (±0.5 °C), four samples of 26 fruit each were hermetically sealed in 20 L polyethylene chambers. The chambers were equipped with a rubber septum, an internal mini fan for air homogenization, and two sachets (20 g) of potassium permanganate (99 %).

2.2.1. Respiratory quotient (RQ), O_2, CO_2 and ethanol levels

After 24 h of hermetically sealing the chambers, the pO_2 and pCO_2 were monitored with a gas analyzer model PA 7.0 (WITT-Gasetechnik GmbH & Co KG, Witten, Germany), and the internal ethanol concentration was monitored with a portable gas analyzer model X-am® 5000 (Drägerwerk AG & Co, Lübeck, Germany). The RQ was determined as the ratio between consumed pO_2 and produced pCO_2. The gaseous measurements were accomplished every hour until the detection of ethanol that indicates of transition from aerobic to anaerobic fruit respiration, anaerobic compensation point (ACP). All gases were measured at storage temperature, 9 °C.

2.2.2. Atmosphere with CO_2 absorption

The minimum pO_2, at the starting point of ethanol synthesis in the fruit, was also evaluated with CO_2 absorption carried out with two sachets of 100 g calcium hydroxide inside each chamber. Under these conditions, pO_2 and ethanol emission were monitored as mentioned previously. The atmosphere with CO_2 absorption was established after the analysis described previously without CO_2 absorption.

2.2.3. Fruit quality analyses

At harvest and every week during storage, after determining the ACP, fruit samples were analyzed for skin and pulp color, pulp firmness, soluble solids (SS), titratable acidity (TA) and SS/TA ration, as described below.

2.3. Experiment 2. validation of DCA conditions for 'Palmer' mango

The ACP determined in the previous year was used to establish the

DCA conditions for 'Palmer' mangos produced in the following winter growing season in the next year in the SFV. Medium-sized fruit were harvested at physiological maturity and stored at 9 °C ± 0.5 °C with 90–95 % relative humidity for 60 days under refrigerated atmosphere (RA) or DCA. Each storage condition was composed of four replications of 24 fruit. Fruit under RA were stored in plastic trays, and those under DCA were stored in hermetically sealed 20 L polyethylene chambers. Each chamber contained one sachet with 100 g of calcium hydroxide and other with 150 g of potassium permanganate to absorb CO_2 and ethylene, respectively.

2.3.1. Establishment and maintenance of DCA conditions

A continuous humidified flow system (500 mL min^{-1}) was adopted for the maintenance of the DCA. Compressed air (21 %) and high purity (99.99 %) gas cylinder (White Martins Gases Industriais Ltda, Brazil) were the sources of O_2 and N_2, respectively. Both O_2 and N_2 were manually mixed to obtain the minimum pO_2 required to maximally inhibit 'Palmer' mango aerobic respiration, as determined in the Experiment 1.

The safe pO_2 at the beginning of DCA was set at 3.5 kPa, which was determined as the average ACP (3.2 kPa) plus 10 % (0.3 kPa). In that case, the safe pO_2 takes into account the 10 % variability in the ACP levels observed at harvest in fruit produced in the same growing season in previous year. The O_2, CO_2 and ethanol levels in the DCA chambers were monitored twice a day throughout storage, as described above (Fig. 1). The pO_2 was maintained throughout storage at 3.5 kPa because the ethanol production remained below 500 μL L^{-1} (Weber et al., 2020; Thewes et al., 2021). The fruit were analyzed at harvest (0), 15, 30, 45 and 60 days of storage, as well as plus seven days of shelf life at 25 °C (± 0.5 °C).

2.4. Fruit quality analyses

2.4.1. Mass loss

Mass loss was calculated by multiplying the difference between the initial mass and the mass at the end of the storage by 100 and dividing by the initial mass. Mass loss was expressed as percentage.

2.4.2. Respiration rate

The respiration rate was determined considering the difference in CO_2 partial pressure inside the chamber and ambient conditions, fruit weight and time the chamber remained hermetically closed for CO_2 accumulation. The measurement was carried out with a gas analyzer model PA 7.0 (WITT-Gasetechnik GmbH & Co KG, Alemanha). The results were expressed in mg CO_2 kg^{-1} h^{-1}.

2.4.3. Color

The skin and pulp color were evaluated middle portion of the fruit, with a colorimeter model CR 400 (Konica Minolta, Tokyo, Japan). The values were expressed as the hue angle (°h), which varies from 0° to 360°, where 0/360° represents red, 90° represents yellowish green, 180° represents turquoise blue and 270° represents violet (McGuire, 1992).

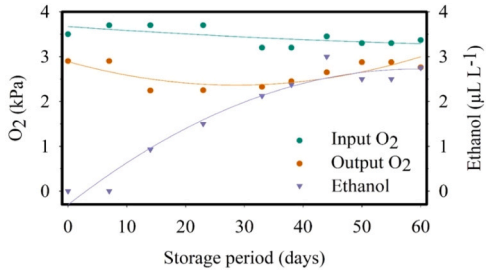

Fig. 1. Input and output O_2 partial pressure and ethanol concentration in DCA chambers used to store 'Palmer' mangos at 9 ± 0.5 °C for 60 days.

2.4.4. Pulp firmness

Pulp firmness was determined with a texture analyzer model TA. XTplus (Stable Micro Systems, Godalming, United Kingdom). The epidermis of the equatorial region was removed and two measurements per fruit were carried out with a 6 mm diameter stainless steel probe and a penetration distance of 10 mm, adopting a test speed of 2.00 mm s^{-1}. The firmness was expressed in Newtons (N).

2.4.5. Soluble solids (SS), titratable acidity (TA) and SS/TA ratio

Pulp samples were taken from the equatorial region of the fruit for juice processing. Soluble solid (SS) was evaluated in 1 mL of fruit juice, using a digital refractometer model PAL-1 (Atago, Tokyo, Japan) with automatic temperature compensation. The results were expressed as a percentage.

Titratable acidity (TA) was assessed using an automatic digital titrator model Titrino Plus 848 (Metrohm, Herisau, Switzerland). Titration was performed in 1 mL of the fruit juice diluted in 50 mL of distilled water, using a 0.1 N NaOH solution, until pH 8.1. The results were expressed as percentage of citric acid.

SS/TA ratio was calculated for each sample by dividing the SS value by its respective TA value.

2.5. Statistical analysis

The experiments were carried out in a completely randomized design. In the experiment 1, the weeks of storage were compared, and the growing seasons were analyzed separately. In the experiment 2, we followed a split-plot arrangement, with two atmospheric conditions in the plot and five storage times in the subplot.

Physicochemical quality data obtained in both experiments were submitted to analysis of variance (ANOVA) ($p \leq 0.05$), and the treatments were compared by the Tukey's test ($p \leq 0.05$), using the *ExpDes.pt* R package.

Data were expressed as means ± standard deviations. Before applying ANOVA, the arc sin transformation was performed on the percentage data.

A principal component analysis (PCA) was applied to data in the Experiment 2, to summarize the main findings of this study in a bi-dimensional graph composed of two principal components that explain most of data variance.

3. Results

3.1. Definition of ACP for the establishment of DCA conditions

Over seven weeks of cold storage, 'Palmer' mangos harvested in both growing seasons showed the same climacteric respiration behavior with an increase in respiratory quotient (RQ) due to the consumption of O_2 and production of CO_2 (Supplementary Fig. 1). Two hours after sealing the fruit inside the 20 L chambers, internal partial pressure of gases in the chamber were 10.55–14.59 kPa and 10.58–14.10 kPa of O_2 and 0.25–0.70 kPa and 0.25–0.43 kPa of CO_2, for fruit harvested in summer and winter, respectively (Supplementary Fig. 2–D). Twenty-four hours after closing the chambers, internal O_2 ranged from 0.20 to 1.10 kPa in the summer and 1.75–4.13 kPa in the winter, whereas internal CO_2 ranged from 8.45 to 11.45 kPa in the summer and 5.80–6.88 kPa in the winter, according to the weekly gas analyses (Supplementary Fig. 2A–D).

After closing the chambers, ethanol production took place earlier in fruit at more advanced ripening at weeks 5, 6, and 7 of storage, compared to fruit at earlier ripening at the 1st, 2nd, 3rd, and 4th weeks of storage Supplementary Fig. 2E, 2F). Fruit at early ripening (1–4 weeks of storage) had higher rates of ethanol production in the summer, than in the winter growing season (Supplementary Fig. 2E, 2F).

In the summer, the ACP ranged from 0.3 to 0.9 kPa of O_2 during the first four weeks of storage (Fig. 2A). In the winter, the ACP ranged from

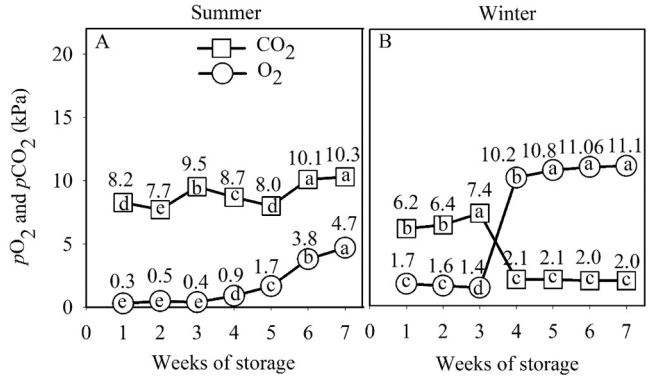

Fig. 2. Internal partial pressures of O_2 and CO_2 at the anaerobic compensation point (ACP) for 'Palmer' mangos produced in the summer (A) and winter (B) growing seasons and stored at 9 °C for seven weeks. Values are represented as mean ± standard error (n = 4). Partial pressures of O_2 and CO_2 were identified at the beginning of ethanol production in the fruit. For each growing season, the weekly O_2 or CO_2 mean values followed by the same letter are statistically equal at according to the Tukey test ($p \leq 0.05$).

1.75 to 1.44 kPa during the first three weeks of storage (Fig. 2B). In the last weeks of storage, the ACP increased in fruit from both growing seasons, but with higher levels for fruit harvested in winter than in summer (Fig. 2A, B). Summer-harvested fruit had lower ACP (pO_2), than winter-harvested fruit, during 7 weeks of storage at 9 °C (Fig. 2A, B). The internal levels of O_2 and ethanol (ACP) in the chambers with CO_2 absorption had similar behavior to that observed in the atmosphere without CO_2 absorption, in both growing seasons (data not shown).

Mango skin and pulp values showed significant changes during storage (Table 1). Pulp firmness did not change during storage in summer-harvested fruit, but it decreased in winter-harvested fruit from 66.19 N at harvest to 48.52 N at the end of seven weeks of storage (Table 1). For fruit harvested in summer the apparent discrepancy arises from the relatively high standard deviation of firmness values, which reflects the natural variability of mango pulp texture, even within the same treatment and storage period. Although numerical differences such as from 56.96 N to 37.37 N were observed, statistical analysis (ANOVA and Tukey's test, $p \leq 0.05$) showed that these changes were not significant. During storage, SS increased and TA decreased, resulting in increasing SS/TA ratio in fruit from both growing seasons (Table 1). The SS were low at harvest, but increased during seven weeks of cold storage, reaching values of 13.78 % and 12.53 % in summer and winter produced mangos, respectively. It is also important to notice that after seven weeks of cold storage, the fruit still had high flesh firmness of 37.37 N and 48.52 N in summer and winter produced mangos, respectively, which suggest that SS content could still reach higher values if the fruit had reached the ready-to-eat ripening stage, known to improve consumer acceptability.

3.2. Validation of DCA conditions for 'Palmer' mango

In the second year of the study, using 'Palmer' mangos harvested in the winter growing season, the DCA reduced fruit weight loss, compared to RA after 30 days of storage (Figs. 3A, 3B). The greatest weight loss was observed at 60 days of cold storage, with 8.3 % for fruit stored under RA and 3.7 % for fruit stored under DCA (Fig. 3A). After shelf life, weight loss increased to 12 % in fruit stored under RA and 7.8 % in fruit stored under DCA (Fig. 3B).

In the second year of the study, the respiration rate of winter-harvested mango was higher in RA than in DCA at 15 days of cold storage, as well as at 30 + 7 and 60 + 7 days of shelf life at 25 °C (Fig. 4A, B). Fruit stored under DCA showed a decline in the respiration rate during cold storage (Fig. 4A, B).

DCA delayed skin color changes from 30 to 60 days and pulp color changes from 15 to 45 days of cold storage, compared to RA (Fig. 4C, E). There were no significant differences for skin and pulp color, when the fruit were exposed to the shelf-life conditions, except at 15 + 7 days of shelf life for skin color, with higher value in DCA (Fig. 4D, F).

In the validation of DCA conditions, accomplished in the second year with mangos produced in the winter growing season, DCA was efficient on maintaining higher mango pulp firmness only from 45 to 60 days of cold storage, compared to RA (Fig. 4G). After 7 days of shelf life, all fruit stored in DCA and RA showed equal pulp firmness (Fig. 4H).

The different storage conditions significantly affected SS, TA and the SS/TA ratio. Fruit stored under RA showed higher SS, compared to fruit kept in DCA for 15–60 days (Fig. 5A). After shelf life, there was no statistical difference for the SS content between the storage conditions at 15 + 7 and 45 + 7 (Fig. 5B). TA was higher in DCA stored fruit only at 60 days of cold storage, compared to RA stored fruit (Fig. 5C). After shelf life, DCA stored fruit showed higher TA only at 30 + 7 and 60 + 7 days, compared to RA stored fruit (Fig. 5D). The SS/TA ratio increased more markedly in fruit stored under RA, than under DCA (Fig. 5E). After shelf life, no statistical differences were observed between RA and DCA stored fruit for SSC/TA ratio at 15 + 7 and 45 + 7 days of storage plus shelf life (Fig. 5F).

The principal component analysis (PCA) was applied to the data obtained during cold storage and after the shelf life (Fig. 6A, B). In the PCA applied to the data obtained during cold storage, the eigenvalues of the covariance matrix indicated that the first two principal components accounted for more than 77 % of the total variance (Fig. 6A). The PC1 explained 61.25 %, whereas PC2 explained 15.80 % of the variance in the dataset (Fig. 6A). PC1 was positively correlated with SS and weight

Table 1
Physicochemical quality of 'Palmer' mangos produced in the summer and winter growing seasons and stored in refrigerated atmosphere at 9 °C for seven weeks.

Fruit quality parameter	Week of storage						
	1	2	3	4	5	6	7
	Summer growing season						
Skin color (°hue)*	101.60^{b}	101.58^{b}	110.14^{ab}	114.89^{a}	113.97^{a}	112.36^{ab}	107.78^{ab}
Pulp color (°hue)	104.47^{a}	103.45^{a}	102.08^{a}	102.34^{a}	102.28^{a}	100.90^{a}	101.04^{a}
Pulp firmness (N)	56.96^{a}	55.07^{a}	49.14^{a}	40.98^{a}	47.46^{a}	41.91^{a}	37.37^{a}
SS (%)	8.98^{e}	10.68^{d}	11.68^{cd}	12.35^{bc}	13.08^{abc}	13.28^{ab}	13.78^{a}
TA (g 100 g^{-1})	0.74^{a}	0.75^{a}	0.71^{a}	0.60^{ab}	0.65^{a}	0.50^{bc}	0.43^{c}
SS/TA	12.24^{b}	14.36^{b}	16.58^{b}	20.68^{b}	20.25^{b}	27.63^{a}	32.07^{a}
	Winter growing season						
Skin color (°hue)	118.98^{a}	108.62^{bc}	118.25^{a}	115.60^{ab}	105.60^{c}	107.51^{c}	105.07^{c}
Pulp color (°hue)	105.16^{a}	101.81^{b}	101.97^{b}	100.21^{b}	100.01^{b}	100.08^{b}	99.74^{b}
Pulp firmness (N)	66.19^{a}	62.05^{ab}	61.32^{ab}	60.93^{ab}	56.37^{abc}	53.30^{bc}	48.52^{c}
SS (%)	5.88^{c}	6.75^{bc}	8.50^{b}	10.78^{a}	11.60^{a}	12.28^{a}	12.53^{a}
TA (g 100 g^{-1})	1.26^{a}	1.26^{a}	1.13^{a}	0.84^{a}	1.21^{a}	0.85^{a}	0.76^{a}
SS/TA	4.85^{c}	5.67^{c}	8.06^{bc}	13.30^{ab}	10.93^{abc}	14.90^{a}	16.74^{a}

*Means followed by the same letter in each row are statistically equal according to the Tukey's test ($p \leq 0.05$).

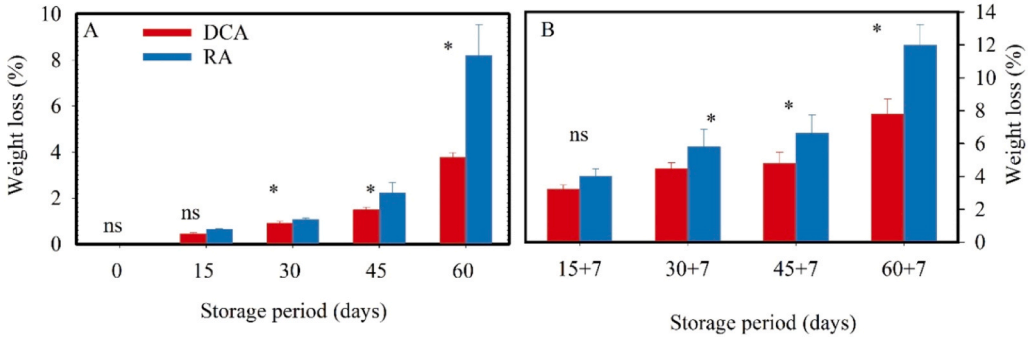

Fig. 3. Weight loss of 'Palmer' mangos produced in the winter growing season and stored under RA and DCA conditions for 60 days at 9 °C (A) plus seven days of shelf life at 25 °C (B). Means followed by asterisk (*) are statistically different according to the F test (n = 4). ns: non-significant.

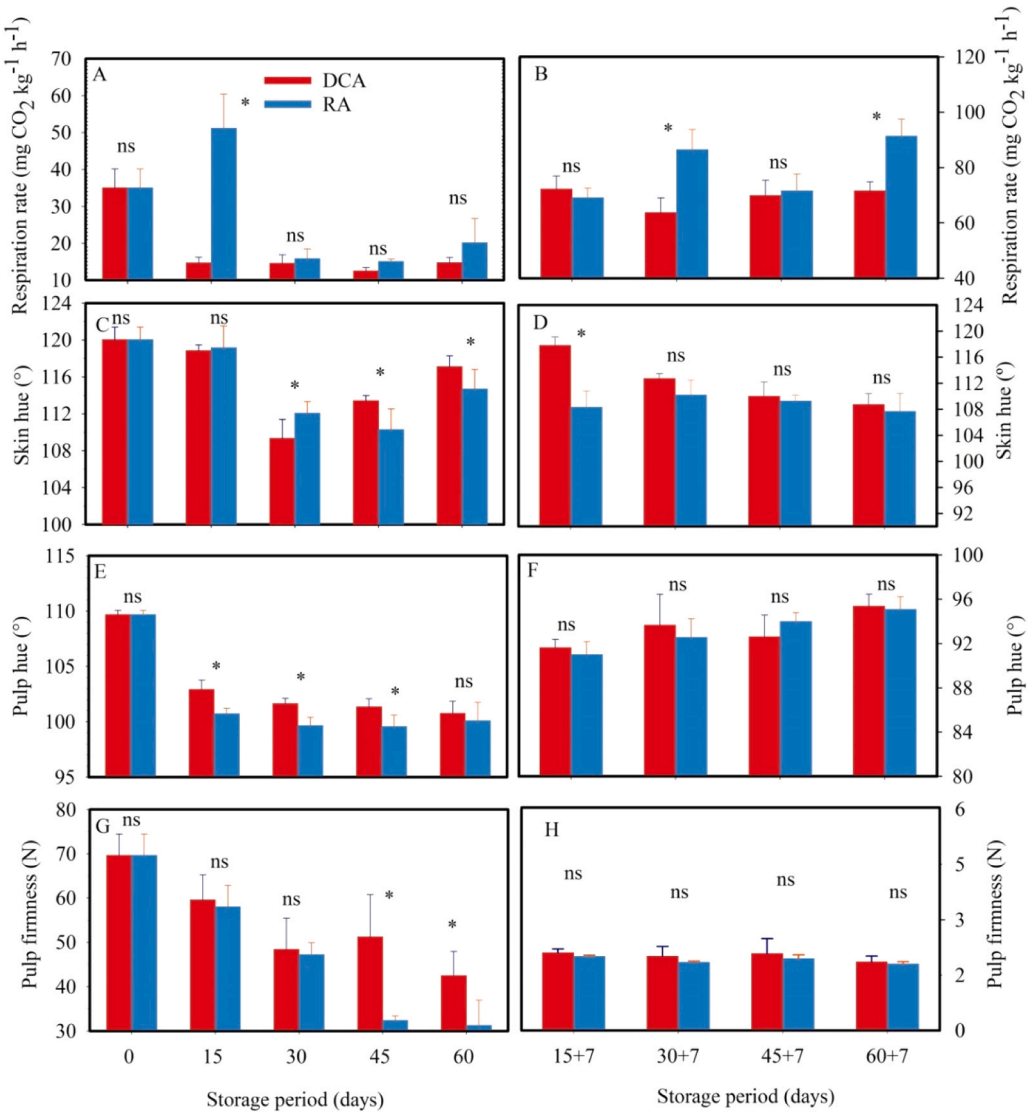

Fig. 4. Respiration rate (A and B), skin color (C and D), pulp color (E and F), and pulp firmness (G and H) of 'Palmer' mangos produced in the winter growing season and stored under DCA and RA for 60 days at 9 °C (A, C, E, G) plus 7 days of shelf life at 25 °C (B, D, F, H). Means followed by ns (non-significant) are statistically equal, according to the F test. Means followed by asterisk (*) are statistically different according to the F test (n = 4).

loss, and negatively correlated with skin and pulp color, respiration rate, pulp firmness, and TA (Fig. 6A). Fruit in both storage conditions at 30, 45 and 60 days of cold storage showed the highest correlation with the positive PC1 axes (Fig. 6A). The RA samples were scattered further to the right of the plot, indicating more advanced ripening stage (Fig. 6A).

After 7 days of shelf life at 25 °C, the PCs 1 and 2 accounted for more than 81 % of the total variance (Fig. 6B). The PC1 explained 59.83 %, whereas PC2 explained 22.13 % of the variance in the dataset (Fig. 6B).

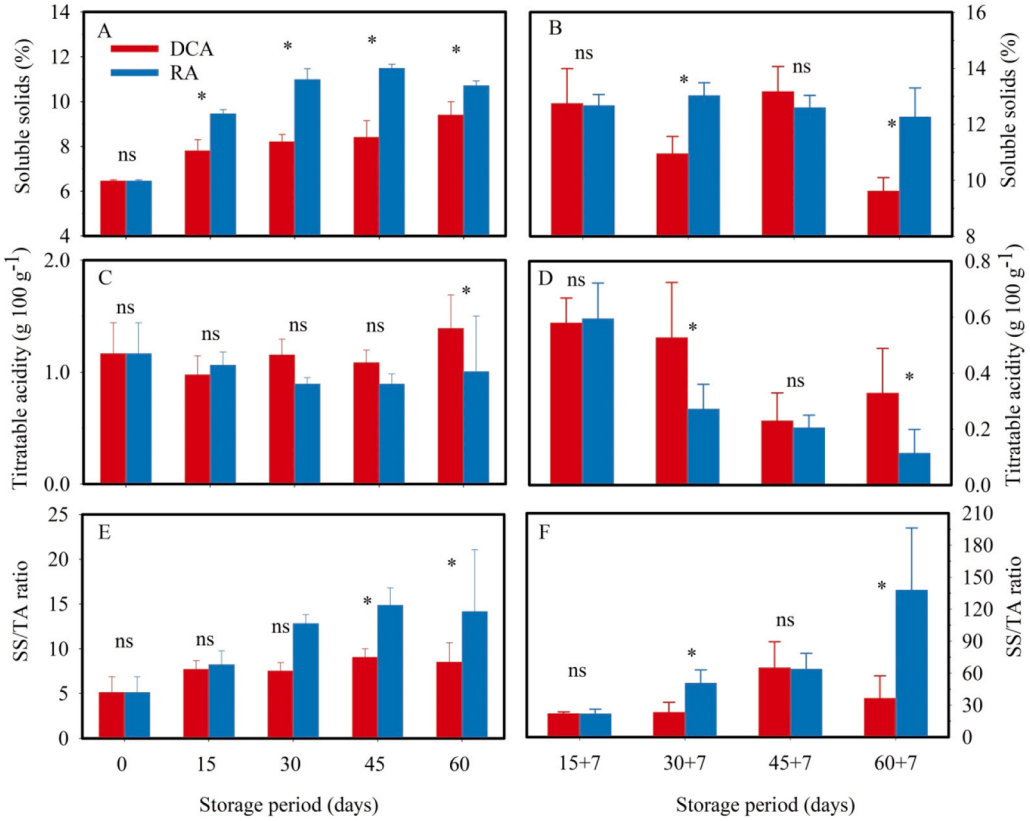

Fig. 5. Soluble solids (A and B), titratable acidity (C and D) and SS/TA ratio (E and F) of 'Palmer' mangos produced in the winter growing season and stored under DCA and RA for 60 days at 9 °C (A, C, E) plus 7 days of shelf life at 25 °C (B, D, F). Means followed by ns (non-significant) are statistically equal, according to the F test. Means followed by asterisk (*) are statistically different according to the F test (n = 4).

PC1 was positively correlated with skin color, firmness and TA, and negatively correlated with respiration rate, weight loss and pulp color (Fig. 6B). Fruit kept in RA after 30 + 7, 45 + 7 and 60 + 7 days of shelf life, as well as fruit kept in DCA after 60 + 7 days of shelf life showed the highest correlation with the negative PC1 axes (Fig. 6B). At each evaluation date, RA samples were always scattered further to the left of the plot, indicating more advanced ripening stage, compared to DCA samples (Fig. 6B).

4. Discussion

4.1. Definition of ACP for the establishment of DCA conditions

The static CA does not take into account the variations on the minimum O_2 levels required to maximally inhibit fruit aerobic respiration after harvest (Teixeira and Durigan, 2011; Teixeira et al., 2018; Bender et al., 2021; Dos Santos et al., 2023). Therefore, identifying the minimum O_2 levels throughout storage/shipping as a function of fruit ripening is essential to effectively inhibit fruit metabolism and increase postharvest life (Dos Santos et al., 2023). Using the lowest possible O_2 levels without triggering anaerobic respiration is important to achieve maximum inhibition of aerobic respiration and, consequently, changes in fruit ripening (Singh and Zaharah, 2015; Dos Santos et al., 2023).

The respiration rate of 'Palmer' mangos showed climacteric behavior of reducing O_2 consumption, increasing CO_2 production and consequently increasing the respiratory quotient (RQ) during seven weeks of storage at 9 °C (Supplementary Fig. 1). The gradual increase in CO_2 production was also observed in 'Keitt' and 'Tommy Atkins' mangos (Bender et al., 2021; Santos et al., 2023). The RQ represents the ratio between the volume of CO_2 produced and O_2 consumed (CO_2/O_2) by the fruit, which is close to 1.0 under normoxia conditions, where O_2 levels are adequate for fruit aerobic respiration (Saltveit, 2020). However, even in normoxia conditions, the RQ value can vary slightly above and below 1.0, implying that sugars are the most important carbohydrate used in the respiration metabolism (Goyette et al., 2012). In the present study, RQ exhibited small fluctuations in both harvests, with approximately 1.0 at the beginning of storage and approximately 1.8 in the last week of storage. Measuring RQ is important because it indicates the metabolism that is occurring in the fruit cells and, in addition, it can be indicative of the compounds that are being produced by the metabolic activity (Pesis, 2005; Wright et al., 2015). Therefore, based on the variation in RQ throughout storage, it is possible to estimate the minimum level of O_2 tolerated by the fruit, as the factor that most affects the variation in RQ during storage is the partial pressure of O_2 in the storage atmosphere (Thewes et al., 2021). RQ is a well consolidated method for monitoring real time metabolism in apples (Weber et al., 2015), with studies also in progress for mangoes.

In the São Francisco Valley, the summer growing season was characterized by higher temperatures (22.4 °C to 34.1 °C), lower relative humidity (31.7–88.2 %), and higher global solar radiation (18.0 MJ m^{-2} to 22.1 MJ m^{-2}), whereas the winter growing season was characterized by lower temperatures (19.3 °C to 30.5 °C), higher relative humidity (34.3–92.2 %), and lower global solar radiation (14.4 MJ m^{-2} to 18.5 MJ m^{-2}). These contrasting environmental conditions directly influence fruit physiology and explain the differences in the minimum O_2 requirements observed between the two growing seasons. Indeed, the results showed that 'Palmer' mangos produced in the summer can tolerate O_2 levels below 1.0 kPa during the first four weeks, whereas mangos produced in the winter can tolerate O_2 levels below 2.0 kPa during the first three weeks of storage at 9 °C. Therefore, whenever an extremely low pO_2 is used, it is of fundamental importance to adopt a method for determining the minimum level of O_2 tolerated by the fruit

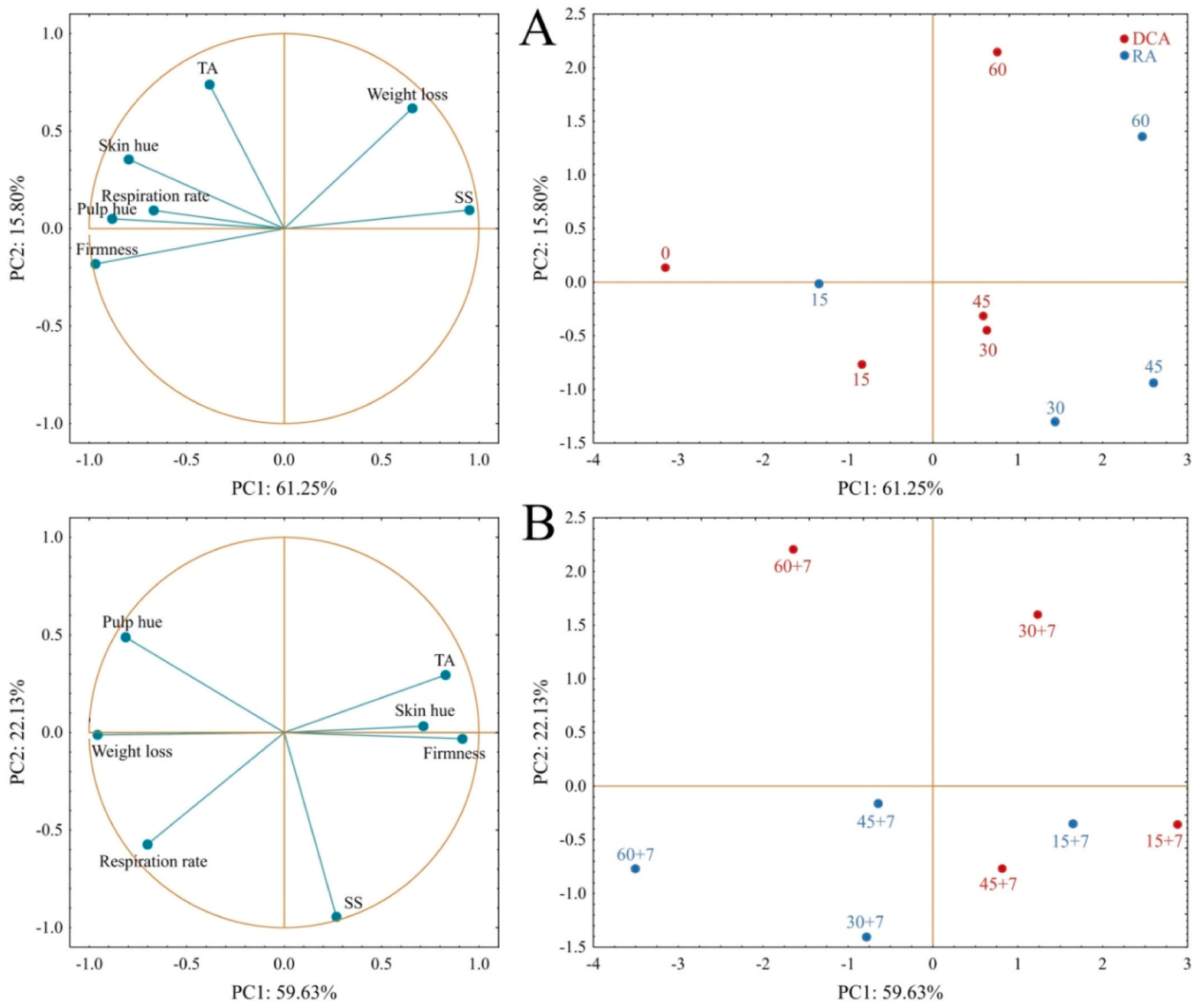

Fig. 6. Principal component analysis for 'Palmer' mangos produced in the winter growing season and stored under DCA and RA for 60 days at 9 °C (A) plus 7 days of shelf life at 25 °C (B). Red circles are DCA samples and blue circles are RA samples at 0, 15, 30, 45, and 60 days of cold storage (A), or at 15, 30, 45, and 60 days cold storage plus 7 days of shelf life (B).

to avoid losses during storage, mainly due to the production of anaerobic compounds such as acetaldehyde, ethyl acetate and ethanol (Thewes et al., 2021). Ethanol detection was an efficient approach for monitoring 'Palmer' mango aerobic/anaerobic respiration, which was used to determine the minimum levels O_2 required to maximally inhibit fruit ripening and senescence. This approach has also proven effective for monitoring aerobic/anaerobic respiration of 'Tommy Atkins' mango (Dos Santos et al., 2023).

Ethanol production can change, depending on fruit O_2 requirements during long storage/shipping periods. Indeed, our results showed that 'Palmer' mangos produced ethanol at much higher pO_2 in the last weeks of storage, compared to the first weeks of storage. This was possibly due to anaerobic respiration or accelerated metabolism in response to advanced ripening and senescence (Porat and Fallik., 2008). However, higher O_2 levels were observed in the last weeks of storage of fruit harvested in winter than in summer. These results imply that different pO_2 requirements during storage may be related to the different environmental conditions in which the fruit were produced (Weber et al., 2015; Bessemans et al., 2016). As observed for 'Tommy Atkins' mangos (Dos Santos et al., 2023), the ideal pO_2 identified in our study for storing/shipping 'Palmer' mangos is very different from the pO_2 recommended in other studies for other mango cultivars (Teixeira and Durigan, 2011; Teixeira et al., 2018; Ntsoane et al., 2019; Bender et al., 2021; Ikwan et al., 2021). Therefore, our study highlights important results to ensure the highest efficiency of DCA to store/ship 'Palmer' mangos for the longest possible time using this technology.

Although studies have shown that high pCO_2 can also affect fruit respiration rate (Mathooko, 1996), our results have showed that CO_2 levels accumulated during the transition from aerobic to anaerobic respiration had no effect on ethanol production by the fruit. Furthermore, pO_2 demands were not changed in fruit stored with or without CO_2 absorption, suggesting possible commercial conditions for maritime transport with control of pO_2 and pCO_2 or just pO_2.

4.2. *Validation of DCA conditions for 'Palmer' mango*

The DCA with the safe minimum pO_2 used to inhibit aerobic respiration was very efficient on delaying 'Palmer' mango ripening changes during 60 days of storage at 9 °C. This result is very promising, considering the longest shipping time required to export mangos from

countries like Brazil to distant markets like in Asia, which requires approximately 45 days.

Water loss is the main cause of fruit weight loss after harvest, promoting browning, softening and loss of flavor, which results in reduced fruit quality and consumer acceptance (Lara et al., 2014). Temperature management is the most important factor that maintains fruit quality (Trindade et al., 2015), as with a reduction in temperature there is a reduction in the speed of physical and biochemical processes in fruit, such as transpiration, respiration and ethylene production, which are responsible for ripening and senescence. However, many studies have shown that temperature control alone is not sufficient to maintain fruit quality for longer periods. Thus, associated with temperature and humidity control, monitoring and controlling gases in the storage atmosphere has shown promising results to maintain postharvest quality of mangos, as it was also observed in our study with reduced fruit weight loss under DCA conditions. According to Nunes et al. (2007), weight loss higher than 9 % results in strong effects on fruit internal and external appearance, making the fruit unacceptable for commercialization.

Respiration is the main physiological process after harvesting and consists of the oxidation of complex molecules, generating energy and simpler molecules. The higher the respiration rate of a fruit, the more perishable it will be (Koblitz, 2008). The initial increase in respiration rate recorded at 15 days in RA, compared to DCA, may be related to the need for energy production to maintain metabolic processes, as this is the main physiological process that fruit undergo after harvesting (Tonutti and Bonghi, 2014). In our study, DCA was efficient in inhibiting the respiration rise observed in RA stored fruit at 15 days of storage, which represents a respiration peak characteristic of ripening in climacteric fruit. Similar results were observed in 'Tommy Atkins' mangos stored under DCA, which maintained lower respiration rate throughout storage (Dos Santos et al., 2023). This result suggests that low pO_2 suppresses respiration and ethylene production in fruit (Thewes et al., 2015). Low pO_2 decreases respiration because O_2 is the substrate for respiration and acts in the electron transport chain (Prange et al., 2015). Furthermore, the reduction in respiration rate and ethylene production with the reduction in pO_2 can be explained by the low activity of ACC oxidase, since ACC oxidase activity is oxygen dependent (Génard and Gouble, 2005; Asoda et al., 2009). In addition, the low pO_2 resulted in a small amount of ethanol production in fruit, which can be beneficial for maintaining fruit quality. Studies have found that compounds produced during anaerobic respiration, such as ethanol, can inhibited fruit metabolism (Asoda et al., 2009; Jin et al., 2013; Weber et al., 2016; Weber et al., 2020). Studies using DCA for the storage of apples found that low pO_2 induces ethanol production through the activation of the enzyme alcohol dehydrogenase (ADH) (Weber et al., 2020). These authors found that ethanol produced by the fruit or applied externally influenced the fruit's metabolism, inhibiting respiration rate and ethylene biosynthesis. In our study, the ethanol produced by 'Palmer' mangos may have inhibited fruit respiration rise under DCA, which was also observed in 'Tommy Atkins' mango (Dos Santos et al., 2023).

'Palmer' mangos kept in DCA showed better maintenance of skin and pulp color than fruit stored in RA, which was more evident after 45 days of storage at 9°C. The better maintenance of skin and pulp color in fruit kept under DCA with low pO_2 may be related to the reduction in ethylene production through inhibition of 1-amino-cyclopropane-1-carboxylic acid (ACC) synthase and ACC oxidase activities. Skin and pulp color changes are highly related to ethylene, because this ripening hormone triggers the expression and activity of many enzymes involved chlorophyll breakdown and/or carotenoids synthesis in different fruits (Wills et al., 1998).

The DCA showed effect on maintaining higher 'Palmer' mango pulp firmness only after 45 days of storage, compared to RA condition. Interestingly, dos Santos et al. (2023) have also shown that DCA maintains higher 'Tommy Atkins' mango pulp firmness only after 45 days of storage, compared to RA condition. These results suggest that DCA should only be used to transport mangos to markets that require shipping times greater than 45 days. In this case, using DCA or CA to store or transport mangos for less than 45 days may not have beneficial effects on maintaining fruit quality, but would make the fruit production more expensive.

The fruit stored under DCA for 45 days had slightly greater pulp firmness than fruit stored under RA for 30 days, inferring that DCA increase mango postharvest life for at least 15 days. Greater differences in pulp firmness between fruit stored in DCA and RA were observed after 45 days of storage, which suggests that DCA was more efficient in increasing the postharvest life of mangos over longer storage periods. The loss of firmness occurs mostly in response to ethylene, because it increases the expression and activity of hydrolytic enzymes, such as amylase, cellulase, pectin methylesterase and polygalacturonase, which degrades starch and the cell wall, converting starch into sugars, leading to loss of turgor and consequently softening of the fruit (Payasi et al., 2009; Singh et al., 2013). Therefore, low pO_2 reduces the expression and activity of ethylene-dependent enzymes that are responsible for fruit softening, which was observed in our study. The positive effect of DCA in extending the postharvest life of mango can be used to reach current markets with fruit harvested at more advanced maturity and higher quality, as well as to export the fruit to new markets that were limited due to long shipping time (Vilvert et al., 2022; Dos Santos et al., 2023; Vilvert et al., 2023).

In general, the SS content increased gradually during storage regardless of the storage condition, as an effect of fruit ripening. However, fruit stored under RA had higher SS content, compared to those stored under DCA. During mango ripening, starch is hydrolyzed into simple sugars, mainly glucose, fructose and sucrose, increasing the SS content and providing the fruit with a sweeter taste. Therefore, the results suggest that this hydrolysis process was delayed in fruit stored under DCA. This effect occurs due to the low pO_2 around the fruit stored under DCA (Dos Santos et al., 2023), which reduces metabolic processes, such as those involved in the breakdown of starch into sugars (Singh et al., 2013). TA was higher in fruit stored in DCA, compared to fruit stored in RA, which was possibly due to the inhibition of organic acids oxidation in the tricarboxylic acid cycle in response to low pO_2 condition, as previously described in other fruits (Saquet, 2019). The observed changes in SS and TA resulted in a faster increase in the SS/TA ratio in RA stored fruit, compared to DCA stored fruit, showing that DCA slowed down 'Palmer' mango ripening during 60 days of storage at 9 °C.

5. Conclusions

'Palmer' mangos produced in the summer and winter growing seasons required different minimum O_2 levels to maximally inhibit aerobic respiration, ranging from 0.3 to 4.7 kPa and 1.75–11.15 kPa, respectively, throughout storage. In the São Francisco Valley, the summer growing season was characterized by higher temperatures, lower relative humidity, and higher global solar radiation, compared with the winter growing season.

Storage under DCA efficiently delayed ripening of 'Palmer' mangos, reducing mass loss, respiration rate and softening, and maintaining color, soluble solids, and titratable acidity for up to 60 days at 9 °C. Compared to RA, DCA extended the postharvest life of the fruit by approximately 15 days, which is sufficient to support marine transport to distant markets.

Despite these benefits, DCA did not alter the natural ripening process once the fruit were transferred to shelf life at 25 °C, which is desirable to ensure high eating quality for consumers.

CRediT authorship contribution statement

Sergio Tonetto de Freitas: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data

curation, Conceptualization. **Luana Ferreira dos Santos:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Bruna Parente de Carvalho Pires:** Validation, Methodology, Investigation, Formal analysis. **Jasciane da Silva Alves:** Validation, Methodology, Investigation, Formal analysis. **João Claudio Vilvert:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Tassiane Alves de Souza:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Daniel Alexandre Neuwald:** Writing – review & editing, Writing – original draft, Methodology, Conceptualization. **Luciano Sobral Fraga Junior:** Validation, Software, Methodology.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.postharvbio.2025.113951.

Data availability

Data will be made available on request.

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