



The response of 'Monalisa' apples to high CO₂ storage conditions, harvest maturity and 1-MCP treatment

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ABSTRACT

This study aimed to determine the effects of harvest maturity, 1-MCP treatment and storage conditions with high CO₂ partial pressures on 'Monalisa' apples physicochemical quality and susceptibility to physiological disorders and decay during long-term storage, plus 7 d of shelf life at 22 °C. The study was composed by two experiments. In Experiment 1, fruit were harvested in one growing season (2011) at the same maturity stage and were treated or not treated with 1-MCP (1 µL L⁻¹). In Experiment 2, fruit were harvested in two growing seasons (2019 and 2020), at two maturity stages. In both experiments, all fruit were stored under CA with four CO₂ partial pressure (0.5, 1.5, 3.0 and 4.5 kPa) and regular air (RA, standard of comparison) for 6 or 7 months at 0.8 °C, plus 7 d shelf life at 22 °C. CA was very effective on delaying fruit ripening, senescent disorders and decay incidences, regardless of the CO₂ partial pressure. However, under CA, 'Monalisa' apples were very susceptible to CO₂ injury, expressed as dark flesh browning and cavities that were exacerbated with increasing CO₂ partial pressures. Therefore, 'Monalisa' apples should be stored under CA with CO₂ no higher than 0.5 kPa. The response of 'Monalisa' apples to high CO₂ is more pronounced in late harvested fruit, which were also more prone to develop senescent flesh browning, cracking and rough skin. 1-MCP application had no effect on 'Monalisa' apple susceptibility to CO₂ damages, while it reduced fruit softening and acidity loss in both RA and CA storages.

1. Introduction

The breeding program of the Agricultural Research and Rural Extension Company of Santa Catarina (EPAGRI) has developed several new apple cultivars that are more adapted to the Brazilian climate, especially to subtropical humid climate of Southern Brazil (Denardi et al., 2019). Monalisa is one of these new cultivars originated from crosses between 'Gala' and 'Malus 4' (Denardi et al., 2013). This new cultivar has very interesting characteristics for the organic apple growers, as it is resistant to apple scab and Glomerella leaf spot (Denardi et al., 2013). On-tree maturation of both 'Monalisa' and 'Gala' apples fruit are at the same time, and therefore, fruit of both cultivars could be stored in the same room if they had the same responses to low temperature and controlled atmosphere regimes. However, the response of 'Monalisa' apples to controlled atmosphere (CA) conditions has not been

reported yet, especially regarding its tolerance to high CO₂ partial pressures.

CA storage is generally employed for long-term quality maintenance of apples. In CA conditions, O₂ and CO₂ partial pressures are reduced and increased, respectively, to reduce fruit metabolism and ethylene production (Argenta et al., 1994; Saquet et al., 1997; Thewes et al., 2021). Under such conditions, the tolerance to low O₂ and high CO₂ depends on the cultivar (Li et al., 2022). Although 'Gala', a parental of 'Monalisa', is highly tolerant to CO₂ partial pressures up to 3.0 kPa (Brackmann et al., 2008, 1996; Saquet et al., 1997), there is no information about 'Monalisa' apples tolerance to CO₂ during CA storage. The use of inadequate CO₂ partial pressures during storage can result in several symptoms of CO₂ injury, such as skin browning, flesh browning and cavities in sensitive cultivars, including Fuji (Argenta et al., 2002), Empire (Burmeister and Dille, 1995), Braeburn (Lau, 1998) and Pink

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Lady (de Castro et al., 2008, 2007; Li et al., 2022; Saquet et al., 1997). The tolerance to high CO₂ partial pressures during storage can be affected by harvest maturity, ethylene management with 1-MCP (de Castro et al., 2007; Lau, 1998; Thewes et al., 2017), and O₂ partial pressure (Beaudry, 1999; Brackmann et al., 2015) during storage.

One of the main causes of internal physiological disorders in apples is the use of inadequate CO₂ partial pressure during storage (Argenta et al., 1994; Brackmann et al., 2015; Lumpkin et al., 2015; Saquet et al., 1997). Although increasing CO₂ partial pressures can help to reduce fruit metabolism, inappropriate high partial pressures can cause abnormal metabolic activity and physiological disorders incidence in susceptible cultivars (Mathooko, 1996a).

There are a few possible approaches to increase apple fruit tolerance to high CO₂ partial pressures. One approach is to determine the harvest maturity required to reduce fruit susceptibility to CO₂ injury. Studies have shown that apple fruit tolerance to CO₂ injury during storage can be reduced by late harvest (Argenta et al., 2002; Lau, 1998; Thewes et al., 2017). Another possible approach is to determine the effect of 1-MCP on reducing apple fruit metabolism and increasing tolerance to CO₂ injury during storage. Although both approaches have the potential to affect apple fruit tolerance to high CO₂ partial pressures, there are no studies accomplished with 'Monalisa' apples. The 1-MCP is known to reduce fruit metabolism by inhibiting ethylene action and responses (Blankenship and Dole, 2003; Watkins, 2008). However, the impact of 1-MCP on apple quality depends on the cultivar and harvest maturity (Jung and Watkins, 2011; Thewes et al., 2017). In some apple cultivars, 1-MCP treatment can increase fruit susceptibility to CO₂ injury (Argenta et al., 2010). Therefore, 'Monalisa' apple tolerance to high CO₂ partial pressure during CA storage can be affected by ethylene action management with 1-MCP and/or maturity at harvest.

This study was carried out to determine the effects of storage conditions with high CO₂ partial pressures and possible interactions with harvest maturity or 1-MCP treatment on 'Monalisa' apples physico-chemical quality and susceptibility to physiological disorders and decay during long-term storage, plus 7 d of shelf life at 22 °C.

2. Material and methods

2.1. Fruit sources and sample performing

'Monalisa' apples were planted in an orchard located at the EPAGRI experimental station, Caçador, Santa Catarina, Brazil (26°46'19.9"S, 50°55'12.7"W). Orchard management practices were performed according to the recommendations for apple production in Southern Brazil (EPAGRI, 2018). The trees were grafted on 'Marubakaido' rootstocks with a M9 interstocks.

Fruit were harvested at the mid-height of the inner and outer canopy on both tree-row sides. Immediately after harvest, fruit were transported to the Postharvest laboratory, where fruit with similar size were sampled to compose replications of 25 fruit that were held on fiberboard trays during the storage in regular air (RA, 20.9 kPa O₂) or controlled atmosphere (CA) conditions.

2.2. Experiments performed

Two experiments were performed during three growing seasons (2011, 2019 and 2020) to determine the effect of RA and CA with different CO₂ partial pressures on fruit quality, as well as on physiological disorders and decay incidences after long-term storage plus 7 days of shelf life at 22 °C. In these experiments, the interactions between storage atmosphere and harvest maturity or 1-MCP treatment were investigated, as described below.

2.2.1. Experiment one: storage atmosphere x 1-MCP treatment

Fruit were harvested on February 8th in 2011 and subjected to the following storage atmospheres for six months: [1] RA; [2] CA – 1.5 kPa

O₂ + <0.5 kPa CO₂; [3] CA + 1.5 kPa CO₂; [4] CA + 3.0 kPa CO₂; and [5] CA + 4.5 kPa CO₂. At harvest, fruit subjected to each storage atmosphere were treated with 1-MCP (≈ 1 μL L⁻¹). Untreated fruit were used as a control treatment.

2.2.2. Experiment two: storage atmosphere x harvest maturity

Fruit from two consecutive growing seasons were used for this experiment (2019 and 2020). The harvests were performed on February 1st and 13th in 2019 and, February 6th and 17th in 2020, for early and late harvested fruit. After harvest, fruit of both maturity stages were stored for seven months in the following conditions: [1] RA; [2] CA – 1.5 kPa O₂ + <0.5 kPa CO₂; [3] CA + 1.5 kPa CO₂; [4] CA + 3.0 kPa CO₂; and [5] CA + 4.5 kPa CO₂.

2.3. 1-MCP treatment

Fruit were treated with 1-MCP (1 μL L⁻¹) within 24 h after harvest, which was accomplished in a sealed steel container (1 m³) for 12 h at ambient temperature. The 1-MCP gas was generated by mixing cyclodextrin-1-MCP powder EthylBloc™ (AgroFresh Inc. Spring-house, USA) and water, and its concentration inside the container was checked as described previously (Mattheis et al., 2005). Untreated fruit remained in RA at the same temperature as 1-MCP-treated fruit.

2.4. Atmosphere conditions

Apple fruit were put into stainless steel chambers (0.150 m³) with a plexiglass lid for the establishment of each CA conditions described above. These chambers were allocated inside a cold room with temperature at 0.8 ± 0.8 °C and relative humidity (RH) between 92 and 95%. Temperature and RH were monitored as described by Argenta et al. (2022). The CA treatments were established within 54 h after fruit cooling and were maintained as described by Argenta et al. (2022). Fruit stored under RA conditions were kept in the same cold room with the same temperature and RH as described for fruit stored under CA conditions.

2.5. Fruit quality and physiological disorder analyses

An initial analysis to characterize fruit maturity was undertaken one day after harvest (Supplementary Table 1). Fruit analyses were accomplished at harvest and after 6- or 7-months storage, plus 7 d of shelf life at 22 °C.

Maturity of 25 individual apples was determined 24 h after harvest by the analyses of flesh firmness, starch index (SI), soluble solids content (SSC), and titratable acidity (TA). For the post-storage assessment of flesh firmness, physiological disorders and fungal decay incidences, an amount of three replications of 25 fruit were evaluated from each harvest maturity and postharvest treatment. Ethylene production, respiration rate, TA and SSC were evaluated in four replications of eight fruit. Flesh firmness, starch index (1–9 scale), SSC and TA were assessed as described by Argenta et al. (2022), while ethylene production and respiration rate were assessed as described by Mattheis et al. (2005). External and internal disorders were visually assessed using subjective scales of severity, where a score of one indicates the absence of disorders. Internal disorders were assessed from four transverse slices across the fruit. Incidence and severity of fungal decay, CO₂ injury, skin browning ('scald-like'), shriveling, bitter pit, senescent flesh browning and fruit cracking were assessed according to the area of fruit surface or cortex cross-section affected or the number of lesions per fruit as described by Argenta et al. (2022, 2020). Scores for CO₂ injury were 1, ≤30% of flesh with well-defined dark brown areas (brown-heart); 2, 31–60%; or 3, >60%. CO₂ injury scores were also given with reference to the development of light brown dry cavities from the dissection of affected tissues: 1, 1 cavity; 2, 2–5 cavities; and 3, >5 cavities. Fruit affected by rough skin was scored as 1, 1–30% of the fruit surface with

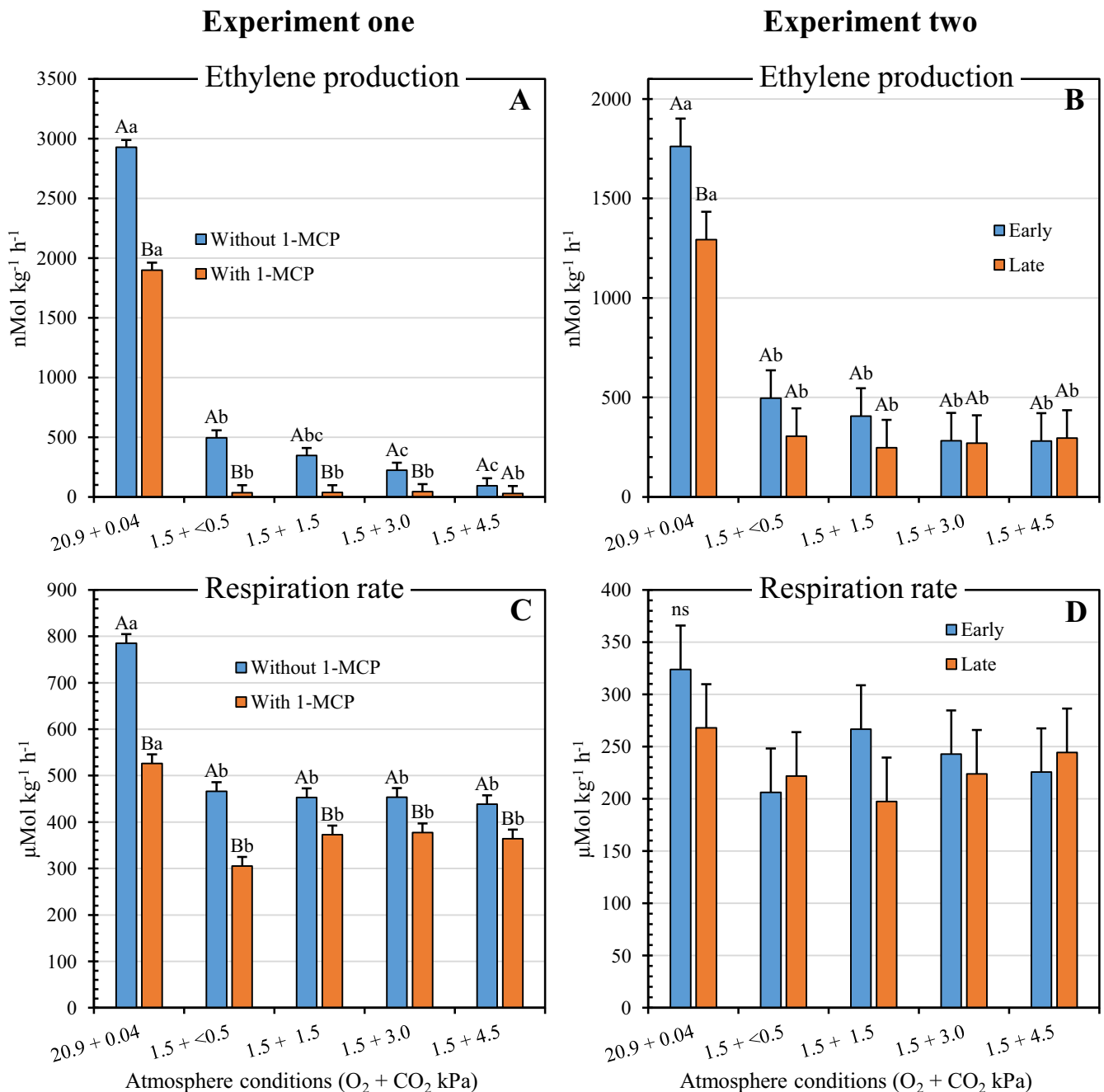


Fig. 1. Ethylene production (A and B) and respiration rate (C and D) of 'Monalisa' apples after 6 (A and C) or 7 (B and D) months storage at 0.8 °C under regular air (20.9 kPa O₂) or controlled atmosphere (1.5 kPa O₂) with four CO₂ partial pressures (0.5, 1.5, 3.0 and 4.5 kPa) plus 7 days of shelf life at 22 °C. Fruit were treated or not with 1-MCP (experiment one, A and C) and from an early and late harvested (experiment two, B and D). Different lower case letters show statistical difference among storage atmospheres, and different uppercase letters show statistical difference between fruit treated and not treated with 1-MCP or harvest maturities by Tukey's test, at 5% probability. Error bars mean standard error.

light roughness; 2, 31–60% of the fruit surface with light to deep roughness; or 3, >60% of the cortex with light to deep roughness. Data of all physiological disorders were presented as severity.

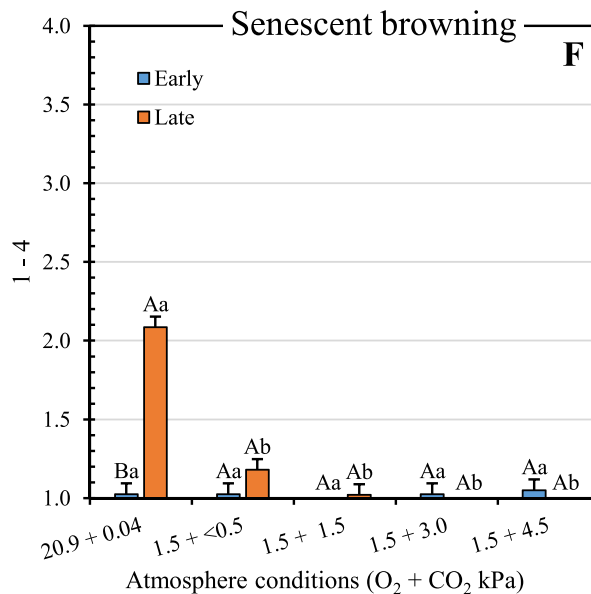
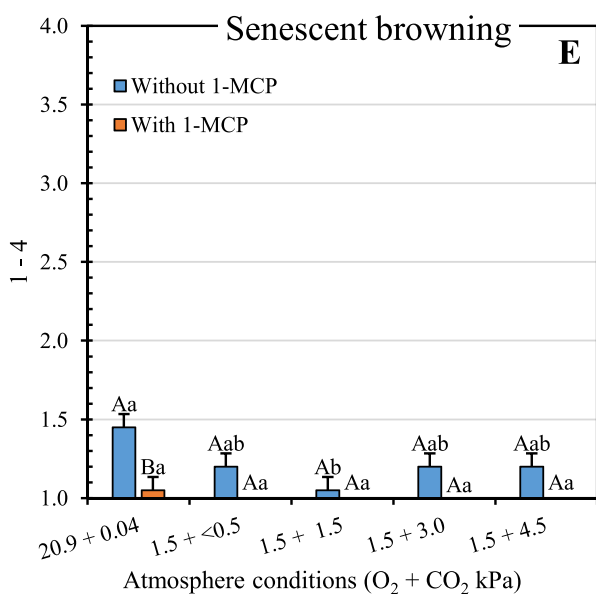
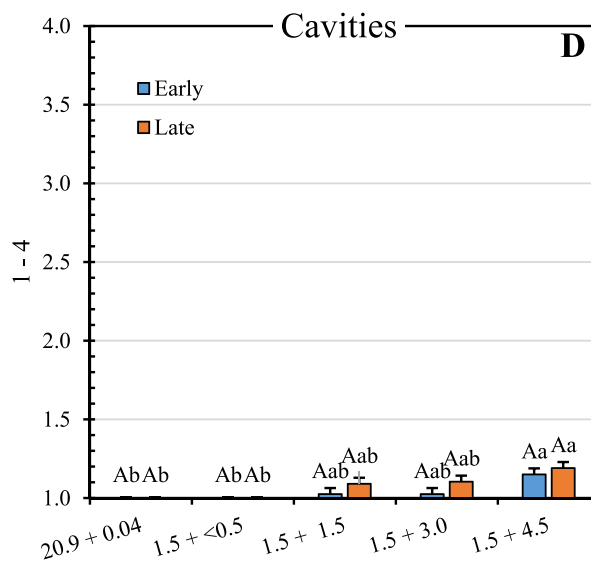
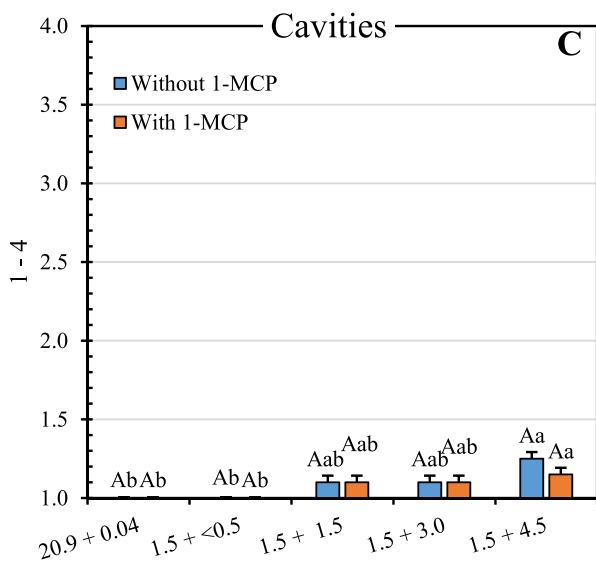
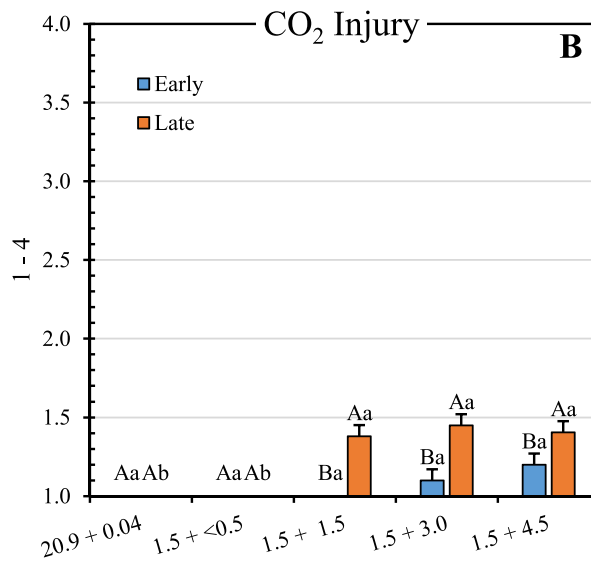
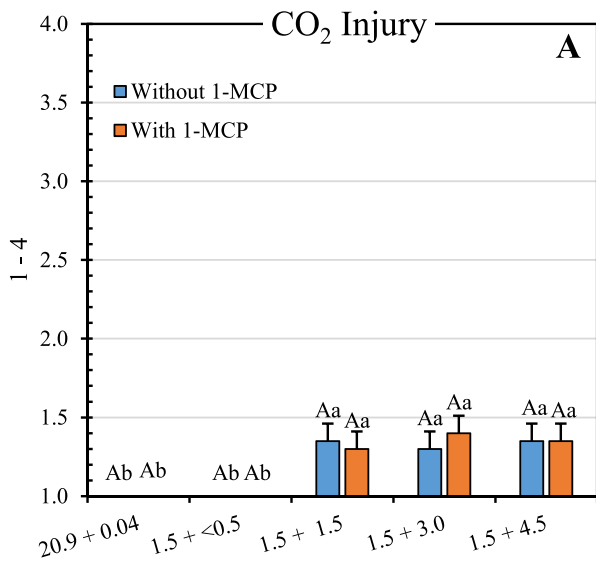
2.6. Statistical analysis

Each experiment was analyzed separately. In the Experiment 2, data from both growing seasons (2019 and 2020) were averaged due to similar fruit responses to the storage treatments. All data were subjected to the analysis of variance (ANOVA) and mean values were compared by the Tukey test ($p < 0.05$). Physiological disorders and fungal decay data

were transformed by the $\text{arc.sin}\sqrt{x/100}$ to have the normal distribution required for the analysis of variance. The data were also subjected to the Principal Component Analysis (PCA) using MetaboAnalyst® 5.0 software (<https://www.metaboanalyst.ca/>). All data were standardized (mean = 0 and variance = 1 for each variable) before performing the PCA.

Experiment one

Experiment two



(caption on next page)

Fig. 2. CO₂ injury expressed as a well-defined flesh dark browning (CO₂-Browning, A and B) and cavities (C and D) and senescent diffuse flesh browning (Senescent browning, E and F) in ‘Monalisa’ apples after storage under RA and CA with four CO₂ partial pressures (0.5, 1.5, 3.0 and 4.5 kPa), either without or with 1-MCP treatment (experiment one) and early and late harvested (experiment two), plus 7 days of shelf life at 22 °C. Different lower case letters show statistical difference among storage atmospheres, and different uppercase letters show statistical difference between fruit treated and not treated with 1-MCP or harvest maturities by Tukey’s test, at 5% probability. Error bars mean standard error. Data are means of severity (1 - 4).



Fig. 3. ‘Monalisa’ apple fruit grown in Southern Brazil with symptoms of CO₂ injury (internal well defined dark browning and cavities) as a response to high CO₂ partial pressure during storage under CA.

3. Results and discussion

3.1. Ethylene production and respiration rate

Fruit stored under RA had the highest ethylene production in both experiments, regardless of the 1-MCP treatment and harvest maturity (Fig. 1A and B). In the first experiment, fruit stored in CA with 3 and 4.5 kPa CO₂ had lower ethylene production, when not treated with 1-MCP, without differing from fruit stored under CA with 1.5 kPa CO₂ (Fig. 1A). Oxygen availability is necessary to ethylene synthesis (Yang and Hoffman, 1984), which explains the lower ethylene production in CA stored fruit. Additionally, high CO₂ partial pressures is also known to play an important role on inhibiting autocatalytic ethylene production (Mathooko, 1996b). However, there was no effect of high CO₂ partial pressures on ethylene production when fruit were treated with 1-MCP (Fig. 1A). This result agrees with previous studies suggesting that high CO₂ can inhibit ethylene production by affecting ACC oxidase activity (Rothan and Nicolas, 1994; Wild et al., 1999), as well as by binding to the ethylene receptors (Ahammed and Li, 2022), in that last case, competing for ethylene responses inhibition with 1-MCP.

In all storage conditions, fruit treated with 1-MCP had lower ethylene production, except in CA with 4.5 kPa CO₂ (Fig. 1A), which is probably due to the ethylene action inhibition effect of high CO₂ (Mathooko, 1996b) and also its effect on ethylene synthesis (de Wild et al., 2005). On the other hand, late harvested fruit had lower ethylene production when fruit were stored under RA (Fig. 1B), which is probably due to advanced ripening of the fruit. These results suggest that late harvested fruit stored under RA have probably passed the climacteric peak, when ethylene production has already declined, which was not observed in CA stored fruit, regardless of the CO₂ partial pressure. Similarly, previous studies have shown that late harvested ‘Galaxy’ apples also have reduced ethylene production after long-term CA storage, compared to apples harvested at optimal maturity (Thewes et al., 2017), which was only observed in RA stored ‘Monalisa’ apples in our study.

The results obtained in first experiment show that fruit stored in RA conditions, as well as fruit not treated with 1-MCP have higher ethylene production, which resulted in higher respiration rate (Fig 1A, C). However, the respiration rate was not affected by atmosphere conditions and harvest maturity in the second experiment (Fig. 1D). The reduced respiration in 1-MCP treated fruit is due to ethylene action inhibition by

this compound, which reduced ethylene-dependent metabolic activities, such as the ones involved in fruit respiration. It is well reported that 1-MCP treatment reduces respiration rate of apples after storage (Argenta et al., 2022; Schmidt et al., 2020; Thewes et al., 2020, 2017; Weber et al., 2017). The impact of CA on respiration rate is mainly via reduction of O₂ available, as CO₂ partial pressure had no effect on respiration rate of ‘Monalisa’ apples (Fig. 1C). Therefore, as expected, CA storage reduced respiration of ‘Monalisa’ apples after storage (Argenta et al., 2022; Brackmann et al., 2008).

3.2. Physiological disorders

The occurrence of physiological disorders during and after storage of apples can be triggered by several factors, one of which is the use of inadequate CO₂ partial pressures to store susceptible cultivars. ‘Monalisa’ apples stored in CA with high CO₂ partial pressures (> 0.5 kPa) developed CO₂ injury in the flesh (Fig. 2A), which was more prone to occur in late harvested fruit (Fig. 2B). The CO₂ injury is characterized by well-defined dark browning areas in the apple flesh (Fig. 3). Although 1-MCP treatment had no effect on CO₂ injury occurrence in ‘Monalisa’ apples (Fig. 2A), it may increase fruit susceptibility to CO₂ injury in ‘Cripps Pink’ (de Castro et al., 2008, 2007), ‘Empire’ (Lee et al., 2012), and ‘Fuji’ apples (Weber et al., 2017).

Increasing CO₂ partial pressures in CA storage, resulted in higher cavities incidence in the fruit, being the highest incidence observed at 4.5 kPa CO₂, which differed from fruit stored with 0.5 kPa CO₂ (Fig. 2C and D). The 1-MCP application and harvest maturity did not affect the occurrence of cavities in ‘Monalisa’ apples (Fig. 2C). These results show that cavities are a symptom of CO₂ injury that follows the development of brown tissue in ‘Monalisa’ fruit (Fig. 2A–D). Previous studies have reported that the first symptom of CO₂ injury is the formation of brown areas in the flesh, which further evolve to cavities (Argenta et al., 2000; Lau, 1998). These results evidenced that ‘Monalisa’ apples are very sensitive to high CO₂ partial pressures in CA storage, occurring CO₂ injuries when the CO₂ partial pressure is higher than 0.5 kPa. Therefore, ‘Monalisa’ apples should not be stored under the same CA conditions recommended for ‘Gala’ apples that tolerate CO₂ partial pressures up to 3.0 kPa without developing injuries (Brackmann et al., 2008, 1996; Saquet et al., 1997).

Senescent-like flesh browning was more pronounced in late harvested fruit not treated with 1-MCP and stored under RA (Fig. 2E, F). This physiological disorder was not significantly affected by storage atmosphere (RA vs CA), as well by CO₂ partial pressures, when fruit were treated with 1-MCP or harvested at early maturity, which has also been previously reported for ‘Galaxy’ (Thewes et al., 2017) and ‘Luiza’ apples (Argenta et al., 2022). In contrast, other studies have suggested that high CO₂ partial pressure may increase senescent-like browning in ‘Gala’ apples (Brackmann et al., 2008; Saquet et al., 1997), which was not observed in ‘Monalisa’ apples.

Another physiological disorder associated to advanced apple ripening is fruit cracking, which also had higher incidence in late harvested fruit not treated with 1-MCP and stored in RA (Fig. 4A and B). CO₂ partial pressures had no effect on fruit cracking incidence in both experiments. Indeed, previous studies have shown that CA reduces fruit cracking incidence in ‘Gala’ apples, regardless of the CO₂ partial pressure (Brackmann et al., 2008; Saquet et al., 1997). Similarly, 1-MCP treatment has also been reported to reduce fruit cracking incidence in apples (Lee et al., 2016). A similar result was observed for skin browning incidence (scald-like), which was higher in RA stored fruit in both

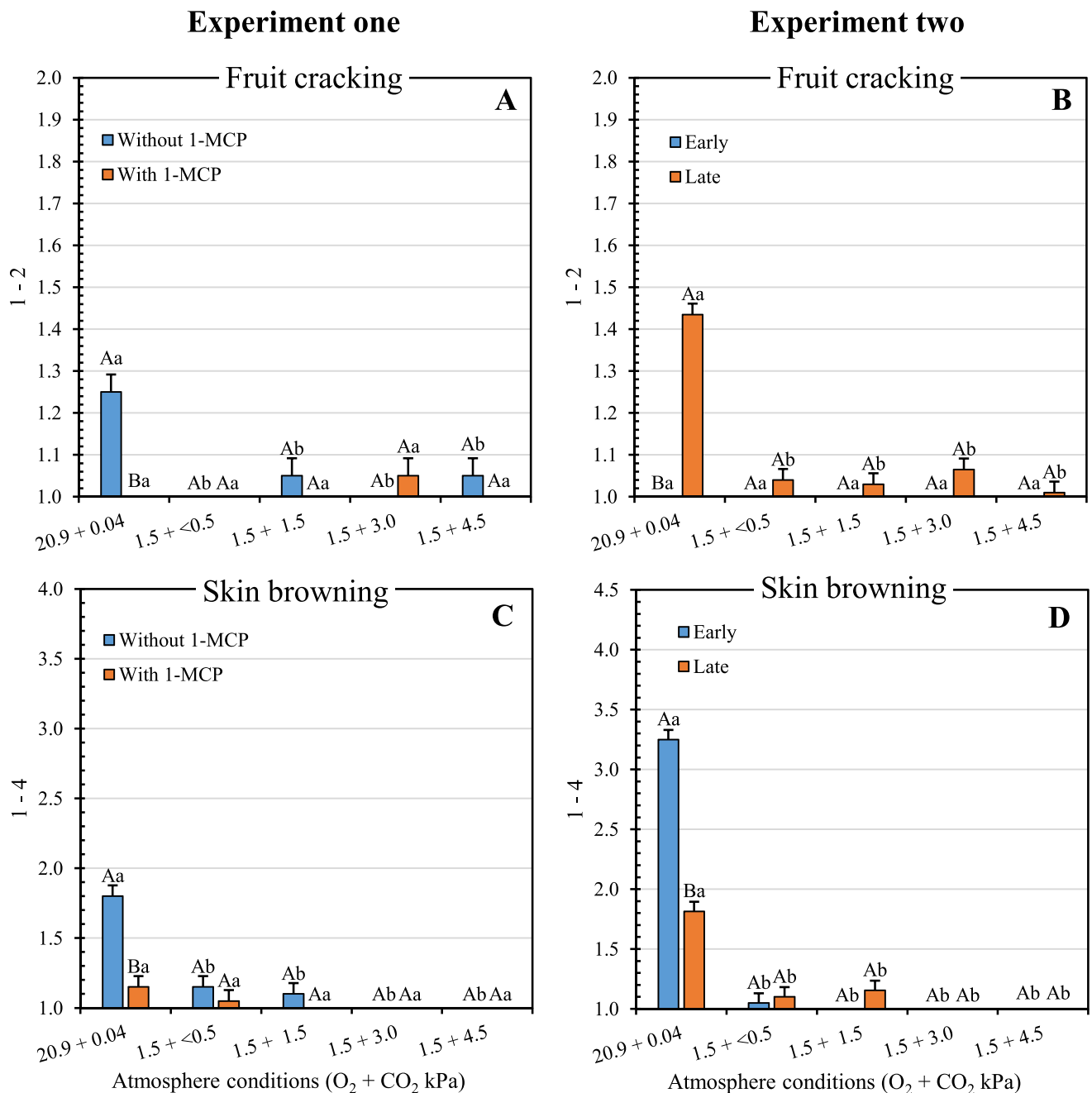


Fig. 4. Fruit cracking (A and B) and skin browning (‘scald-like’) (C and D) of ‘Monalisa’ apples after storage under RA and CA with four CO₂ partial pressures (0.5, 1.5, 3.0 and 4.5 kPa), either without or with 1-MCP treatment (experiment one) and early and late harvested (experiment two), plus 7 days of shelf life at 22 °C. Different lower case letters show statistical difference among storage atmospheres, and different uppercase letters show statistical difference between fruit treated and not treated with 1-MCP or harvest maturities by Tukey’s test, at 5% probability. Error bars mean standard error. Data are means of severity (1 - 2 or 1 - 4).

experiments (Fig. 4C and D). Late harvested fruit and 1-MCP treatment resulted in lower skin browning incidence in RA stored fruit. The different CO₂ partial pressures in CA had no effect on skin browning incidence (Fig. 4C and D).

Rough skin was affected by storage atmosphere conditions, 1-MCP treatment and harvest maturity (Fig. 5A and B). 1-MCP treatment increased the occurrence of rough skin, when fruit were stored under CA with 3 and 4.5 kPa CO₂ (Fig. 5A). This result demonstrates that there is a combined effect of 1-MCP and high CO₂ partial pressures on increasing ‘Monalisa’ apples susceptibility to rough skin. Late harvested fruit had higher rough skin incidence, regardless of the atmosphere conditions (Fig. 5B). Rough skin decreases fruit visual quality and has not been

described for other apple cultivars yet.

3.3. Decay incidence

The decay incidence was not affected by CO₂ partial pressures in both experiments (Fig. 5C and D). However, fruit stored under CA had lower decay incidence if harvested at early maturity and treated with 1-MCP. Indeed, previous studies have also shown that apple susceptibility to decay is highly affected by fruit maturity at harvest, as observed for ‘Luiza’ (Argenta et al., 2022), ‘Galaxy’ (Thewes et al., 2017), ‘Golden Smoothee’ (Vilanova et al., 2014), and ‘Honeycrisp’ apples (Watkins et al., 2005). When fruit were treated with 1-MCP, both RA and CA

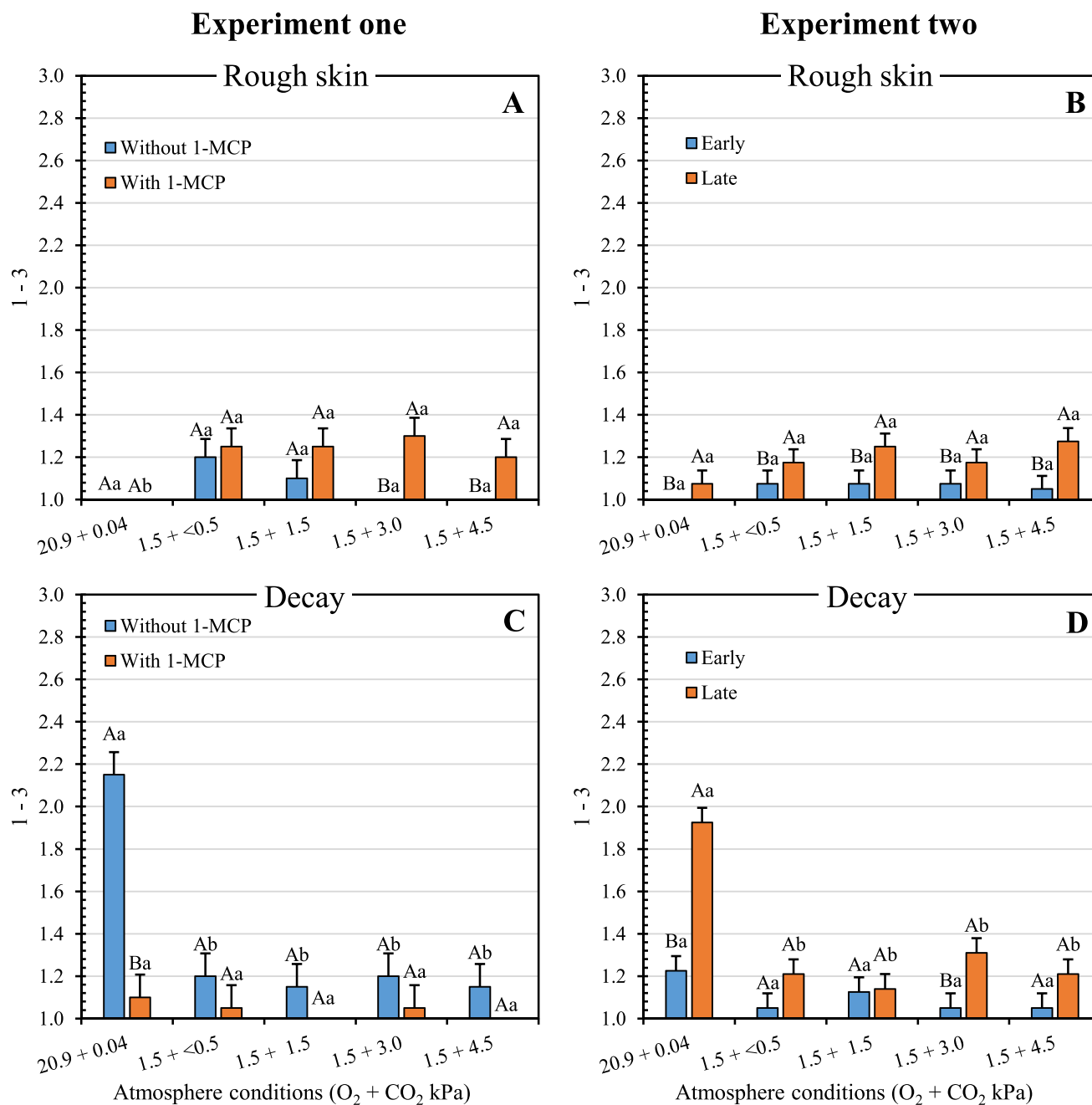


Fig. 5. Rough skin (A and B) and decay (C and D) of 'Monalisa' apples storage under RA and CA with four CO_2 partial pressures (0.5, 1.5, 3.0 and 4.5 kPa), either without or with 1-MCP treatment (experiment one) and early and late harvested (experiment two), plus 7 days of shelf life at 22 °C. Different lower case letters show statistical difference among storage atmospheres, and different uppercase letters show statistical difference between fruit treated and not treated with 1-MCP or harvest maturities by Tukey's test, at 5% probability. Error bars mean standard error. Data are means of severity (1 - 3).

stored fruit had the same low decay incidence (Fig. 5C), which can also be attributed to ripening inhibition by 1-MCP, as demonstrated by the low ethylene production and respiration rate (Fig. 1 and 8). The reduction of decay incidence by CA and 1-MCP is likely related to the delay of fruit ripening and reduced skin browning incidence (scald-like), which can weaken skin tissues and favor pathogen infection in the fruit.

3.4. Flesh firmness

Flesh firmness is one of the most important eating quality attributes considered by consumers (Bonany et al., 2013; Harker et al., 2008), which is highly affected by storage atmosphere, 1-MCP treatment and

harvest maturity (Fig. 6A and B). In the first experiment, when fruit were not treated with 1-MCP, CA storage resulted in higher flesh firmness, but when fruit were treated with 1-MCP, there was no difference between RA and CA (Fig. 6A). Therefore, 1-MCP treatment resulted in greater apple flesh firmness, regardless of the storage atmosphere (Fig. 6A). The higher flesh firmness retention in response to CA and 1-MCP was due to the observed lower ethylene production and fruit metabolic activity (Fig. 1A), which inhibited fruit ripening and ethylene dependent and independent cell wall degrading enzyme activities that are responsible for fruit softening after harvest (Gwanpua et al., 2016; Payasi et al., 2009).

Late harvested fruit had lower flesh firmness than early harvested

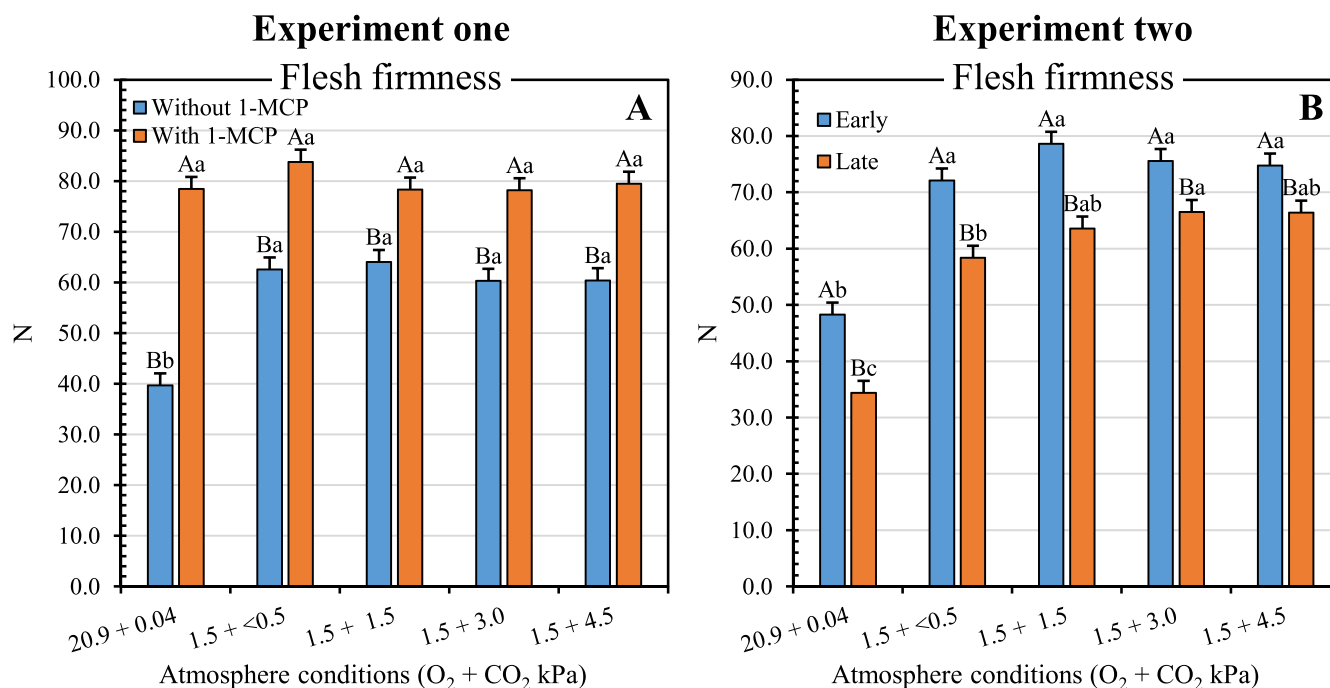


Fig. 6. Flesh firmness (A and B) of ‘Monalisa’ apples after storage under RA and CA with four CO₂ partial pressures (0.5, 1.5, 3.0 and 4.5 kPa), either without or with 1-MCP treatment (experiment one) and early and late harvested (experiment two), plus 7 days of shelf life at 22 °C. Different lower case letters show statistical difference among storage atmospheres, and different uppercase letters show statistical difference between fruit treated and not treated with 1-MCP or harvest maturities by Tukey’s test, at 5% probability. Error bars mean standard error.

fruit after both CA and RA storage conditions. However, CA storage effectively maintained higher flesh firmness than RA storage of fruit harvested at both maturity stages (Fig. 6B). The CO₂ partial pressure affected flesh firmness only in late harvested fruit (Fig. 6B), being firmness higher at 3.0 kPa of CO₂ than at 0.5 kPa CO₂. The effect of CO₂ partial pressure on flesh firmness is determined by several factors, such as pre-harvest (Watkins and Liu, 2010) and postharvest environmental conditions (Brackmann et al., 2015, 2008; Saquet et al., 1997). According to our results, ‘Monalisa’ apple harvest maturity also affected fruit responses to CO₂ partial pressures during storage, showing that late harvested fruit stored with 3.0 kPa CO₂ had the highest flesh firmness after storage. However, this combination also resulted in higher physiological disorders incidence in the fruit (Fig. 2). In addition, the CO₂ partial pressure had no effect on ethylene production in late harvested fruit (Fig. 1B), which suggests that the higher flesh firmness observed in fruit stored under CA with 3.0 kPa CO₂ can be attributed to ethylene action inhibition due to high CO₂ (Mathooko, 1996b).

3.5. Titratable acidity and soluble solids

Fruit treated with 1-MCP had higher titratable acidity (TA) in all storage conditions, except for CA with 4.5 kPa CO₂, where 1-MCP had no effect on TA (Fig. 7A). The strongest effect of 1-MCP on acidity maintenance was observed in RA stored fruit, which was similar to the 1-MCP effect observed on respiration rate in the same fruit (Fig. 1C and 8). These results were also observed in other apple cultivars, where the highest effect of 1-MCP on acidity maintenance was in RA stored fruit (Argenta et al., 2022; Watkins, 2008). In fruit not treated with 1-MCP, the CO₂ partial pressures had no effect on TA (Fig. 7A). However, in 1-MCP treated fruit, the highest TA was observed in CA stored fruit with 1.5, 3.0 and 4.5 kPa CO₂ (Fig. 7A). The harvest maturity had no effect on TA of ‘Monalisa’ apples stored either in RA or CA (Fig. 7B). CA fruit had higher TA than RA fruit, regardless of the harvest maturity (Fig. 7B). The TA behavior observed in ‘Monalisa’ apples was different than observed in other cultivars, such as ‘Gala’ (Thewes et al., 2017) and ‘Luiza’ apples

(Argenta et al., 2022), where early harvested fruit maintained higher acidity after long-term storage.

The SSC were higher in CA than in RA storage, regardless of the CO₂ partial pressure and 1-MCP treatment (Fig. 7C). This can be attributed to the lower respiration rate observed in CA stored fruit, compared to RA stored fruit (Fig. 1C). Late harvested fruit only showed higher SSC than early harvested fruit in CA storage with 4.5 kPa CO₂ (Fig. 7D). Accordingly, previous studies have shown that late harvested fruit usually have higher SSC after storage than early harvested fruit (Argenta et al., 2022; Lu et al., 2012; Thewes et al., 2017). However, in our study with ‘Monalisa’ apples, this behavior was dependent on the CA storage condition. Harvest and storage conditions favoring higher SS/TA ratio are desirable to improved fruit eating quality and consumer acceptance (Harker et al., 2008).

3.6. Overview of ‘Monalisa’ apple quality in response to harvest maturity, 1-MCP treatment and storage at high CO₂ partial pressures

According to the results obtained in the Principal Component Analysis (PCA), in both experiments, the PC 1 and PC 2 explained more than 80% of the overall data variability (Fig. 8). According to PC 1, in both experiments, the data are mostly separated in response to RA and CA storage, regardless of the 1-MCP treatment and harvest maturity (Fig. 8A and C). On the other hand, in both experiments, CO₂ partial pressures are separated across PC 2. This evidences that the main factors affecting fruit quality is the employment of CA, instead of RA, being the CO₂ partial pressure the most important factor on ‘Monalisa’ apple quality, when CA storage was employed. These results support the idea that CA storage plays an important role on determining ‘Monalisa’ apple quality. However, adequate CO₂ partial pressures must be applied to avoid physiological disorders incidence due to high CO₂ levels, such as CO₂ injury and cavity formation in the fruit (Fig. 8B and D).

The response of ‘Monalisa’ apples to high CO₂ partial pressures during storage are more influenced by the harvest maturity than by 1-MCP treatment (Fig. 8B and D). This result is supported by the strong

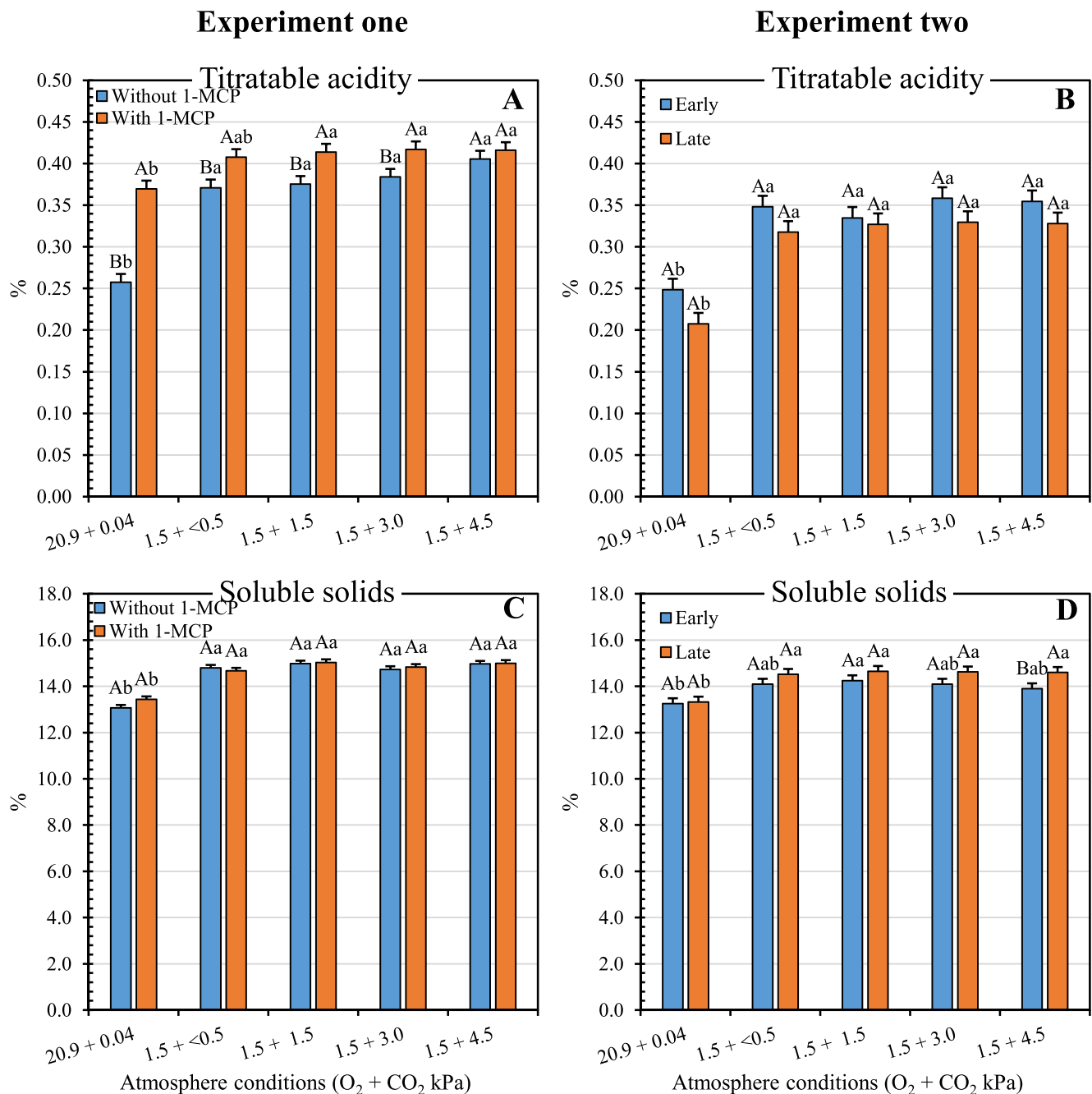


Fig. 7. Titratable acidity (A and B) and soluble solids (C and D) of ‘Monalisa’ apples after storage under RA and CA with four CO₂ partial pressures (0.5, 1.5, 3.0 and 4.5 kPa), either without or with 1-MCP treatment (experiment one) and early and late harvested (experiment two), plus 7 days of shelf life at 22 °C. Different lower case letters show statistical difference among storage atmospheres, and different uppercase letters show statistical difference between fruit treated and not treated with 1-MCP or harvest maturities by Tukey’s test, at 5% probability. Error bars mean standard error.

relationship observed between late harvested fruit stored under CA, with high CO₂ partial pressures, and the incidence CO₂ injury (browning and cavity). However, the incidence of skin browning is correlated to early harvested fruit stored under RA, which is probably a result of the high ethylene production and respiration rate (Fig. 8C and D). In addition, according to the TA and flesh firmness results, ‘Monalisa’ apples maintained better eating quality in response to early harvest, 1-MCP treatment and CA storage with 0.5 kPa CO₂ (Fig. 8). Therefore, the storage of ‘Monalisa’ apples should be performed under CA with low CO₂ partial pressure, which does not allow storing this cultivar together with ‘Gala’ apples due to the risk of physiological disorders incidence, especially the ones related to high CO₂ partial pressures.

4. Conclusions

‘Monalisa’ apples have high susceptibility to CO₂ injury in CA storage and to ‘scald-like’ and senescent flesh browning in regular air (RA). CO₂ injury is exacerbated with increasing CO₂ partial pressures in CA storage. Therefore, ‘Monalisa’ apples should not be stored under CA conditions with CO₂ higher than 0.5 kPa.

Late harvested ‘Monalisa’ apples are more susceptible to CO₂ injury, skin browning (‘scald-like’), senescent flesh browning, fruit cracking, decay, and rough skin.

1-MCP treatment at harvest improves retention of flesh firmness and acidity in ‘Monalisa’ apples stored in CA and RA conditions. Moreover,

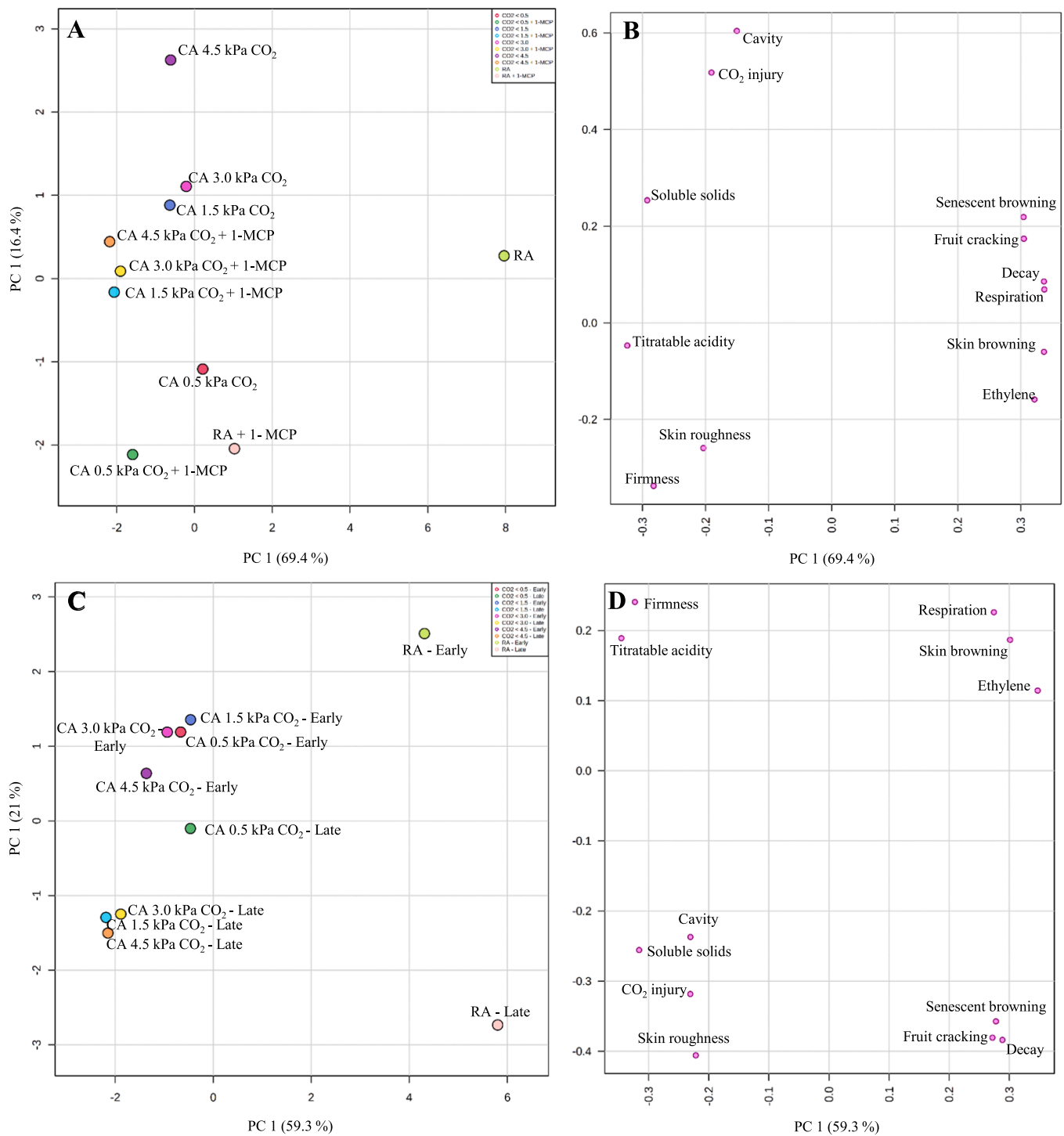


Fig. 8. Principal component analysis showing the two first principal components of experiment one (A and B) and experiment two (C and D) of ‘Monalisa’ apples after storage under RA and CA with four CO₂ partial pressures (0.5, 1.5, 3.0 and 4.5 kPa) plus 7 days of shelf life at 22 °C.

1-MCP substantially reduces senescent related disorders and fungal decay incidence in fruit stored in RA, while it does not affect CO₂ injury in fruit stored in CA.

CRediT authorship contribution statement

Fabio Rodrigo Thewes: Conceptualization, Methodology, Formal analysis, Data curation, Visualization, Writing – original draft, Writing – review & editing. **Luiz Carlos Argenta:** Conceptualization, Methodology, Formal analysis, Data curation, Visualization, Writing – original

draft, Writing – review & editing, Funding acquisition, Project administration, Resources, Supervision. **Rogério de Oliveria Anese:** Writing – review & editing. **Mayara Cristiana Stanger:** Writing – review & editing. **Sérgio Tonetto de Freitas:** Writing – review & editing.

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.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Data availability

All data are showed in the manuscript.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.scienta.2023.112038](https://doi.org/10.1016/j.scienta.2023.112038).

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