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Harvest and post harvest

Maintenance of 'Luiza' apple fruit quality as affected by postharvest practices

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Abstract - This study was carried out to examine the response of 'Luiza' apple to different storage atmospheres, durations, and chemical inhibition of ethylene action by 1-MCP. Analysis of fruit quality and physiological disorder incidence were performed every two months during eight months of storage for Exp. 1 and 2, and after eight months for Exp. 3. Both CA storage and 1-MCP treatment reduced fruit ethylene production and respiration and prevented the rapid fruit softening, flesh browning incidence and fungal decay of 'Luiza' apple. The combination of 1-MCP treatment before storage in CA provided an additional benefit in firmness retention after simulated marketing conditions at 22°C. Based on the time to reach a firmness of 53 N, the storage life of 'Luiza' apple is less than four months in air-storage and more than six months under CA-storage. 'Luiza' fruit did not develop symptoms of CO_2 injury when stored under high CO_2 partial pressures (up to 4.5 kPa). However, we observed increased flesh browning and fungal decay incidence for CA-stored fruit between six and eight months of storage. Therefore, the storage potential of 'Luiza' apple fruit may be limited to six months under CA-storage (1.5 kPa O_2 and 2.5 kPa CO_2).

Index terms:. *Malus* × *domestica* Borkh., flesh firmness, decay, storability, flesh browning.

Manutenção da qualidade de maçã 'Luiza' em função de práticas pós-colheita

Resumo-Este estudo foi realizado para examinar a resposta da maçã 'Luiza' a diferentes atmosferas e tempos de armazenamento, e a inibição química da ação do etileno pelo 1-MCP. As análises da qualidade dos frutos e da incidência de distúrbios fisiológicos foram realizadas a cada dois meses, durante oito meses de armazenamento para Exp. 1 e 2, e após oito meses para Exp. 3. Tanto o armazenamento em AC quanto o tratamento com 1-MCP reduziram a produção de etileno e a respiração dos frutos, impediram o rápido amolecimento dos frutos e reduziram a incidência de escurecimento da polpa em maçã 'Luiza'. A combinação do tratamento com 1-MCP na colheita e no armazenamento, em AC proporcionou benefício adicional na retenção da firmeza após o armazenamento mais sete dias a 22 °C. Com base no tempo para atingir firmeza de 53 N, o potencial de armazenamento da maçã 'Luiza' é inferior a quatro meses em ar refrigerado e superior a seis meses em AC. Maçãs 'Luiza' não desenvolveram sintomas internos de dano por CO_2 quando armazenadas sob altas pressões parciais de CO_2 (até 4,5 kPa). No entanto, houve aumento do escurecimento da polpa e incidência de podridões para maçãs armazenadas em AC por seis a oito meses. Por isso, o potencial de armazenamento do fruto da macieira 'Luiza' pode ser limitado a seis meses sob armazenamento em AC (1.5 kPa O_2 e 2.5 kPa CO_2).

Termos para indexação: *Malus* × *domestica* Borkh., firmeza da polpa, podridão, conservação, escurecimento.

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Introduction

The new apple cultivar 'Luiza' is resistant to Glomerella Leaf Spot, the main disease for 'Gala' apple in Southern Brazil (DENARDI et al., 2019). 'Luiza' has a sweet, crisp, and juicy taste and was generated by crossing 'Imperatriz' (♀) (Gala × Mollie's Delicious) with 'Cripps Pink' (♂) (Golden Delicious × Lady Williams) (DENARDI et al., 2019). When grown under southern Brazilian conditions, optimal harvest for 'Luiza' is between the last week of January and the second week of February, similarly to 'Gala' strains (MAGRIN et al., 2017; DENARDI et al., 2019).

'Luiza' apple fruit show a simultaneous increase of ethylene production and starch degradation during maturation, typical of early maturing cultivars at the beginning of summer. However, the rate of flesh firmness decrease is lower than other early maturing cultivars like 'Gala' during on-tree maturation (ARGENTA; MONDARDO, 1994; PLOTTO et al., 1995; MAGRIN et al., 2017). As 'Luiza' is a new cultivar, there is a lack of literature regarding its storage potential under different storage practices, such as cold air (21 kPa O_2), controlled atmosphere (CA), and 1-methylcyclopropene (1-MCP) application. Furthermore, the behavior of 'Luiza' apple during ripening and its susceptibility to decay incidence and CO_2 injury during storage is unknown.

Under air storage, which uses low temperature and high relative humidity, apple cultivars such as 'Gala' and 'Fuji' can only maintain quality for 4-6 months (ARGENTA; DENARDI, 1994; BRACKMANN et al., 1996). After long-term storage under air, fruit usually show a loss in flesh firmness and acidity and have high physiological disorders and decay incidences. CA storage, which uses low O_2 and high CO_2 partial pressures, can extend the storage period for up to 7-9 months (THEWES et al., 2015). The application of 1-MCP, an ethylene action inhibitor, increases the storage potential in air and CA by maintaining fruit quality (WATKINS, 2008). The storability of apple cultivars and the responses to chilling, low O_2 and high CO_2 storage atmospheres, and ethylene inhibition by 1-MCP vary greatly among apple cultivars (WATKINS et al., 2016). Furthermore, apple cultivars differ in susceptibility to physiological disorders during storage (WATKINS; MATTHEIS, 2019). Decay and physiological disorders, such as flesh browning and superficial scald, are the leading cause of apple losses (KADER, 2005; ARGENTA et al., 2021), influenced by harvest maturity, storage condition (temperature, O_2 and CO_2 partial pressure), and ethylene management.

The objective of this study was to examine the response of 'Luiza' apple to storage technologies, chemical inhibition of ethylene action, and storage duration. These studies can guide recommendations to the apple industry regarding the optimal storage treatments for new and established apple cultivars.

Material and Methods

Fruit source and sampling

Two commercial orchards established near Fraiburgo (Latitude 27° 1' 36'' S, Longitude 50° 55' 19'' W), Santa Catarina, Southern Brazil, were used for the study. Trees of 'Luiza' apple, grafted on 'Marubakaido' rootstock with a M9 interstock, were planted in 2007 at 0.9 × 4.0 m (Orchard 1) and in 2015 at 0.7 × 3.5 m spacing (Orchard 2). Trees of both orchards were trained to a central leader. Orchard practices (mineral fertilization, weed, insect and disease control, pruning, and growth regulator for dormancy release) followed the recommendations for integrated production for Southern Brazil (EPAGRI, 2018).

Fruit of a similar size that were representative of each orchard and the inner- and outer-canopy of both tree-row sides were harvested at mid-canopy height. In the laboratory, visually unblemished fruit were randomly selected to prepare homogeneous samples of 25 fruit held on fiberboard trays.

Experiments

Three experiments were carried out over three growing seasons. Experiments were conducted according to a completely randomized design. Fruit for the three experiments were treated with or without 1-methylcyclopropene (1-MCP) and stored in air (~21 kPa O_2) or controlled atmosphere (CA) with low O_2 (pO_2) and high CO_2 (pCO_2) partial pressure.

Experiment 1: Fruit were sampled at early harvest maturity (3 Feb. 2014 and 7 Feb. 2019) from Orchard 1 over two years and subjected to four postharvest treatments: 1: air (untreated control), 2: 1-MCP exposure and then regular atmosphere (Air+MCP), 3: CA (1.5 kPa O_2 and 2.5 kPa CO_2), and 4: 1-MCP exposure and then CA (CA+MCP). Fruit were stored for 2 to 8 months.

Experiment 2: Fruit from Orchard 2 were harvested on 6 and 17 Feb. 2020. These dates represented an early (harvest 1) and advanced (harvest 2) maturity. Following harvest, fruit were treated with or without 1-MCP and stored in air for 2 to 8 months.

Experiment 3: Fruit from Orchard 2 were treated with or without 1-MCP and then stored in air or CA with 1.5 kPa O_2 and four CO_2 partial pressures (<0.5, 1.5, 3.0 or 4.5 kPa) for eight months. Air storage was used as a standard of comparison.

1-MCP treatment

Within 24 h after harvest, fruit were exposed to ≈ 1 µL L^{-1} 1-MCP in a sealed steel container (1 m^3) 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 treatment container was checked

as described previously (MATTHEIS et al., 2005). Untreated fruit remained in air at the same temperature as 1-MCP-treated fruit.

Storage

Fruit were moved to cold storage room within two hours of 1-MCP treatment and cooled to 0.8ºC within 48 h of harvest. Fruit samples for air storage were placed on fiberboard trays and packed in cardboard boxes lined with perforated low-density polyethylene bags (20 µm = 10 µm per wall). Fruit samples for CA storage were held on fiberboard trays and enclosed in 0.150 m^3 stainless steel chambers with a plexiglass lid.

Low pO_2 and high pCO_2 atmosphere in CA chambers were established within 48 h after fruit cooling and maintained using compressed N_2, air, CO_2 and a CO_2 scrubber. Concentrations of O_2 and CO_2 were monitored and adjusted at 120 min intervals by an automatic CA system equipped with a dedicated manager software, an O_2 and CO_2 analyzer (Isolcell, Laives, Italy), CO_2 scrubber and VPSA N_2 generators (NeuTec, Lana, Italy). Hydrated lime [80 g of $Ca(OH_2)$ per kg fruit] was placed inside chambers to help maintain the CO_2 concentration below 0.05 kPa.

The temperature and relative humidity (RH) of storage atmospheres were measured automatically every five minutes using calibrated Pt100 and HUMICAP® sensors (Vaisala Inc. Finland) connected to a transmitter (HMT3307, Vaisala Inc. Finland) and to a dedicated analytical software (Kalfritec, Joinville, Brazil). The storage temperature was 0.8 ± 0.8 °C, and relative humidity (RH) ranged between 92 to 95 % for 90 % of the recorded data, based on box plot analysis.

After storage, fruit were held for one or seven days at 22 ± 1 °C before ripening and quality analysis.

Fruit ripening and quality assessment

Ripening and quality of 25 individual apples was determined 24 h after each harvest date by analyses of respiration, ethylene production, firmness, soluble solids content (SSC), titratable acidity (TA), starch index (SI) and fruit weight. For the post harvest assessment of fruit firmness, physiological disorders, and fungal decay, an amount of 75 (Experiment 1) or 50 (Experiments 2 and 3) fruit were evaluated from each harvest date, postharvest treatment, storage duration and length of shelf life combination. For ethylene production, respiration, TA and SSC measurements, four replicates of eight fruit were evaluated. Flesh firmness, starch index (1-9 scale), SSC and TA were assessed as previously described (ARGENTA et al., 2020). Assessments of ethylene and CO_2 production were performed using eight apples placed in 4 L jars through which compressed air flowed at 22ºC, as previously described (MATTHEIS et al., 2005). External and internal disorders were visually assessed using subjective scales of severity, where a score of 1 indicates the absence of disorders. Internal disorders were assessed from four transverse slices across the fruit. The severity of disorders was recorded according to the area of fruit surface or cortex cross-section affected or the number of lesions per fruit. Assessment of fungal decay, skin browning (a superficial scald-like disorder), shriveling, bitter pit, moldy core rot, and core browning were as previously described (ARGENTA et al., 2020). Flesh browning was assessed on a cross section of fruit at the equatorial region. Fruit affected by this disorder was scored as 2, 1–30 % of cortex with diffuse light browning; 3, 30–60% of cortex with diffuse light browning; or 4, >60% of cortex with diffuse light to dark browning (Fig 4). Background color was measured using color chart (1 to 5 scale) (ARGENTA et al., 2010).

Statistical analyses

Each experiment was analyzed separately. Data from both years in Experiment 1 were pooled, as the fruit response to storage treatments was similar for most variables (e.g. firmness, TA, fungal decay and flesh browning). Physiological and physicochemical data of Experiments 1 and 2 were subjected to regression analysis using the Equation Dynamic Fit Wizard of SigmaPlot software Version 14 (Systat Software Inc., San Jose, USA). Statistical models for each variable and treatment were initially selected by examining the Akaike information criterion (AIC) and then its validation by analyses of the determination coefficient and regression residuals. Additionally, treatment means for these variables were compared by Fisher's least significant difference LSD test ($\alpha = 0.05$) at each storage duration. The physicochemical data of Experiment 3 were subjected to analysis of variance (ANOVA) using R (TEAM-R-CORE, 2020) to determine main effects and interactions, and means were compared by Tukey test ($P \leq 0.05$).

Physiological disorders and fungal decay did not fit normal distribution by Shapiro-Wilk test. Thus, the non-parametric Kruskal-Wallis test for multiple comparisons of treatments and storage time was performed using R (TEAM-R-CORE, 2020) and the add-on package 'Agricolae' (MENDIBURU, 2017).

Results and Discussion

Maturity at harvest

'Luiza' apples harvested in 2014, 2019 and 2020 (harvest 1) showed adequate maturity for long-term storage (MAGRIN et al., 2017). Apple harvested in 2014 had a lower flesh firmness than other seasons, which could be associated with a larger fruit size and pre-harvest factors, such as environmental conditions

and crop load (Table 1). In 2020, flesh firmness was similar for both harvest dates, however, fruit picked at the later harvest (H2) were riper than earlier harvested fruit (H1), verified by high starch index, low titratable acidity (TA), and high soluble solids content (SSC). These results confirm that the maturation pattern of 'Luiza', which shows a slow decline in flesh firmness (~2.2 N/week) during on-tree maturation (MAGRIN et al., 2017) compared to 'Gala', which has a reduction in flesh firmness by more than 5 N a week (ARGENTA; MONDARDO, 1994; ARGENTA et al., 1995).

Table 1. Starch index (SI), flesh firmness, soluble solids content (SSC), titratable acidity (TA) and fruit weight of 'Luiza' apples from orchard one (experiment 1) and orchard 2 (experiments 2 and 3). Values are mean and standard deviation of 25 fruit.

Experiment	Year	Harvest time	SI (1-9)	Firmness (N)	SSC (%)	TA (%)	Weight (g)
1	2014	3 Feb. (H1)	6.4±1.6	77.5±3.3	13.8±0.2	0.238±0.031	138±6
1	2019	7 Feb. (H1)	5.4±1.8	80.2±5.8	12.4±0.3	0.295±0.034	120±8
2 and 3	2020	6 Feb. (H1)	5.5±2.0	81.5±5.6	13.5±0.3	0.345±0.029	113±8
2 and 3	2020	17 Feb. (H2)	8.3±1.2	80.4±5.2	14.2±0.2	0.287±0.036	122±9

Physiological and quality changes during storage in air and CA

CA-stored fruit showed a lower ethylene production and respiration rate during the whole storage and shelf life period compared to air-stored fruit, especially when treated with 1-MCP (Figure 1). Treatment with 1-MCP also reduced respiration rate and ethylene production by fruit throughout the eight months of air storage. Fruit treated with 1-MCP and then stored in air, and fruit stored in CA both with and without 1-MCP, showed similar respiration rates until six months of storage (Figure 1). After six-month storage, there was an increase in ethylene production and respiration in 1-MCP treated fruit stored in air. These results are similar to those of Mattheis et al. (2005), which found a reduced ethylene synthesis in 'Gala' apple treated with 1-MCP and stored under air until five months of storage compared to air-stored fruit without 1-MCP. The resuming of ethylene production in 1-MCP treated fruit stored in air might result from regenerated ethylene receptors (SISLER, 2006). However, the increasing ethylene production was not associated with significant changes in flesh firmness (Figure 2 A).

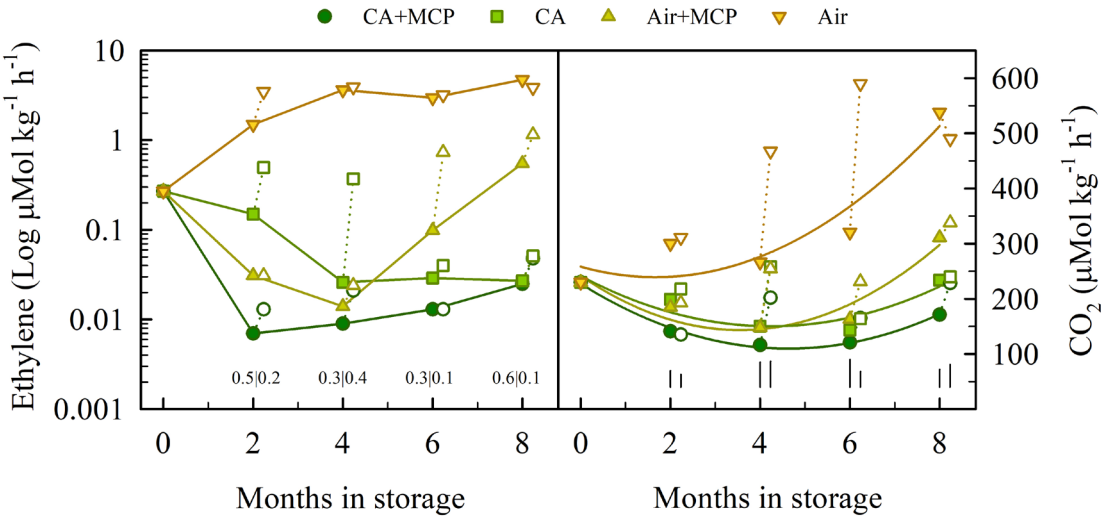

Figure 1. Ethylene production and respiration rate of 'Luiza' apples at harvest and after storage. Fruit were treated (+MCP) or not treated with 1-MCP and then stored at 0.8ºC in air or controlled atmosphere (CA) for two to eight months. Fruit held 1 d (filled symbols) or 7 d (open symbols) at 22°C after removal from storage. Inserted numbers or vertical lines are Fishers' LSD values, α= 0.05. Data of two years were pooled for this analysis (Experiment 1). Lines of statistical models (Table 2) are presented when fitted (P< 0.05) to CO_2 production data by regression analysis.

Flesh firmness reduction during storage adjusted to exponential and sigmoidal models, except for 1-MCP CA-stored fruit, which retained a flesh firmness similar to harvest for eight months (Figure 2A). Apple stored in air without 1-MCP had the highest reduction in flesh firmness from harvest. Although 1-MCP treated fruit in CA had the highest flesh firmness after storage, CA-stored fruit without 1-MCP kept a flesh firmness of more than 74 N after eight months of storage, which is above the lowest level of consumer acceptance (HARKER et al., 2008). Interestingly, 1-MCP fruit stored in air had the same flesh firmness retention of untreated fruit under CA. 'Luiza' apples harvested with advanced maturity (H2) showed a slightly higher rate of flesh firmness reduction than fruit harvest earlier (H1) (Figure 2B). This harvest maturity effect occurred during six months of storage for fruit without 1-MCP and between the sixth and eighth months for 1-MCP treated fruit, mainly after shelf life. The change in firmness retention in response to 1-MCP and CA for 'Luiza' apples is similar to that described for 'Gala' apple (BAI et al., 2005; MATTHEIS et al., 2005). 'Luiza' fruit untreated with 1-MCP and stored in air showed a similar loss in flesh firmness as reported for 'Gala', however, in untreated CA-stored 'Luiza' fruit, softening occurred at a slightly lesser rate than 'Gala' (BAI et al., 2005; MATTHEIS et al., 2005). Firmness is the dominant factor of consumer acceptance of apples (HARKER et al., 2008). There is a substantial increase in acceptance by consumers when fruit firmness increases from ~36N to ~62N, and smaller improvements in acceptance with a fruit firmness higher than 62 N (HARKER et al., 2008). Additionally, there is an abrupt increase in mealy apple percentage as firmness decreases below 50 N (HARKER et al., 2002). Considering a minimum firmness threshold of 62 N to deliver to the market, the storage potential of 'Luiza' apple is between two to four months when untreated with 1-MCP and stored under air, and eight months when treated with 1-MCP and stored in CA (Figure 2).

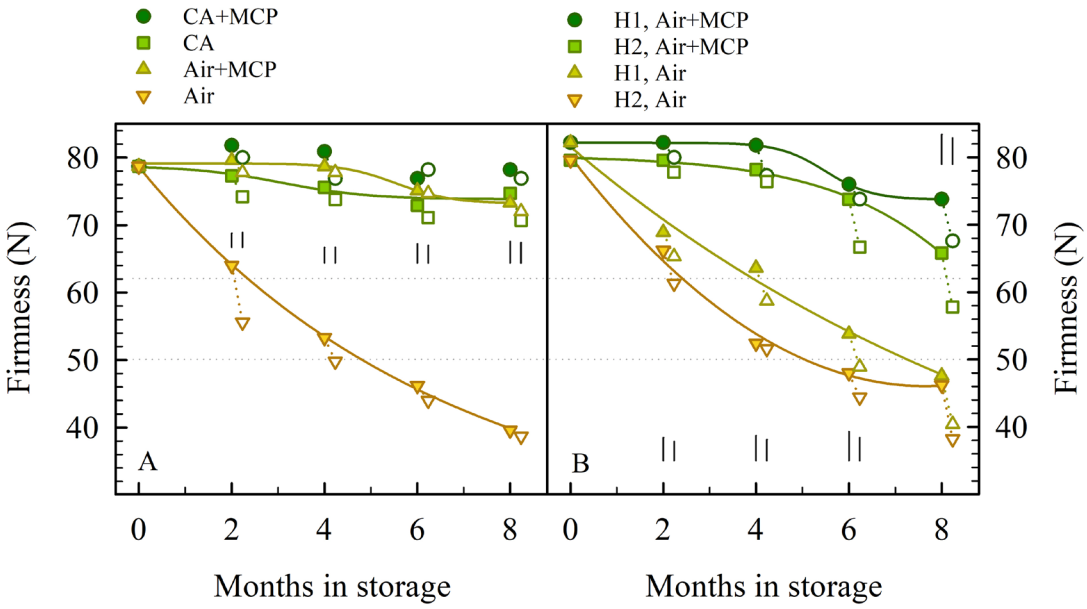

Figure 2. Flesh firmness of 'Luiza' apples at harvest and after storage. Fruit were treated (+MCP) or not treated with 1-MCP and then stored at 0.8ºC in air or controlled atmosphere (CA) for two to eight months. Fruit held 1 d (filled symbols) or 7 d (open symbols) at 22°C after removal from storage. Left graphic (A): Fruit of one harvest maturity were stored in air or CA. Data of two years were pooled for this analysis (Experiment 1). Right graphic (B): Fruit of early- (H1) and advanced (H2) maturity were stored in air (Experiment 2). Lines of statistical models (Table 2) are presented when fitted ($P< 0.05$) to data by regression analysis. Inserted vertical lines are Fishers' LSD values ($\alpha= 0.05$) for treatment effects in each assessment date.

Both CA and 1-MCP reduced the loss of TA during storage, however, CA was more effective in TA retention than 1-MCP (Figure 3A). The effect of 1-MCP on TA maintenance was more pronounced in air-stored fruit. In Experiment 2, fruit of both harvest maturities showed similar rates of acidity loss throughout storage, although earlier harvested fruit retained a higher TA (Figure 3B).

Dynamic changes in SSC during storage varied between harvest maturities and post harvest practices. SSC increased slightly during the first months of storage for all storage treatments (Figure 3C), excluding later harvested fruit stored in air, where SSC decreased steadily throughout the storage period (Figure 3D). CA-stored fruit retained a higher SSC than air-stored fruit untreated with 1-MCP (Figure 3C). In earlier harvest fruit, 1-MCP treated fruit retained a higher SSC than untreated fruit, whereas there was no difference in treated and untreated fruit for the later harvest (Figure 3D).

The highest SSC/TA ratio after long term storage was in air stored fruit (Figure 3E) and later harvested fruit (Figure 3F). The increase in SSC/TA ratio in all treatments throughout storage was mainly due to the reduction in TA.

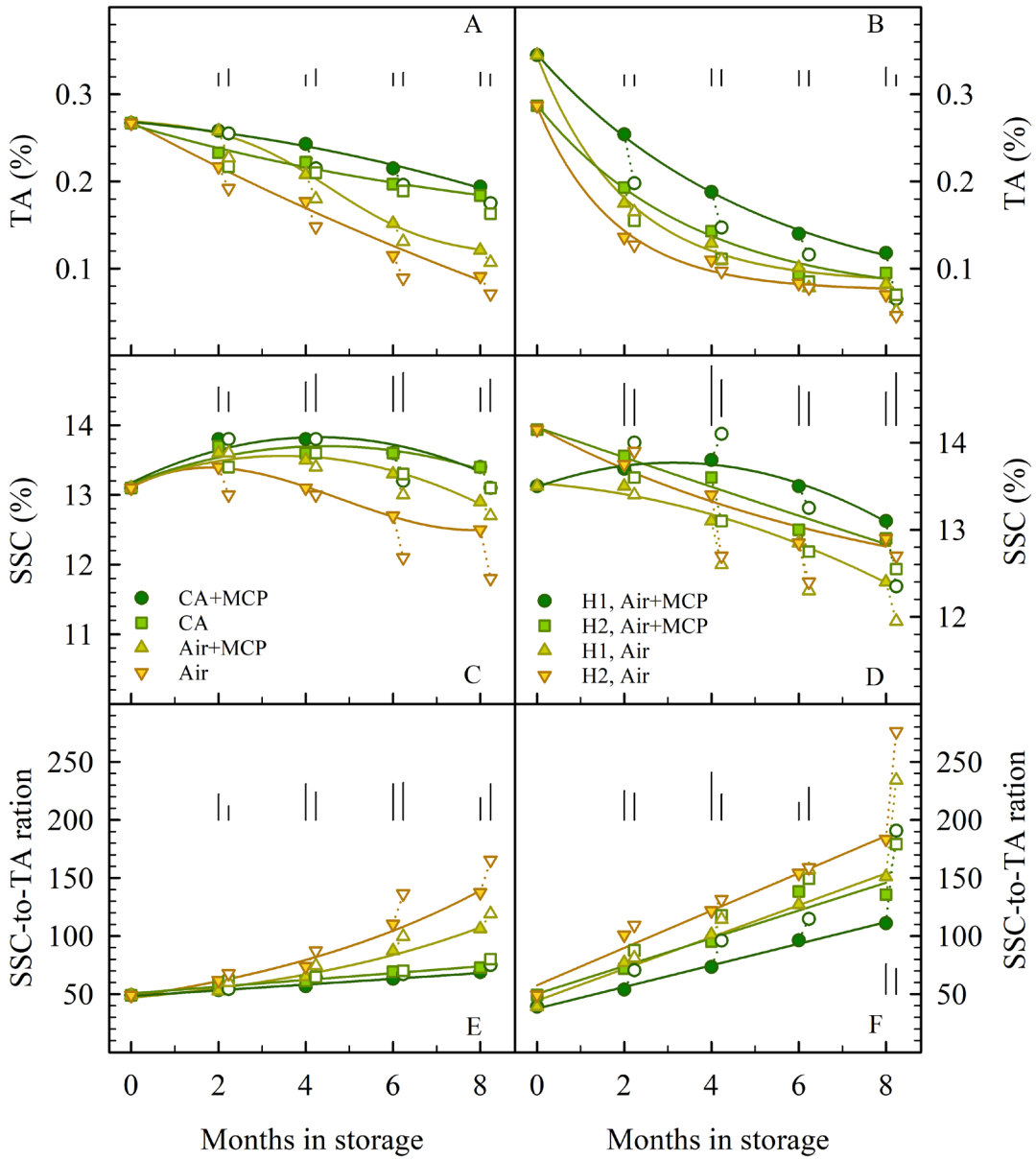

Figure 3. Titratable acidity (TA), soluble solids content (SSC) and SSC-to-TA ration of 'Luiza' apples at harvest and after storage. Fruit were treated (+MCP) or not treated with 1-MCP and then stored at 0.8ºC in air or controlled atmosphere (CA) for two to eight months. Fruit held 1 day (filled symbols) or 7 days (open symbols) at 22°C after removal from storage. Left graphic (A): Fruit of one harvest maturity were stored in air or CA. Data of two years were pooled for this analysis (Experiment 1). Right graphic (B): Fruit of early- (H1) and advanced (H2) maturity were stored in air (Experiment 2). Lines of statistical models (Table 2) are presented when fitted ($P< 0.05$) to data by regression analysis. Inserted vertical lines are Fishers' LSD values ($\alpha= 0.05$) for treatment effects in each assessment date.

Table 2. Statistical models of variables change as a function of storage time fitted by regression analysis. Air: atmosphere of air (21 kPa O_2), CA: Controlled atmosphere, MCP (1-methylciclopropene), H1: early harvest, H2: late harvest.

Variable and year	Treatment		R^2
Ethylene	Air	$f = 0.54+0.521x$	0.87**
	CA	$f = 0.27-0.082x+0.0065x^2$	0.98*
	Air+MCP	$f = 0.28-0.18x+0.026x^2$	0.97*
	CA+MCP	$f = (0.27*0.125)/(0.125+x)$	0.98***
Respiration			
2014 - 2019	Air	$f =258.3–22.8x+6.8x^2$	0.87*
	CA	$f = 241.6–43.1x+5.1x^2$	0.82*
	Air+MCP	$f = 241.9–55.5x+7.8x^2$	0.91*
	CA+MCP	$f = 227.4–50.8x+5.5x^2$	0.99***
Firmness (N)			
2014 -19	Air	f= 23. 8+54.8exp(-0.15x)	0.99***
	CA	f = 73.8+4.9/(1+exp(-(x-3.07)/-0.94))	0.89*
	Air+MCP	f = 73.2+5.9/(1+exp(-(x-5.55)/-0.59))	0.98**
	CA+MCP	ns	
2020	H1, Air	$f = 80.1-8.9x+0.58x^2$	0.99***
	H2, Air	f = 14.6+67exp(-0.088x)	0.99*
	H1, Air +MCP	f = 73.7+8.4/(1+exp(-(x-164.8)/-14.9))	0.99***
	H2, Air +MCP	f = 80.3/(1+exp(-(x-11.08)/-2.04))	0.99***
Acidity (%)			
2014 -19	Air	f = -0.34+0.61exp(-0.044x)	0.99**
	CA	f = 0.12+0.14exp(-0.106x)	0.89*
	Air+MCP	f = 0.113+0.16/(1+exp(-(x-4.55)/-1.23))	0.98***
	CA+MCP	f = 0.29/(1+exp(-(x-10.98)/-4.6))	0.99***
2020	H1, Air	f = 0.074+0.21exp(-0.56x)	0.99**
	H2, Air	f = 0.064+0.22exp(-0.28x)	0.99**
	H1, Air+MCP	f = 0.083+0.26exp(-0.49x)	0.99**
	H2, Air+MCP	f = 0.054+0.29exp(-0.19x)	0.99***
SST (%)			
2014 -19	Air	$f = 13.1+0.35x-0.12x^2+0.0083x^3$	0.99**
	CA	$f = 13.15+0.24x-0.034x^2$	0.93***
	Air+MCP	$f = 13.16+0.23x-0.027x^2$	0.81***
	CA+MCP	$f = 13.17+0.31x-0.036x^2$	0.86***
2020	H1, Air	$f = 13.53-0.035x-0.013x^2$	0.98***
	H2, Air	f = 12.0+2.17exp(-0.12x)	0.96***
	H1, Air+MCP	$f = 13.49+0.18*x-0.029x^2$	
	H2, Air+MCP	f = -4.64+18.8exp(-0.0093x)	0.96***
SST/TA			
2014 -19	Air	f = 12.7+35.3exp(0.159x)	0.98**
	CA	f = 27.4+19.5exp(0.175x)	0.99**
	Air+MCP	f = 50.6+2.91x	0.96***
	CA+MCP	f = 48.34+2.48x	0.98***
2020	H1, Air	f = 44.4+13.7x	0.99***
	H2, Air	f = 57.6+16.08x	0.98***
	H1, Air+MCP	f = 50.24+11.96x	0.94***
	H2, Air+MCP	f = 37.6+9.3x	0.99***

Only statistically significant models are presented at $P < 0.001$ (***), $P < 0.01$ (**), $P < 0.05$ (*).

For Experiment 1 and 2, decay incidence was lower in 1-MCP treated fruit than untreated fruit during air storage (Table 3 and 4). CA-stored fruit had lower incidences of decay than air-stored fruit after eight months of storage plus shelf life (Table 3). The application of 1-MCP provided no benefit to decay incidence in CA-stored fruit. In Experiment 2, 1-MCP reduced decay incidence in fruit of advanced maturity stored for six and eight months plus a seven-day shelf life (Table 4). Longer storage durations can increase the opportunity for postharvest infection and fungal development in fruit (KIM and XIAO, 2006; NERI et al., 2009). Decay occurrence in 'Luiza' fruit was lower than that found in 'Gala' and 'Fuji' apples stored in CA under commercial conditions (ARGENTA et al., 2021). These authors reported that losses caused by fungal decay during storage ranged from 8.4% to 17.6% for 'Gala' apples, depending on the year. The difference in decay incidence during storage between 'Luiza' and 'Gala' apples can be related to the genetic characteristics of 'Luiza'. However, fruit harvested in this experiment were from a young orchard (< 8 years). Previous studies have demonstrated that postharvest fruit decay incidence increases with orchard age (SPOTTS et al., 2009). Furthermore, in this work, apples were cooled immediately after harvest. Another important cause of commercial apple loss during storage is moldy core rot. In this study, moldy core rot did not increase during the storage period (Table 5 and 6), probably because infections occur only during pre-harvest. In Experiment 2, there were no differences among treatments and harvest dates for moldy core incidence (data not shown). In relation to storage atmosphere (Experiment 1), after eight months plus 1 d of shelf life, only fruit in air with 1-MCP showed moldy core incidences, but the incidence did not differ to fruit stored for eight months plus 7 d of shelf life.

Flesh browning severity after 7 d of shelf life increased with storage duration in Experiments 1 and 2 (Table 3 and 4). After eight months of air storage plus 1 d of shelf life, fruit treated with 1-MCP showed higher flesh browning than untreated fruit. However, after 7 d of shelf life, 1-MCP-treated fruit had no incidences of flesh browning compared to 7.8% in untreated fruit (Table 3). Untreated fruit also had a higher flesh browning incidence than 1-MCP treated fruit after eight months of CA storage and a 7 d of shelf life (Table 3). These results indicate that chemical inhibition of ethylene action was effective in reducing flesh browning after eight months plus 7 d of shelf life in air and CA stored fruit, but did not have an effect on fruit stored for six months under CA.

'Luiza' apples at late harvest had a higher flesh browning severity than earlier harvested fruit after eight months of air storage plus 1 d of shelf life (Table 4). After eight months of storage plus a 7 d of shelf life, flesh browning incidence did not differ between harvest maturities in untreated fruit. In contrast, in 1-MCP treated fruit, flesh browning incidence differed between harvest maturities. In earlier harvested fruit, 1-MCP application reduced flesh browning incidence, while there was no difference in flesh browning incidence in treated and untreated fruit of advanced harvest maturity. These results are consistent with Magrin et al. (2017), which reported a higher flesh browning incidence in 'Luiza' fruit with an advanced harvest maturity. Flesh browning symptoms, like observed in 'Luiza' apples (Figure 4), may be a senescence-related disorder or a response to chilling injury (WATKINS AND MATTHEIS, 2019). These symptoms are frequently associated with a loss in membrane integrity and the oxidation of phenolic compounds (FRANCK et al., 2007).

In this study, core browning (Figure 5) was observed only after eight months of storage plus 7 d of shelf life in Experiment 1 (Table 5). CA-stored fruit without 1-MCP had the lowest core browning severity compared to the other storage treatments. 'Luiza' apple developed a peel disorder typified by a superficial browning (skin browning) after eight months of storage plus 7 d of shelf life (Table 5 and 6). In Experiment 1, there was no difference in skin browning among treatments. In Experiment 2, skin browning incidence was higher in earlier harvested fruit than in fruit of advanced maturity. However, this disorder was reduced in earlier harvest fruit by 1-MCP application. Fruit cracking only occurred in earlier harvested fruit in Experiment 2 after eight months of air storage and 7 d of shelf life (Table 6).

Table 3. Severity and incidence of fungal decay and flesh browning in 'Luiza' apple treated (+MCP) or not treated with 1-MCP at harvest then stored in air or controlled atmosphere (CA, 1.5 kPa O_2 and 2.5 kPa CO_2) at 0.8 °C for 2 to 8 months followed by 1 and 7 days at 22°C. Data of 2014 and 2019 were pooled (n=75).

Month	Air	Air+MCP	CA	CA+MCP	Air	Air+MCP	CA	CA+MCP
	Severity*				Incidence (%)			
	Decay							
	1 day shelf life							
2	1.01aA**	1.00aA	1.00aA	1.01aA	1.4	0.0	0.0	1.4
4	1.03aA	1.00aA	1.00aA	1.00aA	1.4	0.0	0.0	0.0
6	1.03aA	1.03aA	1.00aA	1.00aA	1.4	1.4	0.0	0.0
8	1.03aA	1.01aA	1.03aA	1.02aA	2.9	1.4	1.4	2.2
	7 day shelf life							
2	1.01bA	1.00bA	1.02aA	1.00A	1.1	0.0	1.1	0.0
4	1.00b	1.00b	1.00a	1.00	0.0	0.0	0.0	0.0
6	1.03abA	1.03abA	1.04aA	1.00A	2.2	2.2	2.2	0.0
8	1.09aA	1.04aAB	1.01aB	1.00B	6.7	4.3	1.1	0.0
	Flesh browning							
	1 day shelf life							
2	1.00a	1.00b	1.00b	1.00	0.0	0.0	0.0	0.0
4	1.00a	1.00b	1.00b	1.00	0.0	0.0	0.0	0.0
6	1.00a	1.00b	1.00b	1.00	0.0	0.0	0.0	0.0
8	1.03aB	1.14aA	1.04aB	1.00B	1.4	11.4	4.3	0.0
	7 day shelf life							
2	1.00b	1.00	1.00b	1.00b	0.0	0.0	0.0	0.0
4	1.02bA	1.00B	1.00bB	1.00bB	2.2	0.0	0.0	0.0
6	1.06bA	1.00B	1.04bA	1.04aA	3.3	0.0	4.4	4.4
8	1.09aA	1.00B	1.10aA	1.01abB	7.8	0.0	10.0	1.1

* Scales of 1 to 3 for decay and 1 to 4 for flesh browning

** Means of severity followed by different lower-case letter in each column and different upper-case letter in each row are different according to Kruskal-Wallis test (α = 0.05).

Figure 4. 'Luiza' apple fruit grown in southern Brazil without flesh browning (top left) and with light (top middle), moderate (top right) and severe (bottom) flesh browning symptoms.

Table 4. Severity and incidence of fungal decay and flesh browning in 'Luiza' apple treated (+MCP) or not treated with at harvest then stored in air at 0.8 °C for 2 to 8 months followed by 1 and 7 days at 22°C. Fruit harvested at early (H1) and advanced (H2) maturity. 2020 season.

Month	Air		Air+MCP		Air		Air+MCP	
	H1	H2	H1	H2	H1	H2	H1	H2
	Severity*				Incidence (%)			
	Decay							
	1 day shelf life							
2	1.00 aA**	1.04 bA	1.00 aA	1.04 aA	0.0	4.0	0.0	4.0
4	1.00 aA	1.08 bA	1.00 aA	1.00 aA	0.0	4.0	0.0	0.0
6	1.04 aA	1.08 abA	1.06 aA	1.08 aA	2.0	8.0	4.0	4.0
8	1.02 aB	1.16 aA	1.06 aB	1.08 aAB	2.0	12.0	4.0	8.0
	7 day shelf life							
2	1.00 bA	1.00 bA	1.00 aA	1.04 aA	0.0	0.0	0.0	4.0
4	1.04 bA	1.04 bA	1.00 aA	1.08 aA	4.0	4.0	0.0	4.0
6	1.06 abB	1.16 aA	1.00 aB	1.04 aB	6.0	14.0	0.0	4.0
8	1.14 aA	1.26 aA	1.04 aB	1.04 aB	14.0	20.0	2.0	4.0
	Flesh browning							
	1 day shelf life							
2	1.00 a	1.00 a	1.00 a	1.00 b	0.0	0.0	0.0	0.0
4	1.00 a	1.00 a	1.00 a	1.00 b	0.0	0.0	0.0	0.0
6	1.02 aA	1.00 aA	1.00 aA	1.00 bA	2.0	0.0	0.0	0.0
8	1.00 aB	1.08 aA	1.04 aB	1.12 aA	0.0	8.0	4.0	12.0
	7 day shelf life							
2	1.00 b	1.00 b	1.00 b	1.00 b	0.0	0.0	0.0	0.0
4	1.00 b	1.00 b	1.00 b	1.00 b	0.0	0.0	0.0	0.0
6	1.02 bA	1.02 bA	1.00 bA	1.02 bA	2.0	2.0	0.0	2.0
8	1.22 aA	1.18 aA	1.04 aB	1.12 aA	12.0	12.0	4.0	12.0

* Scales of 1 to 3 for decay and 1 to 4 for flesh browning.
** Means of severity followed by different lower-case letter in each column and different upper-case letter in each row are different according to Kruskal-Wallis test ($\alpha = 0.05$).

Figure 5. 'Luiza' apple fruit grown in southern Brazil with core browning symptoms.

Table 5. Severity and incidence of moldy core rot, skin browning, fruit cracking and core browning in 'Luiza' apple treated (+MCP) or not treated with 1-MCP at harvest then stored in air or controlled atmosphere (CA, 1.5 kPa O_2 and 2.5 kPa CO_2) at 0.8 °C for 2 to 8 months followed by 1 and 7 days at 22°C. Data of 2014 and 2019 were pooled (n=75).

Month	Air	Air+MCP	CA	CA+MCP	Air	Air+MCP	CA	CA+MCP
	Severity*				Incidence (%)			
				Moldy core rot				
				1 day shelf life				
2	1.03aA**	1.04abA	1.01aA	1.01aA	1.4	2.9	1.4	1.4
4	1.00a	1.00b	1.00a	1.00a	0.0	0.0	0.0	0.0
6	1.00a	1.00b	1.00a	1.00a	0.0	0.0	0.0	0.0
8	1.00aB	1.09aA	1.00aB	1.00aB	0.0	7.1	0.0	0.0
				7 day shelf life				
2	1.00aB	1.02aA	1.00.B	1.00aB	0.0	2.2	0.0	0.0
4	1.01aA	1.00aA	1.00.A	1.02aA	1.1	0.0	0.0	2.2
6	1.00a	1.00a.	1.00	1.00a	0.0	0.0	0.0	0.0
8	1.00aA	1.04aA	1.00.A	1.01aA	0.0	2.2	0.0	1.1
				Skin browning				
				1 day shelf life				
8	1.00	1.00	1.00	1.00	0.0	0.0	0.0	0.0
				7 day shelf life				
8	1.02A	1.00A	1.01A	1.01A	1.1	0.0	1.1	1.1
				Fruit Cracking				
				1 day shelf life				
8	1.00	1.00	1.00	1.00	0.0	0.0	0.0	0.0
				7 day shelf life				
8	1.00	1.00	1.00	1.00	0.0	0.0	0.0	0.0
				Core Browning				
				1 day shelf life				
8	1.00	1.00	1.00	1.00	0.0	0.0	0.0	0.0
				7 day shelf life				
8	1.06A	1.02A	1.01B	1.02A	5.7	2.2	1.1	2.2

* Scales of 1 to 2 for fruit cracking, 1 to 3 for moldy core rot and core browning and 1 to 4 for Skin browning.
** Means of severity followed by different lower-case letter in each column and different upper-case letter in each row are different according to Kruskal-Wallis test (α = 0.05).

Controlled atmosphere (CA) with increased CO_2 partial pressures (pCO_2)

In Experiment 3, flesh firmness was higher in fruit treated with 1-MCP than untreated fruit, regardless of harvest maturity and storage atmosphere (Table 7). CA-stored fruit retained higher flesh firmness than air storage. Increasing pCO_2 from 0.5 to 4.5 kPa in CA storage did not improve retention of flesh firmness. This response of 'Luiza' apple fruit to pCO_2 in CA differs from other apple cultivars like 'Gala', which retains a higher flesh firmness in CA with a pCO_2 of 3 to 5 kPa compared to 0 to 1 kPa (BRACKMANN et al., 1996).

Table 6. Severity and incidence of skin browning, fruit cracking in 'Luiza' apple treated (+MCP) or not treated with 1-MCP at harvest then stored in air at 0.8 ºC for 8 months followed by 7 days at 22ºC. Fruit harvested at early (H1) and advanced (H2) maturity. 2020 season.

Month	Air		Air+MCP		Air		Air+MCP	
	H1	H2	H1	H2	H1	H2	H1	H2
	Severity*				Incidence (%)			
	Skin browning							
8	1.12A**	1.00B	1.04B	1.00B	12.0	0.0	4.0	0.0
	Fruit Cracking							
8	1.02aA	1.00A	1.00A	1.00A	2.0	0.0	0.0	0.0

*Scales of 1 to 2 for fruit cracking and 1 to 4 for Skin browning.
**Means of severity followed by different upper-case letter in each row are different according to Kruskal-Wallis test (α = 0.05).

Table 7. Flesh firmness, background color, soluble solid content, and titratable acidity of 'Luiza' apple fruit after storage in air (O_2=21 kPa) or in controlled atmosphere (O_2 =1.5 kPa) with different concentrations (kPa) of CO_2. Fruit harvested at early (Harvest 1) and advanced (Harvest 2) maturity, 2020 season, were exposed (MCP) or not exposed (C) to 1-methylcyclopropene within 24 h after harvest. Fruit held 7 d at 22ºC following 8 months storage.

Treatment		Harvest 1			Harvest 2			Average		
O_2	CO_2	C	MCP	Average	C	MCP	Average	C	MCP	
		Firmness (N)								
21	<0.5	42.7	67.9	55.3bA	39.1	70.8	55.0bA	40.9bB	69.4bA	
1.5	<0.5	68.0	75.3	71.6aA	63.5	70.6	67.0aB	65.7aB	73.0abA	
1.5	1.5	68.3	79.7	74.0aA	62.6	71.0	66.8aB	65.4aB	75.3aA	
1.5	3.0	71.0	79.4	75.2aA	61.8	72.8	67.3aB	66.4aB	76.1aA	
1.5	4.5	66.1	81.4	73.7aA	60.0	73.3	66.7aB	63.0aB	77.4aA	
		Background color (1-5)								
21	<0.5	5.0	5.0	5.0aA	5.0	5.0	5.0aA	5.0aA	5.0aA	
1.5	<0.5	4.0	3.4	3.7bB	4.4	4.1	4.3bA	4.2bA	3.7bB	
1.5	1.5	3.6	3.6	3.6bB	4.1	4.4	4.3bA	3.8bA	4.0bA	
1.5	3.0	3.2	3.6	3.4bB	4.3	4.3	4.3bA	3.7bA	3.9bA	
1.5	4.5	3.8	3.9	3.8bB	4.3	4.5	4.4bA	4.0bA	4.2bA	
		Titratable acidity (%)								
21	<0.5	0.075	0.106		0.072	0.092		0.073	0.099	0.086c
1.5	<0.5	0.139	0.142		0.135	0.136		0.137	0.139	0.138b
1.5	1.5	0.150	0.169		0.139	0.161		0.144	0.165	0.155a
1.5	3.0	0.154	0.161		0.141	0.159		0.148	0.160	0.154a
1.5	4.5	0.159	0.179		0.152	0.175		0.155	0.177	0.166a
Average								0.131 B	0.148 A	
		Soluble solids content (%)								
21	<0.5	12.0	12.4		12.6	13.3		12.3	12.8	12.6b
1.5	<0.5	13.5	13.2		13.3	13.5		13.4	13.3	13.6a
1.5	1.5	13.3	13.5		13.5	13.6		13.4	13.6	13.5a
1.5	3.0	13.2	13.3		13.5	13.5		13.4	13.4	13.4a
1.5	4.5	13.2	13.6		13.3	13.6		13.3	13.6	13.4a
Average				13.1B			13.4 A	13.1B	13.4A	

Source	Firmness	Background color	Titratable acidity	Soluble solids content
Treatment	***	***	***	***
Harvest	***	***	ns	**
MCP	***	ns	**	*
T x H	**	**	ns	ns
H x M	ns	ns	ns	ns
T x M	***	**	ns	ns
T x H x M	ns	ns	ns	ns

Means followed by same lower-case letter in each column and same upper-case letter in each row are not different by Tukey test ($p < 0.05$). Lower-case letters compare treatments (storage atmosphere) in each row while upper-case compare harvests (H_1 vs H_2) or 1-MCP exposure (C vs MCP) in each row. ns, *, **, ***: not significant or significant at $p < 0.05$, 0.01 or 0.001, respectively.

Background color increased under air storage for both harvest dates (Table 8). Delayed harvested fruit had a higher background color than earlier harvested fruit, likely due to the advanced stage of ripening. Under CA, increasing CO_2 concentration did not affect background color, regardless of harvest maturity. Similarly, 1-MCP treatment did not prevent skin yellowing regardless of storage atmosphere and harvest maturity. However, for other apple cultivars such as 'Golden Delicious' and 'Gala' the increasing pCO_2 in CA retains higher chlorophyll content (ARGENTA; BRACKMANN, 1996) and background color (SAQUET et al., 1997).

Table 8. Severity of fungal decay, flesh browning and skin browning in 'Luiza' apple fruit after storage in regular atmosphere (O_2=21 kPa) or in controlled atmosphere (O_2=1.5 kPa) with different concentrations (kPa) of CO_2. Fruit harvested at early (Harvest 1) and advanced (Harvest 2) maturity, 2020 season, were exposed (MCP) or not exposed (C) with 1-methylcyclopropene within 24 h after harvest. Fruit held 7 d at 22°C following 8 months storage.

Treatment		Harvest 1			Harvest 2			Average			
O_2	CO_2	C	MCP	Average	C	MCP	Average	C		MCP	
					Fungal decay (1-3)						
21	<0.5	1.10	1.02		1.40	1.06		1.25	aA	1.04	aB
1.5	<0.5	1.10	1.00		1.22	1.06		1.16	bA	1.03	aA
1.5	1.5	1.06	1.02		1.20	1.20		1.13	bA	1.11	aA
1.5	3.0	1.04	1.02		1.18	1.18		1.11	bA	1.10	aA
1.5	4.5	1.00	1.00		1.16	1.18		1.08	bA	1.09	aA
				1.04 B			1.18 A				
					Flesh browning (1-4)						
21	<0.5	1.16	1.18		1.32	1.14		1.24		1.16	1.20 a
1.5	<0.5	1.10	1.04		1.18	1.08		1.14		1.06	1.10 b
1.5	1.5	1.00	1.00		1.14	1.08		1.07		1.04	1.05 b
1.5	3.0	1.00	1.00		1.10	1.06		1.05		1.03	1.04 b
1.5	4.5	1.10	1.00		1.18	1.02		1.14		1.01	1.07 b
Average				1.06 B			1.13 A	1.13	A	1.06	B
					Skin browning (1-4)						
21	<0.5	1.04	1.00		1.04	1.00		1.04	aA	1.00	aB
1.5	<0.5	1.00	1.00		1.00	1.00		1.00	bA	1.00	aA
1.5	1.5	1.00	1.00		1.00	1.00		1.00	bA	1.00	aA
1.5	3.0	1.00	1.00		1.01	1.00		1.01	abA	1.00	aA
1.5	4.5	1.00	1.00		1.00	1.00		1.00	bA	1.00	aA

Source	Fungal Decay	Flesh browning	Skin browning
Treatment	ns	***	***
Harvest	***	***	ns
MCP	***	**	**
T x H	ns	ns	ns
H x M	ns	ns	ns
T x M	**	ns	***
T x H x M	ns	ns	ns

Means followed by same lower-case letter in each column and same upper-case letter in each row are not different by Tukey test ($p < 0.05$). Lower-case letters compare treatments (storage atmosphere) in each row while upper-case compare harvests (H_1 vs H_2) or 1-MCP exposure (C vs MCP) in each row.
ns, *, **, ***: not significant or significant at $p < 0.05$, 0.01 or 0.001, respectively.

There was no significant interaction between pCO_2 in CA and 1-MCP treatment on TA and both higher pCO_2 and 1-MCP treatments improved retention of TA. Therefore, 1-MCP application and CA with higher pCO_2 might impact sensory quality of 'Luiza' apple after long term-storage by maintenance of acidity. Important to notice that TA in 'Luiza' apple fruit is lower than in other apple cultivar such as 'Galas' and 'Red Delicious' (BAI et al., 2005). SSC was not affected by pCO_2 in CA storage while it was slightly increased by 1-MCP treatment.

For Experiment 3, the severity of fungal decay and flesh browning was higher in fruit of an advanced maturity (Table 8), consistent with the results of Experiment 2. Higher decay incidence in later harvested fruit is likely due to advanced fruit ripening (NYBOM et al., 2020) and a longer time in the orchard, which increases the probability of fruit infection. Apple stored under CA without 1-MCP showed a lower fungal decay in relation to air storage. 1-MCP was effective in reducing fungal decay in air storage. Previous studies have reported that the effects of 1-MCP in reducing fungal decay is variable, which can reduce (CAMDELI et al., 2016), increase (JANISIEWICZ et al., 2003) or have no effect (ARGENTA et al., 2021).

'Luiza' apple fruit did not develop symptoms of CO_2 injury when stored under high (up to 4.5 kPa) pCO_2 (Table 8). These results indicate that 'Luiza' apple can be stored together with 'Gala' apples in commercial CA storage rooms, a practice widely employed by warehouses in Brazil. The flesh browning incidence did not have significant interactions among harvest maturities, 1-MCP and pCO_2. However, in Experiment 3, the average flesh browning value for 1-MCP treated fruit was lower than untreated fruit. Fruit under CA had a lower flesh browning incidence than air-stored fruit, indicating that CA can reduce flesh-browning development.

The skin browning disorder occurred only in air-stored fruit without 1-MCP and in CA-stored fruit (1.5 + 3.0 kPa pCO_2). The symptom of skin browning resembles that of superficial scald, and according to Experiments 2 and 3, the effects of harvest maturity, 1-MCP and CA on this disorder is the same of that as superficial scald (WATKINS; MATTHEIS, 2019). However, the effect of CA and 1-MCP on this disorder found in Experiment 1 is not consistent for superficial scald. Both 1-MCP and low pO_2 are well known to reduce superficial scald in apple (BESSEMANS et al., 2016; BUSATTO et al., 2018).

Flesh browning incidence increased in fruit harvested with an advanced maturity (138 DAPF) and a flesh firmness of less than 73 N (MAGRIN et al., 2017). Furthermore, flesh browning incidence increased with storage duration in fruit without 1-MCP (Table 2 and 3), consistent with the expression of senescence disorders (WATKINS; MATTHEIS, 2019). However, this disorder was not consistently reduced through ethylene inhibition by 1-MCP, such as reported in Experiments 1 and 2, suggesting that this disorder may be a response to chilling injury. Apples with flesh browning symptoms, similar to those found in this study (Figure 4), are more frequent in young orchards, with low fruit loads and mineral imbalances (LITTLE AND HOLMES, 2000). Preliminary studies performed in 2011 and 2013 with fruit of Orchard 1 found a high flesh browning incidence (data not show), which can be associated with orchard age, the imbalance of minerals, a low fruit load and the variability of climate among years. Additional studies are required to stablish the role of storage temperature on flesh browning disorder for this cultivar.

Conclusions

The storage life of 'Luiza' was less than four months when stored in air, and six to eight months when stored in CA, based on the time to reach a firmness of 53 N. However, flesh browning and fungal decay incidence increased when the storage period is longer than six months without 1-MCP application.

CA (1.5 kPa O_2 and 2.5 kPa CO_2) storage and 1-MCP treatments prevented the rapid softening, flesh browning and fungal decay in fruit of 'Luiza' apple.

There was an additional benefit of 1-MCP application in CA-stored fruit for the flesh firmness retention of 'Luiza' apple fruit during storage.

'Luiza' apple fruit do not develop internal symptoms of CO_2 injury when stored in high CO_2 partial pressures (up to 4.5 kPa).

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