MARILIA CECILIA DE SOUZA BITTENCOURT

RIPENING MANIPULATION OF 'HASS' AVOCADO WITH ETHYLENE

Thesis submitted to the Plant Sciences Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Doctor Scientiae*.

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ABSTRACT

BITTENCOURT, Marilia Cecilia de Souza, D.Sc., Federal University of Viçosa, July 2024. **Ripening manipulation of 'Hass' avocado with ethylene.** Advisor: Wellington Souto Ribeiro. Co-advisor: Carlos Eduardo M. Dos Santos

The long ripening period of 'Hass' avocado fruits harvested at the beginning of the harvest can be detrimental to sales. Ethylene is capable of anticipating and accelerating the ripening of climacteric fruits and its application at temperatures between 17 and 20 °C is widely used in the industry to accelerate the ripening of 'Hass' avocados. There is no information in the literature about the effectiveness of ethylene when applied at lower temperatures. The main objective of this work was to evaluate the efficacy of ethylene application in accelerating the ripening of 'Hass' avocados harvested at the beginning of the season under treatment temperatures of 8°C and 20°C. In experiment I, freshly harvested fruits (n=192) were treated with 1,000 ppm ethylene for 48 hours at 8°C and then kept for 4 days at 8°C to simulate long-distance refrigerated transport. Subsequently, they were stored for 0, 3, 6, or 9 days at 8 °C, and finally kept at 20 °C to simulate shelf life. In experiment II, freshly harvested fruits (n=192) were kept for 4 days at 8 °C to simulate long-distance refrigerated transport and then stored for 0, 3, 6, or 9 days at 8 °C. After the storage period, they were treated with 100 ppm ethylene for 24 hours at 20 °C and kept at 20 °C and 85% relative humidity to simulate shelf life. In the first experiment, the initial firmness of the fruits was 190 N and, after 4 days on the shelf, it was 4 and 100 N, respectively, for treated and untreated fruits, that were not stored. Optimum firmness for consumption ranges between 4 and 7 N. Fruits treated with ethylene and stored for 3, 6, and 9 days reached 5.5, 1.3, and 1.9 N of firmness in just 3 days at 20 °C, respectively, while the control reached 50.2, 17.7, and 12.9 N. For all storage times, control fruits showed greater weight loss, ranging from 2.3 to 5.5%, while the weight loss of treated fruits ranged from 1.3 to 2.6%. Control fruits that were stored for 0, 3, and 6 days required, respectively, 4.9, 3 and 2.2 days longer to ripen than treated fruits. Application of ethylene at 8 °C accelerates the ripening of 'Hass' avocado by up to 4.9 days, and as the fruit ages from harvest, it takes fewer days to ripen when transferred from 8 °C to 20 °C. In experiment II, the number of days for maturation and firmness were affected by ethylene only for 0 and 3 days of storage. Fruits stored for 0 and 3 days at 8 °C and treated with ethylene took, respectively, 1.7 and 2.0 days less to ripen than control fruits. Avocados not stored and treated with ethylene reached 48.6 N firmness after 6 days on the shelf, while control fruits had 101.2 N in the same period. Avocados stored for 3 days and treated with ethylene reached 11.9 N in just 3 days on the shelf, while control avocados exhibited 122.4 N. Control fruits showed greater weight loss compared to treated fruits in all periods of refrigerated storage evaluated. Application of ethylene at 20 °C accelerates the ripening of ‘Hass’ avocado by up to 2.0 days, and as the fruit ages since harvest, it takes fewer days to ripen when transferred from 8 °C to 20 °C.

Keywords: *Persea americana* Mill.; Ripening control; Ethylene; Cold storage; Pre-conditioning.

RESUMO

BITTENCOURT, Marilia Cecilia de Souza, D.Sc., Universidade Federal de Viçosa, julho de 2024. **Manipulação do amadurecimento de abacate 'Hass' com etileno.** Orientador: Welington Souto Ribeiro. Coorientador: Carlos Eduardo M. dos Santos.

O longo período de maturação dos frutos de abacate 'Hass' colhidos no início da safra pode ser prejudicial para as vendas. O etileno é capaz de antecipar e acelerar a maturação de frutos climatéricas e sua aplicação em temperaturas entre 17 e 20 °C é amplamente utilizada na indústria para acelerar a maturação dos abacates 'Hass'. Não há informações na literatura sobre a eficácia do etileno quando aplicado em temperaturas mais baixas. O principal objetivo deste trabalho foi avaliar a eficácia da aplicação de etileno na aceleração do amadurecimento de frutos de abacate 'Hass' colhidos no início da safra, sob temperaturas de tratamento de 8°C e 20°C. No experimento I, frutos recém-colhidos (n=192) foram tratados com 1.000 ppm de etileno por 48 horas a 8 °C e depois mantidos por 4 dias a 8 °C para simular o transporte refrigerado de longa distância. Posteriormente, foram armazenados por 0, 3, 6 ou 9 dias a 8 °C e, finalmente, mantidos a 20 °C para simular a vida útil. No experimento II, frutos recém-colhidos (n=192) foram mantidos por 4 dias a 8 °C para simular o transporte refrigerado de longa distância e depois armazenados por 0, 3, 6 ou 9 dias a 8 °C. Após o período de armazenamento, foram tratados com 100 ppm de etileno por 24 horas a 20 °C e mantidos a 20 °C e 85% de umidade relativa para simular a vida útil. No primeiro experimento, a firmeza inicial dos frutos era 190 N e, após 4 dias de prateleira, era 4 e 100 N, respectivamente, para frutos tratados e não tratados, que não foram armazenados (0 d armazenamento). A firmeza ótima para o consumo varia entre 4 e 7 N. Os frutos tratados com etileno e armazenados por 3, 6 e 9 dias atingiram 5,5, 1,3 e 1,9 N de firmeza em apenas 3 dias a 20 °C, respectivamente, enquanto o controle atingiu 50,2, 17,7 e 12,9 N. Para todos os tempos de armazenamento, os frutos controle apresentaram maior perda de massa, variando de 2,3 a 5,5%, enquanto a perda de massa dos frutos tratados variou de 1,3 a 2,6%. Os frutos controle armazenados por 0, 3 e 6 dias exigiram, respectivamente, 4,9, 3 e 2,2 dias a mais para amadurecer do que os frutos tratados. A aplicação de etileno a 8 °C acelera a maturação do abacate 'Hass' em até 4,9 dias, e à medida que o fruto envelhece desde a colheita, leva menos dias para amadurecer quando transferido de 8 °C para 20 °C. No experimento II, o número de dias para a maturação e a firmeza foram afetados pelo etileno apenas para 0 e 3 dias de armazenamento. Frutos armazenados por 0 e 3 dias a 8 °C e tratados com etileno levaram, respectivamente, 1,7 e 2,0 dias a menos para amadurecer do que os frutos controle. Os abacates não armazenados e tratados com etileno atingiram 48,6 N de firmeza após 6 dias na prateleira, enquanto os frutos controle tiveram 101,2 N no mesmo período. Os abacates armazenados por 3 dias e tratados com etileno atingiram 11,9 N em apenas 3 dias na prateleira, enquanto os abacates controle exibiram 122,4 N. Os frutos controle apresentaram maior perda de massa em comparação com os frutos tratados em todos os períodos de armazenamento refrigerado avaliados. A aplicação de etileno a 20 °C acelera a maturação do abacate 'Hass' em até 2,0 dias, e à medida que o fruto envelhece desde a colheita, leva menos dias para amadurecer quando transferido de 8 °C para 20 °C.

Palavras-chave: *Persea americana* Mill.; Controle do amadurecimento; Etileno; Armazenamento refrigerado; Pre-condicionamento.

SUMMARY

GENERAL INTRODUCTION

Avocados (*Persea americana* Mill.) are oil-rich climacteric fruits native to Central and South America (CHEN et al., 2009). ‘Hass’ is the main avocado cultivar commercialized worldwide, corresponding to 90% of the total volume of avocados exported internationally (IMBERT, 2015). Brazilian production of ‘Hass’ avocado is increasing due to international demand, and producers have been focusing on the overseas market (ABRAFRUTAS, 2019).

Avocado is grown worldwide in tropical and subtropical regions, but its production is mainly concentrated in the American continent, with Mexico being the largest producer, responsible for approximately 34% of world production, followed by the Dominican Republic and Peru (FAOSTAT, 2020). Brazil is the world's sixth-largest producer, reaching approximately 338 thousand tons in 2022, distributed in 19 thousand hectares of cultivated area, with emphasis on the states of São Paulo and Minas Gerais, which accounted for about 72% of production and 72% of the planted area (IBGE, 2022). The Brazilian Institute of Geography and Statistics points to a growth of 36.12% in planted area and 35.92% in national production since 2001 (IBGE, 2019). In addition, Brazil stands out in terms of productivity, with averages over 15 t/ha, well above the world average productivity of only 9.1 t/ha (SALOMÃO; SIQUEIRA, 2019).

In Brazil, ‘Hass’ avocado production is still small, however, recent forecasts point to an 800% growth only in the last four years (GLOBO RURAL, 2022). In 2013, the commercialization of the cultivar at Ceagesp-SP corresponded to only 1% of the total (SALOMÃO; SIQUEIRA, 2019). The low expression of ‘Hass’ avocados in the domestic market is due, among other factors, to the preference of Brazilians for larger fruits with lower oil content (SOUZA, 2008). Due to its small popularity in Brazil, the national production of ‘Hass’ avocado is almost entirely aimed at the international market, which has a preference for smaller fruits with higher oil content (SALOMÃO; SIQUEIRA, 2019).

Avocado presents a unique and complex physiology. The tree presents a very extensive flowering period of 2 to 3 months, and fruit set is extremely low (LEWIS, 1978). Furthermore, only when the fruit is detached from the tree does it become sensitive to ethylene and ripens (GAZIT; BLUMENFELD, 1970). Thus, fruits of very different ages are obtained at harvest (LEWIS, 1978). This distinctive characteristic makes it possible to stagger the harvest over a long period of time (SALOMÃO; SIQUEIRA, 2019).

Unlike other fruits, avocado accumulates oil rather than sugars, in specialized oil cells called idioblasts (ORTIZ et al., 2004). Based on dry weight, the mesocarp accumulates approximately 60-70% of oils and 10% of carbohydrates (PEDRESCHI et al., 2019). Because avocado does not ripen while attached to the plant, fruit from cultivars such as ‘Hass’ can be kept on the tree for up to six months after reaching physiological maturity, time during which the fruit continues to accumulate oil (SALOMÃO; SIQUEIRA, 2019). Thus, late-season fruits have a much higher concentration of oil than early-season avocados, changing from 9% in the early season to 14% in the late season (HERNÁNDEZ et al., 2016).

The dry matter content, highly correlated with the oil content of the fruit, is commonly used as a maturity index for ‘Hass’ due to its simplicity in estimating the oil content (HOFMAN et al., 2013). In general, ‘Hass’ avocados must have a minimum of 23% dry matter before they can be harvested in order to meet consumer tastes (HERNÁNDEZ et al., 2016). After harvesting at the right physiological maturity stage, the ‘Hass’ avocado ripens within 5 to 10, at a temperature of 18-22 °C (OZDEMIR; TOPUZ, 2004). According to Arpaia et al. (2018), early-season fruits, with lower dry matter content, take longer to ripen than late-season fruits, with higher dry matter content.

Consumers' preference for ripe "ready-to-eat" avocados has presented a challenge to the avocado industry. This is because the natural ripening of Hass avocados is not uniform, and the time required for ripening varies greatly depending on the harvest season (early, mid, and late season). This variability can impact fruit quality and market value (HERNÁNDEZ et al., 2016). Furthermore, market research shows that avocado sales can double or triple if the fruit is offered in a ready-to-eat condition, typically promoted as 'ripe tonight' (YAHIA; WOOLF, 2011). This has led to a system of ethylene ripening ("pre-ripening," "pre-conditioning," or "ethylene conditioning") before stocking, which significantly reduces both the time it takes to ripen and fruit-to-fruit variability in ripening. (GAZIT; BLUMENFELD, 1972).

Pre-conditioning, a practice adopted in many producing countries, is usually performed by treating avocados with 10 to 100 μL L^{-1} ethylene at 17 to 20 °C for one to three days in a high airflow, or preferably forced-air room (YAHIA; WOOLF, 2011). Treatment times vary depending on the time in the season (early, middle, or late harvest) and time of storage before treatment. In general, treatment duration ranges from 48 to 72 h for early season, 24 to 48 h for mid-season, and 12 to 24 h for late season (YAHIA; WOOLF, 2011).

Fruits from different species have been treated with ethylene or its analogs to hasten and standardize ripening and/or enhance fruit quality. The exogenous application of ethylene in climacteric fruits provides a considerable reduction in the period between harvest and the complete ripening of the fruits (BRAZ et al., 2008). In California, the main producing state in the United States, the avocado industry makes extensive use of ethylene to synchronize and control the ripening process (MURPHY; KHILSTADIUS, 1991) and the practice has been adopted in other producing countries to stimulate consumption and increase market penetration of the fruits (CBI, 2017).

The effect of 100 ppm of ethylene applied for 6, 12 and 24 hours on the ripening of ‘Hass’ avocado fruits was studied by Eaks (1966). The author reports that the application of 100 ppm of ethylene for 24 hours leads to the ripening of 'Hass' avocado fruits within 3-4 days after the start of the hormone treatment, both in fruits harvested at the beginning and at the end of the season. This same author verified that the exposure of the fruits to the hormone for 12 hours accelerated the ripening in about one day, when compared to the untreated fruits. According to Gazit and Blumenfeld (1970), ‘Hass’ avocado fruits did not respond to ethylene application immediately after harvest; however, a good response was observed in fruits treated between 25 and 49 hours after harvesting. Adkins (2005) also reports on ethylene effects in ‘Hass’ avocados, verifying that treatment at 100 $\mu L\ L^{-1}$ for 24 h reduced the ripening period in 3 days.

Over the past few years, there has been an increasing demand for 'Hass' avocados in both domestic and international markets, leading to a rise in fruit production and exports in Brazil. However, there is a lack of post-harvest management practices and scientific knowledge specific to this avocado cultivar in the country. While pre-conditioning is a common practice in many avocado-producing regions worldwide, there have been no published studies on its application to 'Hass' avocados grown in Brazil. Furthermore, there is a lack of information in the literature regarding the effectiveness of ethylene treatment at temperatures below 20 °C. The main objective of this work was to evaluate the efficacy of ethylene application in accelerating the ripening of 'Hass' avocados harvested at the beginning of the season under treatment temperatures of 8°C and 20°C, in order to provide practical recommendations during avocado postharvest handling in Southeast Brazil.

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ARTICLE 1 - RIPENING OF EARLY-SEASON 'HASS' AVOCADO WITH ETHYLENE UNDER LOW TEMPERATURE CONDITION

ABSTRACT

The application of ethylene at temperatures between 17 and 20 °C is widely used in the industry to accelerate the ripening of 'Hass' avocados. There is no information in the literature about the effectiveness of ethylene when applied at lower temperatures. The objective was to evaluate the effect of ethylene applied at 8 °C on accelerating the ripening of 'Hass' avocado. Freshly harvested fruits (n=192) were treated with 1,000 ppm ethylene for 48 hours at 8°C and then kept for 4 days at 8°C in order to simulate long-distance refrigerated transport. Subsequently, they were stored for 0, 3, 6 or 9 days at 8 °C, and finally kept at 20 °C to simulate shelf life. A completely randomized design was used, with 3 replications and 2 fruits per replication. The data were subjected to analysis of variance and means were compared by Tukey test ($p \leq 0.05$). The initial firmness of the fruits was 190 N and, after 4 days on the shelf, it was 4 and 100 N, respectively, for treated and untreated fruits, that were not stored. Optimum firmness for consumption ranges between 4 and 7 N. Fruits treated with ethylene and stored for 3, 6 and 9 days reached 5.5, 1.3 and 1.9 N of firmness in just 3 days at 20 °C, respectively , while the control reached 50.2, 17.7 and 12.9 N. For all storage times, control fruits showed greater weight loss, ranging from 2.3 to 5.5%, while the weight loss of treated fruits ranged from 1.3 to 2.6%. Control fruits that were stored for 0, 3 and 6 days required, respectively, 4.9, 3 and 2.2 days longer to ripen than treated fruits. Application of ethylene at 8°C accelerates the ripening of 'Hass' avocado by up to 4.9 days, and as the fruit ages from harvest, it takes fewer days to ripen when transferred from 8°C to 20°C.

KEYWORDS: *Persea americana* Mill.; Ripening; Ethylene; Cold storage.

AMADURECIMENTO DO ABACATE 'HASS' DE INÍCIO DE SAFRA COM ETILENO APLICADO SOB BAIXA TEMPERATURA

RESUMO

A aplicação de etileno sob temperaturas entre 17 e 20 °C é amplamente utilizada na indústria para acelerar o amadurecimento do abacate 'Hass'. Não há informações na literatura sobre a eficácia do etileno quando aplicado sob temperaturas mais baixas. Objetivou-se avaliar o efeito do etileno aplicado a 8 °C na aceleração do amadurecimento de abacate 'Hass'. Frutos recém colhidos (n=192) foram tratados com 1.000 ppm de etileno por 48 horas a 8 °C e, em seguida, mantidos por 4 dias a 8 °C a fim de simular transporte refrigerado de longa distância. Posteriormente, foram armazenados por 0, 3, 6 ou 9 dias a 8 °C, e, finalmente, mantidos a 20 °C para simular a vida de prateleira. Utilizou-se delineamento inteiramente casualizado, com 3 repetições e 2 frutos por repetição. Os dados foram submetidos à análise de variância e as médias comparadas pelo teste de Tukey ($p \leq 0,05$). A firmeza inicial dos frutos era de 190 N e, aos 4 dias de prateleira, era de 4 e 100 N, respectivamente, para frutos tratados e não tratados, que não foram armazenados. A firmeza ótima para consumo varia entre 4 e 7 N. Frutos tratados com etileno e armazenados por 3, 6 e 9 dias atingiram 5,5, 1,3 e 1,9 N de firmeza em apenas 3 dias a 20 °C, respectivamente, enquanto o controle atingiu 50,2, 17,7 e 12,9 N. Para todos os tempos de armazenamento, frutos controle apresentaram maior perda de massa, variando de 2,3 a 5,5%, enquanto a perda de massa dos frutos tratados variou entre 1,3 a 2,6%. Frutos controle que foram armazenados por 0, 3 e 6 dias necessitaram, respectivamente, de 4,9, 3 e 2,2 dias a mais para amadurecer do que frutos tratados. A aplicação de etileno a 8 °C acelera o amadurecimento do abacate 'Hass' em até 4,9 dias e, à medida que o fruto envelhece desde a colheita, leva menos dias para amadurecer quando transferido de 8 °C para 20 °C.

PALAVRAS-CHAVE: *Persea americana* Mill.; Amadurecimento; Etileno; Armazenamento refrigerado.

INTRODUCTION

Avocado (*Persea americana* Mill) is one of the major tropical fruit commercialized worldwide, and cultivar 'Hass' dominates the international trade market. According to the OECD/FAO Agricultural Outlook 2021-2030, avocados are expected to become the second-most traded major tropical fruit by 2030, after bananas (OECD/FAO, 2021). Avocados sales can be doubled or tripled if fruit are offered to the consumer in a ready-to-eat condition, typically promoted as 'ripe tonight' (YAHIA; WOOLF, 2011). Ethylene exposure, known in the avocado industry as pre-conditioning, is a practice adopted in several producing countries to anticipate and synchronize ripening in order to stimulate consumption and increase the fruit's penetration in the market (ARPAIA et al., 2018).

The avocado's distinctive and intricate physiology directly influences post-harvest management. Flowering period can last up to 3 months and fruit set is extremely low (less than 0.1%) (PEDRESCHI et al., 2019). In addition, as fruit does not ripen on the tree, physiologically mature fruit can remain on the tree for many months (HERNÁNDEZ et al., 2016). Consequently, avocados of very different physiological ages hang on the same tree, and, once harvested, time to ripen will vary greatly, reducing with increasing time on the tree (YAHIA; WOOLF, 2011). Furthermore, early-season fruit takes longer to ripen (ARPAIA et al., 2018) and both early-and middle-season fruits are more prone to have a pronounced ripening heterogeneity within a batch and/or consignment than late-season fruit (HERNÁNDEZ, 2016).

Pre-conditioning significantly reduces both the time fruit takes to ripen and fruit-to-fruit variability in ripening (WOOLF et al., 2016). Pre-conditioning is the process of triggering the natural ripening of avocados through the application of ethylene in a ripening room under controlled conditions of temperature, relative humidity, and carbon dioxide levels. Avocado pre-conditioning is often held at 17-20 °C, and ethylene concentration and exposure time is widely dependent on maturity of fruit at harvest and age of fruit from harvest (WOOLF et al., 2016). 'Hass' avocado recommended ripening conditions are 10 to 100 μL L^{-1} ethylene for 48 to 72 h for early-season, 24 to 48 h for mid-season and 12 to 24 h for late-season fruit (YAHIA; WOOLF, 2011). After pre-conditioning, fruit can either be stored under refrigeration to slow ripening or hold at 15-20 °C to promote further ripening.

Under optimum ripening temperatures, 15 to 20 °C (HOPKIRK et al., 1994), 'Hass' avocados will usually ripen within 6 to 15 days after harvest (DAVUR et al., 2023). Various studies have demonstrated reduced ripening time with ethylene treatment. Eaks (1966) registered that the application of 100 μL L^{-1} of ethylene for 24 hours at 20 °C leads to the ripening of fruits within 3-4 days. Treatment of 'Hass' avocado with ethylene at 100 μL L^{-1} for 24 h, under 20 °C, reduced the time to ripen from 13 to 10 days (ADKINS et al., 2005). The application of 40 μL L^{-1} ethylene, also at 20 °C, decreased the number of days to ripe from 14.9 to 10.8 for 'Hass' avocados (ARPAIA et al., 2018).

Brazilian 'Hass' avocado production is mainly destined for exportation, being Argentina the major importer in South America (BARBIERI et al., 2023), where fruit arrives after 4 to 5 days by refrigerated trucks (personal communication). Ethylene can be an important tool in order to meet consumer's demand for ready-to-eat fruit and increase ripening uniformity, especially in the beginning of the season, when fruits take longer to ripe and uneven ripening is more pronounced. Although pre-conditioning with ethylene effectively hastens avocado ripening, there is no information in the literature on its efficacy when applied at temperatures lower than 20 °C. Learning ethylene efficacy under cooler temperatures would broaden pre-conditioning possibilities along the supply chain, avoiding cold chain breakage. This study was designed to determine whether ethylene treatment applied at 8 °C can promote and accelerate the ripening of early harvested 'Hass' avocado fruit.

MATERIAL AND METHODS

Fruit source

Early-season 'Hass' avocado fruit was obtained from a local commercial orchard, at Rio Paranaiba, state of Minas Gerais. The fruit was manually harvested in the early morning following sanitization with peracetic acid and waxing. The fruit was maintained at 8 °C until night, when it was transported in an air-conditioned vehicle to the Federal University of Viçosa Postharvest Physiology Laboratory, in Viçosa, Minas Gerais..

Ethylene fruit ripening and cold storage

'Hass' avocado fruit (n = 192) was shot treated with 0 or 1000 ppm of ethylene in 90 L hermetically sealed buckets at 8 °C. After 48 hours of ethylene treatment, fruit was removed from buckets and kept at 8 °C for 4 days to simulate long distance truck transport. After that, fruit was stored at 8 °C for 0, 3, 6 or 9 days, following shelf-life simulation at 20 °C for 0, 2, 4 or 6 days. The fruits were evaluated at 0, 2, 4 and 6 days of shelf life.

Days to ripe

Number of days to ripe (DTR) was determined from the day fruit was transferred from 8 °C to 20 °C until fruit was fully ripened, after reaching 4-7 N of pulp firmness.

Peel color

The peel color was determined using a Konica Minolta colorimeter (Chroma meter, CR-32). The color was expressed by the rectangular coordinate system L, a* b* according to the Commission internationale de l'éclairage (CIE), where L is expressed as a percentage of brightness values (0% = black and 100% = white), a* represents the colors red (+) or green (-) and b* the colors yellow (+) or blue (-). Based on the a* and b* coordinates, the hue angle and chromaticity parameters were calculated. The reading was done at 3 different points of the fruit's peel.

Pulp firmness

Pulp firmness was evaluated through its resistance to penetration using an analog penetrometer model FT-327, with a cylindrical tip of 8 mm in diameter. Measurements were performed at two opposite points in the equatorial region of the fruit, after removing the peel. The results were expressed in N.

Weight loss

The weight loss was estimated by weighing the fruit individually on an analytical balance throughout the shelf life. Weight loss was calculated (fresh weight basis) using the following equation: Weight Loss (%) = Initial weight - Final weight/ Initial weight × 100.

Total soluble sugars

The amount of total soluble sugars (TSS) was estimated by phenol sulfuric acid reagent method (DUBOIS et al., 1956). To prepare the alcoholic extract, 5 g of avocado pulp was homogenized at 14,000 g with pre-heated EtOH 80% then centrifuged at 2,000 g for 10 minutes. Centrifugation was repeated twice, and the final volume of the alcoholic extract filled to 25 ml. Later, 40 µL of the alcoholic extract, 210 µL of deionized water and 250 µL of 5% phenol was pipetted into a test tube, followed by vortexing. TSS was quantified via colorimetric assay by adding 1.25 mL of concentrated sulfuric acid to the test tube, following heating for 20 minutes at 30 °C. TSS was estimated against a standard curve of 1% sucrose measured at 540 nm. The results were expressed in percentage of fresh mass.

Reducing sugars

The reducing sugar content (RS) was quantified using the dinitrosalicylic acid (DNS) methodology (GONÇALVES et al., 2010) with modifications. The first step was to determine the standard curve, using a 0.2% fructose solution. For quantification, 500 µL of DNS, 200 µL of alcoholic extract and 300 µL of deionized water were pipetted into a test tube following heating for 5 minutes at 100 °C. The tubes were cooled in an ice water bath and 4 mL of deionized water was added to each tube. The readings were carried out in a spectrophotometer at 540 nm and the results expressed in percentage of fresh mass.

Total phenolic content

The content of total phenolic compounds (TPC) was determined by the Folin-Ciocalteu method (FU et al., 2010), with modifications. An aliquot of 200 µL of the alcoholic extract and 1,000 µL of the Folin-Ciocaltieu reagent (1:10) was added to a test tube. After 10 minutes, 800 µL of 7.5% sodium carbonate was added to the mixture, then the tubes were vortexed and kept in the dark for 30 minutes at room temperature. At the end of this step, the absorbance of the samples were quantified in a spectrophotometer at 760 nm, using gallic acid as a standard, and the results expressed in mg per 100 g of fresh mass.

Statistics

The experiment was conducted in a completely randomized design, using three replications, with each experimental unit consisting of 2 fruits. The data obtained was subjected to analysis of variance and the means compared by Tukey's test at 5% probability. Statistical analyzes were performed using the R Core Team program.

RESULTS AND DISCUSSION

Days to ripe

The number of days to ripe was significantly affected by ethylene in all cold storage durations, except for 9 days of storage. Control fruit took 9.2, 6.7 and 5.5 days to ripen at 20 °C after 0, 3 and 6 days at 8 °C, respectively. Ethylene treated fruit stored for 0, 3 and 6 days at 8 °C required 4.9, 3 and 2.2 fewer days to ripen once transferred to 20 °C (Table 1). It was observed that, regardless of the treatment, as the fruit ages from harvest it takes fewer days to ripen once transferred from cold storage to 20 °C.

Table 1. Influence of ethylene applied at 8 °C on days to ripeness of early harvested ‘Hass’ avocados cold stored at 8 °C (CS) for 0, 3, 6 or 9 days prior to final ripening at 20 °C.

	Days to ripe			
	0 d CS	3 d CS	6 d CS	9 d CS
Control	9.2a	6.7a	5.5a	4.3^{ns}
Ethylene	4.3b	3.7b	3.3b	3.2^{ns}

Mean separation using Tukey’s test (P≤0.05). Means with different letters within a storage duration are significantly different from each other.

These findings indicate that the application of 100 ppm of ethylene at 8 °C accelerates the ripening of Hass avocados once transferred to 20 °C. The reduction in the number of days to ripe observed in ethylene-treated fruit can be attributed to the hormone's role in promoting fruit ripening, which involves various biochemical processes such as changes in color, texture, flavor, and aroma (SEYMOUR et al., 2012).

Ethylene has long been known to trigger the ripening of harvested climacteric fruit. In an atmosphere of at least 10 ppm, avocado fruit enter the climacteric phase immediately and soften within days (GAZIT; BLUMENFELD, 1970). Several studies have investigated the use of ethylene to control the ripening of avocado fruit (EAKS, 1966; GAZIT; BLUMENFELD, 1970; EAKS, 1980; ZAUBERMAN, et al., 1988; ADKINS et al., 2005; ARPAIA, 2018). In one study, exposure to 40 ppm of ethylene at 20, and 25 °C substantially reduced ripening time up to 5.6 days across temperatures (ARPAIA et al., 2018). Another study showed that treatment of ‘Hass’ avocado with ethylene at 100 ppm for 24 h at 20 °C reduced the time (days) to ripen from 13 to 10 d (ADKINS et al., 2005). No studies have reported the fruit's response to ethylene under lower temperatures yet.

Various reasons justify ethylene application on ‘Hass’ avocados. One is the consumer’s preference for avocados at or close to a ready-to-eat stage (LEE; COGGINS, 1982; TOKAR, 2007). Early season avocados benefit more from this practice, considering their notable longer ripening period compared to middle to late season (ARPAIA et al., 2018). Ethylene also helps to overcome ripening heterogeneity of 'Hass' avocados (WOOLF at al., 2005), a common defect which creates logistical problems for marketers and inconsistent quality delivery to consumers (HERNÁNDEZ et al., 2016).

While exposure to ethylene can be an advantage due to reduced ripening time and enhanced uniformity, its application might be unnecessary if it follows a sustained period of cold storage. As shown by the data, control fruit cold-stored for 9 days ripened roughly as fast as treated fruit once transferred to 20 °C, with a non significant statistical difference of only 1.1 days. Wolf et al. (2005) states that for fruit stored for 3 to 4 weeks the benefit of adding an ethylene treatment can be low.

Peel color

The peel color parameters a*, Hue angle (H°) and lightness (L*) were significantly affected by ethylene for non-stored fruit (0 d CS) and for fruit stored for 3 days at 8 °C (3 d CS) prior to ripening at 20 °C (Figure 1). Peel color for 6 and 9 d CS were not affected as much (data not shown).

A gradual increase in the a* values is observed over the shelf life for both control and ethylene treated fruit (Figure 1). This increase indicates a shift from green (-a*) towards magenta (+a*) colors, in agreement with the ripening process in 'Hass' avocados, which changes skin color from green to purple/black as fruit ripen (COX et al., 2004). In both storage times (0 d CS and 3 d CS), although the color change pattern was visibly similar, control fruit were significantly slower to switch from green to purple, retaining the green color for a longer time.

Hue angle (H°) and lightness (L*) all decreased with ripening (Figure 1) for both control and treated fruit, but at faster rates for ethylene, specially at 0 d CS. The decrease in L* values represents a shift from lighter to darker colors and in H° values express a transit towards red (red = hue angle of 0°) (MCGUIRE, 1992).

As Hass avocados ripen, they undergo a characteristic color change where the green peel transitions to a purplish or dark color, such as brown or black. This shift is caused by changes in the concentration of pigments in the fruit peel (ASHTON et al., 2006). Over 6 days at 20 °C, ethylene accelerated the rate of color change in treated fruit, leading to the differences observed for the color parameters, specially at 0 d CS. This color change is important as an indicator of ripeness for both industry and consumers. According to Cox et al. (2004), color change in 'Hass' avocados from green to purple, then black, results from an initial decrease in chlorophyll content, followed by an increase in the levels of anthocyanin.

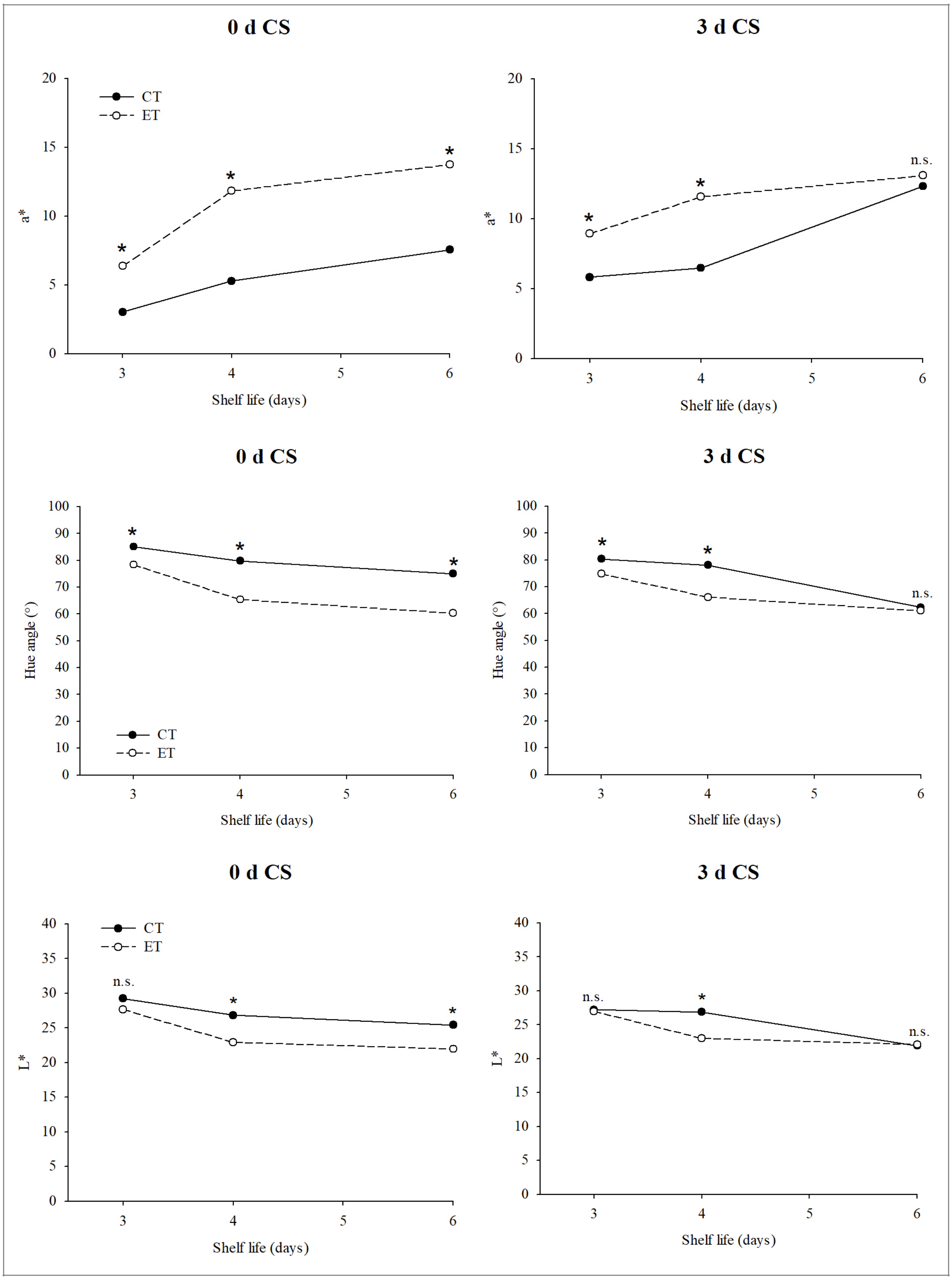

Figure 1. Influence of ethylene applied at 8 °C on peel color parameters (a*, H° and L*) of early harvested 'Hass' avocados stored for 0 and 3 days at 8 °C prior to ripening at 20 °C.

Pulp firmness

Pulp firmness was significantly affected by ethylene throughout shelf life for 0 d CS. For 3 d CS and 6 d CS, differences were found up to 4 and 3 days of shelf life, respectively. No differences between control and ethylene were found for 9 d CS (Figure 2). Non-stored (0d CS) ethylene treated fruit softened from an initial firmness of 190 N to 4 N in 4 days at 20 °C, while at the same time control showed 100.5 N pulp firmness. Ethylene treated fruit stored for 3 and 6 days reached 5.5 and 3.3 N in only 3 days at 20 °C, respectively, while at the same time control reached 50.2 and 17.7 N. Regardless of the treatment, as the fruit ages from harvest, it takes fewer days to soften when transferred to 20 °C.

According to Arpaia et al. (2018), optimum eating firmness for ‘Hass’ avocados ranges from 4.4 to 6.7 N. In our study, non-stored ethylene treated fruit reached eating firmness within 4 days from transferring from 8 °C to 20 °C, while control fruit took longer than 6 days. As consumers generally prefer to buy avocados at or close to a ready-to-eat stage (TOKAR, 2007), pre-conditioning with ethylene can be an advantage if a reduction in ripening time is needed.

Our results suggest that ethylene can be used to promote the ripening of ‘Hass’ avocados when applied during cold transport and/or storage (8 °C) following harvest, and fruits will be ready for consumption within a few days once transferred to room temperature. Nevertheless, if fruit is too be maintained at cold storage/transport for longer than a week after harvest, ethylene application might be unnecessary, as the avocado will have additional time to soften prior to the initiation of final ripening and thus will be at a higher stage of ripeness once transferred to room temperature.

It is well established that avocado fruit softening is the result of the activity of cell wall degrading enzymes with some pre- and postharvest factors affecting the rate of softening (AWAD; YOUNG, 1979; BOWER; CUTTING, 1988). Ethylene plays a key role in ripening of avocados (JEONG; HUBER, 2004), with softening being far more dependent on ethylene than skin color changes. Gwanpua et al. (2018), by modeling ethylene regulated changes in ‘Hass’ avocado, determined that the effect of ethylene on softening enzymes was about 15 times larger than on color degrading enzymes.

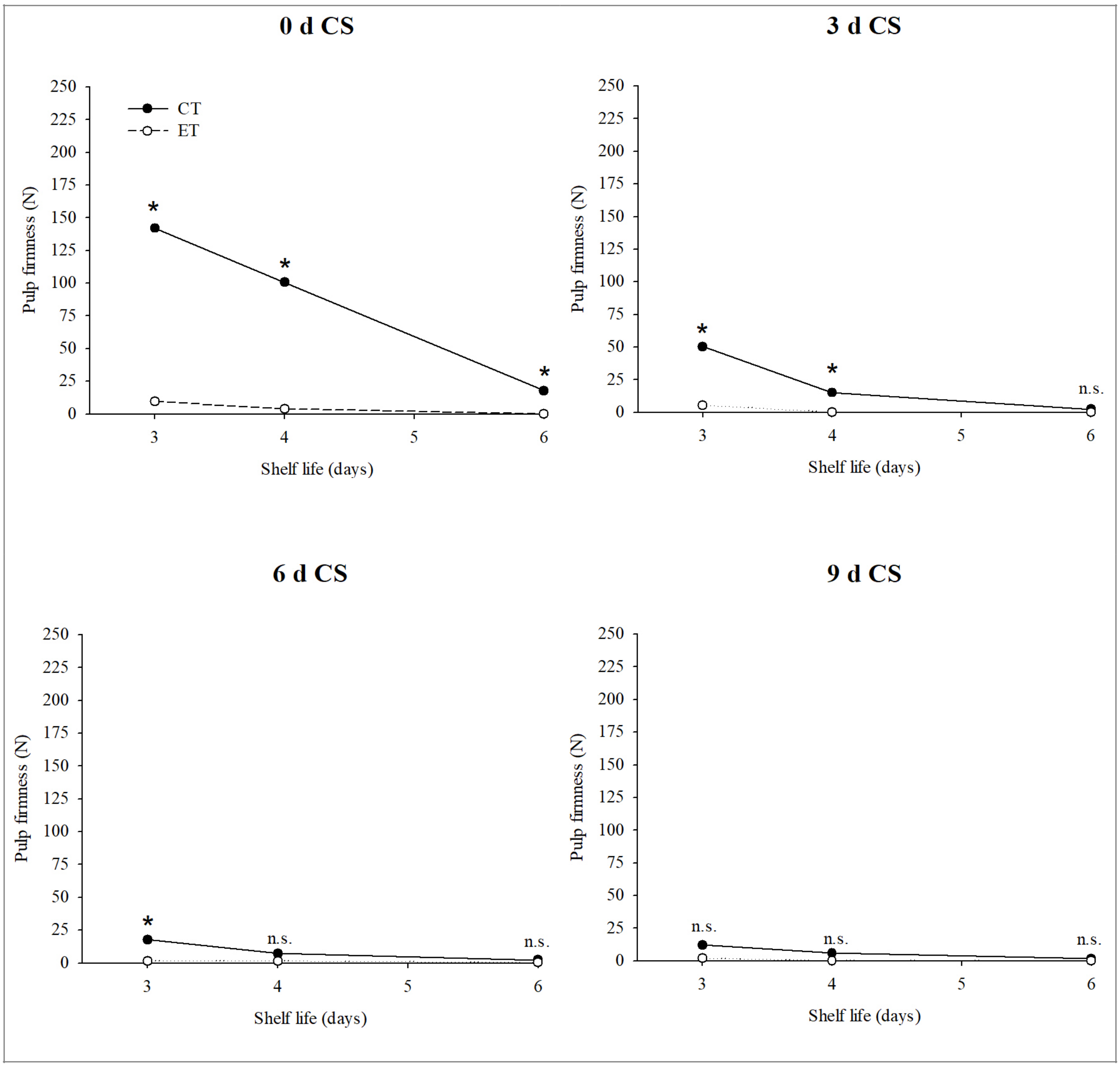

Figure 2. Influence of ethylene applied at 8 °C on pulp firmness of early harvested 'Hass' avocados stored for 0, 3, 6 and 9 days at 8 °C prior to ripening at 20 °C.

Weight loss

Fresh weight loss was significantly affected by ethylene for all cold storage durations throughout shelf life (Figure 3). Control fruit showed significant higher fresh weight loss than ethylene treated fruit during the whole experiment in all cold storage times (0, 3, 6 and 9 d CS). Regardless of the treatment, weight loss increased each day during shelf life. Fresh weight loss for control ranged from 2.3 to 5.5%, while ethylene treated ranged from 1.3 to 2.6%. Visually, shrivel was more pronounced in control fruit by the end of the experiment.

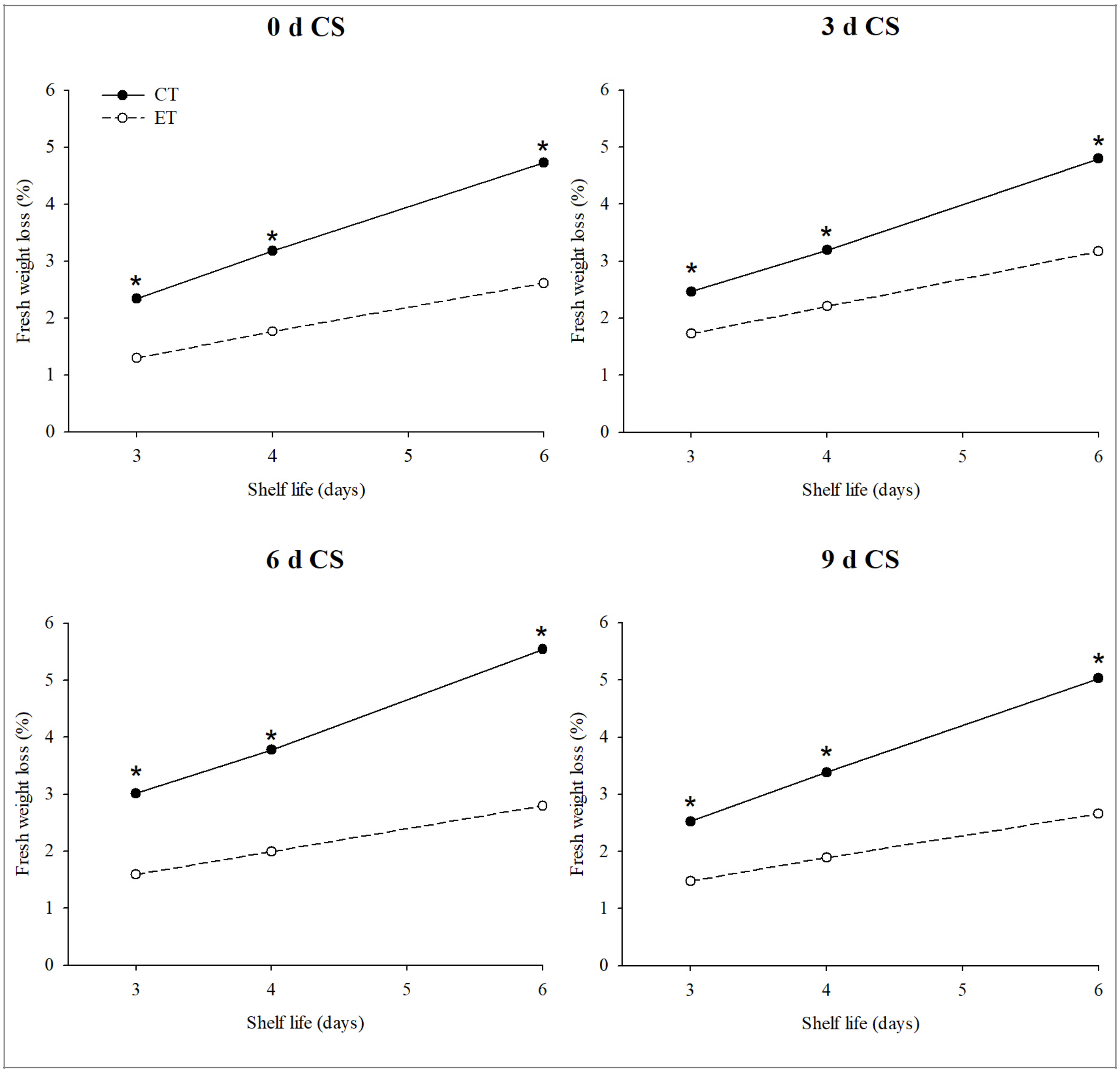

Figure 3. Influence of ethylene applied at 8 °C on fresh weight loss of early harvested 'Hass' avocados stored for 0, 3, 6 and 9 days at 8 °C prior to ripening at 20 °C.

Similar to the findings of this study, weight loss was also suppressed by ethylene in 'Hass' avocado fruit ripened at 20 °C following a 40 ppm ethylene treatment (ARPAIA et al., 2018). On the other hand, Flores et al. (2021) registered a lower weight loss for control fruit (5.28%) compared to fruit treated with 500 (6.05%) or 1000 mg/L (6.74%) of etephon (an ethylene releaser) at the end of 9 days at 20 °C.

As for most crops, weight loss is a significant commercial factor and an issue that should be considered as something to minimize (WOOLF et al., 2016). The use of wax coatings and maintenance of a high relative humidity during storage (85 to 95%) are the main strategies aimed to reduce weight loss in tropical and subtropical fruits (YAHIA; WOOLF, 2011). Considering that both control and ethylene fruit received a waxing treatment shortly after harvest and were kept under controlled temperature and humidity conditions inside of b.o.d. incubators during shelf life, ethylene might have played a role at reducing weight loss. Despite the divergent citations above, the results of this study are consistent and add up to the aforementioned benefits of applying ethylene in ‘Hass’ avocados.

Total soluble sugars and reducing sugars

Both total soluble sugars and reducing sugars were not significantly affected by ethylene for all cold storage durations, nor did they follow any consistent trend during ripening (data not shown).

During the initial phases of growth and development in avocados, sugars constitute over 40% of the mesocarp weight. These sugars progressively rise alongside the rapid expansion of the fruit, followed by a subsequent decrease concurrent with the accumulation of oil (KILARU et al., 2015). Generally, the prevalent sugars in avocados are C7 sugars (mannoheptulose and perseitol), with relatively modest levels of sucrose (PEDRESCHI et al., 2019).

Blakey et al. (2012) reported that hexoses (fructose and glucose) and sucrose were present in low and intermediate concentrations, respectively, and did not exhibit any consistent trend during ripening of ‘Hass’ avocado at 21 °C. Meyer and Terry (2010) also found slight and inconsistent changes in the concentrations of sucrose during ripening. In the present study, calibration curve for total soluble sugars and reducing sugars were generated for sucrose and fructose only, which are important sugars during pre-harvest fruit growth and development (BLAKEY et al., 2012) and not primary as carbon sources or energy store in avocado fruit (MEYER; TERRY, 2010).

Total phenolic content

Total phenolic content in the pulp was significantly affected by ethylene throughout shelf life for 6 and 9 d CS only (Figure 6). For both CS durations, ethylene-treated fruit presented higher total phenolic content than control over 6 days of shelf life. Minor differences were observed for 0 and 3 d CS. Total phenolic content of ethylene treated fruit was significantly higher only at 3 and 6 days of shelf life for 0 d CS and at 6 days of shelf life for 3 d CS.

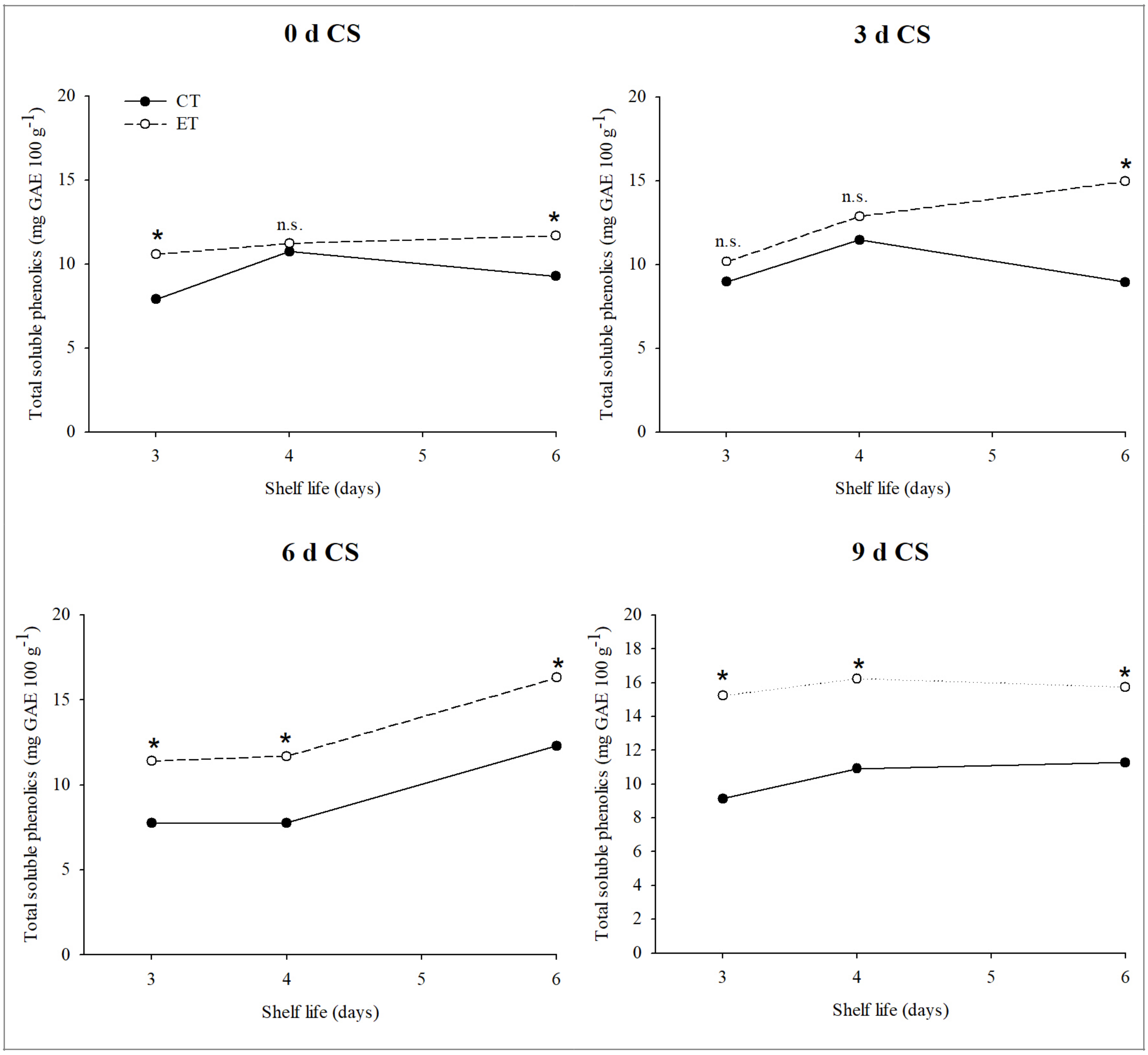

Figure 6. Influence of ethylene applied at 8 °C on total soluble phenolics of early harvested ‘Hass’ avocados stored for 0, 3, 6 and 9 days at 8 °C prior to ripening at 20 °C.

Relatively few researches on phenolic compounds of avocado fruit have been reported to date and none have investigated whether ethylene plays a role in its content. In the present study, treated fruit presented higher total phenolic content over shelf life compared to control, suggesting a possible effect of ethylene on the phenolic compounds biosynthesis pathway. These compounds are generally biosynthesized by the shikimic acid pathway, from which they are produced using intermediates of carbohydrate metabolism (TOMÁS-BARBERÁN; ESPÍN, 2001). It is necessary to further investigate the relationship between ethylene and phenolic compounds content in the pulp of avocados, considering the lack of studies published to date.

Phenolic compounds are plant secondary metabolites that play an important role in fruit quality, affecting appearance, flavor and health-promoting properties (TOMÁS-BARBERÁN; ESPÍN, 2001), which justifies investigating its dynamics in 'Hass' avocados during natural and ethylene induced ripening. According to Golukcu and Ozdemir (2010), total phenol amount is supposed to decrease during ripening. Contrary to this, for 6 d CS total soluble phenolics for both control and treated fruit increased during ripening. The pattern is the same for 9 d CS, except for a minor decrease in ethylene treated fruit at 6 days of shelf life. As for 0 and 3 d CS, there was a slight increase in control fruit from 3 to 4 days of shelf life, and then total phenolic content finally decreased at 6 days of shelf life. The amount of time from harvest to final ripening at 20 °C might have played a role in the different trends observed for each cold storage time and further studies are necessary to establish a pattern between avocado pulp phenolic content and ripening.

CONCLUSIONS

The present study demonstrates that ethylene can be used to promote and accelerate the ripening of early-harvested 'Hass' avocados during cold transport and/or storage at 8 °C. The application of 100 ppm ethylene at 8 °C shortens the ripening time of the fruit once they are exposed to warmer temperatures, which can benefit both producers and consumers by reducing the time from harvest to ripening. While exposure to ethylene can be an advantage due to reduced ripening time and enhanced uniformity, its application might be unnecessary if it follows a sustained period of cold storage. Also, it should be noted that the use of ethylene in avocado storage is not without potential drawbacks. For example, excessive ethylene exposure can lead to over-ripening and reduced shelf life, which can result in significant economic losses for producers. Therefore, it is important to carefully regulate the concentration and duration of ethylene exposure to ensure optimal ripening and quality.

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ARTICLE 2 - RIPENING OF EARLY-SEASON ‘HASS’ AVOCADO WITH ETHYLENE

ABSTRACT

The long ripening period of ‘Hass’ avocado fruits harvested at the beginning of the harvest can be detrimental to sales. Ethylene is capable of anticipating and accelerating the ripening of climacteric fruits. The objective was to evaluate the effect of ethylene applied at 20 °C on accelerating the ripening of ‘Hass’ avocado. Freshly harvested fruits (n=192) were kept for 4 days at 8°C in order to simulate long-distance refrigerated transport and then stored for 0, 3, 6 or 9 days at 8°C. After the storage period, they were treated with 100 ppm ethylene for 24 hours at 20 °C and kept at 20 °C and 85% relative humidity to simulate shelf life. A completely randomized design was used, with 3 replications and 2 fruits per replication. The data were subjected to analysis of variance and the means were compared using the F test ($p \leq 0.05$). The number of days for maturation and firmness were affected by ethylene only for 0 and 3 days of storage. Fruits stored for 0 and 3 days at 8°C and treated with ethylene took, respectively, 1.7 and 2.0 days less to ripen than control fruits. Avocados not stored and treated with ethylene reached 48.6 N firmness after 6 days on the shelf, while control fruits had 101.2 N in the same period. Avocados stored for 3 days and treated with ethylene reached 11.9 N in just 3 days on the shelf, while control avocados exhibited 122.4 N. Control fruits showed greater weight loss compared to treated fruits in all periods of refrigerated storage evaluated. Application of ethylene at 20°C accelerates the ripening of ‘Hass’ avocado by up to 2.0 days, and as the fruit ages since harvest, it takes fewer days to ripen when transferred from 8°C to 20°C.

KEYWORDS: *Persea americana* Mill.; Ripening control; Ethylene.

AMADURECIMENTO DO ABACATE ‘HASS’ DE INÍCIO DE SAFRA COM ETILENO

RESUMO

O longo período de amadurecimento de frutos de abacate ‘Hass’ colhidos no inicio da safra pode ser prejudicial às vendas. O etileno é capaz de antecipar e acelerar o amadurecimento de frutos climatéricos. Objetivou-se avaliar o efeito do etileno aplicado a 20 °C na aceleração do amadurecimento de abacate ‘Hass’. Frutos recém colhidos (n=192) foram mantidos por 4 dias a 8 °C a fim de simular transporte refrigerado de longa distância e, em seguida, armazenados por 0, 3, 6 ou 9 dias a 8 °C. Após o período de armazenamento, foram tratados com 100 ppm de etileno por 24 horas a 20 °C e mantidos a 20 °C e 85% umidade relativa para simular a vida de prateleira. Utilizou-se delineamento inteiramente casualizado, com 3 repetições e 2 frutos por repetição. Os dados foram submetidos à análise de variância e as médias comparadas pelo teste F ($p \leq 0,05$). O número de dias para maturação e firmeza foram afetados pelo etileno apenas para 0 e 3 dias de armazenamento. Frutos armazenados por 0 e 3 dias a 8 °C e tratados com etileno levaram, respectivamente, 1,7 e 2,0 dias a menos para amadurecer do que frutos controle. Abacates não armazenados e tratados com etileno atingiram 48,6 N de firmeza após 6 dias de prateleira, enquanto frutos controle apresentavam 101,2 N no mesmo período. Abacates armazenados por 3 dias e tratados com etileno atingiram 11,9 N em apenas 3 dias de prateleira, enquanto abacates controle exibiam 122,4 N. Frutos controle apresentaram maior perda de peso em comparação com frutos tratados em todos os períodos de armazenamento refrigerado avaliados. A aplicação de etileno a 20 °C acelera o amadurecimento do abacate ‘Hass’ em até 2,0 dias e, à medida que o fruto envelhece desde a colheita, leva menos dias para amadurecer quando transferido de 8 °C para 20 °C.

PALAVRAS-CHAVE: *Persea americana* Mill.; Controle do amadurecimento; Etileno.

INTRODUCTION

Avocado (*Persea americana* Mill) is a rich oil fruit of high economic value in the international trade and ‘Hass’ corresponds to about 90% of the total volume of avocados commercialized worldwide (PEDRESCHI et al., 2019). In Brazil, the ‘Hass’ harvest season usually starts in March, when the fruit reaches around 21% dry matter, and extends through September (BARBIERI et al., 2023). After harvest, the number of days to ripen will depend on the harvest season - early, mid and late (YAHIA & WOOLF, 2011), with early-season fruit requiring longer time to ripen than late-season fruit (ARPAIA et al., 2018).

Brazilian ‘Hass’ avocado production is mainly destined for exportation, being Argentina the major importer in South America (BARBIERI et al., 2023), where fruit arrives after 4 to 5 days by refrigerated trucks (personal communication). When growers are trying to make it to the market at the beginning of the season to get premium prices and compete with other South America exporting countries that have the same harvest calendar, one of the main issues brought by consumers is the long time it takes for fruit to ripen (personal communication). Market research has demonstrated that sales can be doubled or tripled when avocados are offered to consumers in a 'ready-to-eat' condition (YAHIA & WOOLF, 2011). To meet consumer's taste and overcome fruit-to-fruit variability in ripening, an increasingly important commercial tool is the use of ethylene to hasten and synchronize ripening (ARPAIA et al., 2018).

The avocado is a climacteric fruit and exogenous application of ethylene after harvest cause an earlier climacteric with consequent ripening (YAHIA & WOOLF, 2011). Fruits of different species have been treated with ethylene or its analogues in order to promote rapid uniform ripening and/or improve fruit quality aspects (BARRY & GIOVANNONI, 2007). The exogenous application of ethylene in climacteric fruits provides a considerable reduction in the period between harvest and the complete ripening of the fruits (BRAZ et al. 2008). For 'Hass' avocados, ethylene treatment also helps to overcome the "checker-boarding" ripening defect - uneven color and fruit firmness in a batch, a common issue faced by suppliers (HASS AVOCADO BOARD, 2020).

The application of ethylene in a ripening room under controlled conditions of temperature, relative humidity, and carbon dioxide levels is known in the avocado industry as pre-conditioning (ARPAIA et al., 2018). Commercial application of 10 to 100 $\mu L\ L^{-1}$ is recommended, and conditioning time varies depending on harvest season and age of fruit from harvest. According to YAHIA (2011), the most uniform ripening for 'Hass' avocados is achieved when using 48 to 72 h for early season, 24 to 48 h for mid-season, and 12 to 24 h for late season. Avocado pre-conditioning is often held at 17 to 20 °C, relative humidity must be kept high above 85% and conditioning room must be vented to minimize high CO_2 levels, a potential ripening inhibitor (CALIFORNIA AVOCADO COMMISSION, 2020).

Some authors have explored the effects of ethylene on 'Hass' avocado ripening. Eaks (1966) has shown that the application of 100 ppm ethylene for 24 hours leads to the ripening of fruits within 3-4 days after the start of the hormone treatment. The author also states that a 12-hour exposure accelerated the ripening in about one day, when compared to untreated fruits. Adkins (2005) also reports on ethylene effects in 'Hass' avocados, verifying that treatment at 100 $\mu L\ L^{-1}$ for 24 h reduced the ripening period in 3 days.

Although reported in the literature that preconditioning with ethylene effectively hastens avocado ripening, there is no recording on its effects for ‘Hass’ avocados cultivated in the Southeast region of Brazil. This study was designed to test ethylene efficacy applied at 20 °C on accelerating the ripening of early season ‘Hass’ avocado fruit after refrigerated transport and storage.

MATERIAL AND METHODS

Fruit source

Early-season ‘Hass’ avocado fruit was obtained from a local commercial orchard, at Rio Paranaiba, state of Minas Gerais. The fruit was manually harvested in the early morning following sanitization with peracetic acid and waxing. The fruit was maintained at 8 °C until night, when it was transported in an air-conditioned vehicle to the Federal University of Viçosa Postharvest Physiology Laboratory, in Viçosa, Minas Gerais.

Fruit ripening and cold storage

After arriving at the laboratory, ‘Hass’ avocado fruit (n = 192) was kept at 8 °C for 4 days to simulate long distance truck transport - time usually taken to reach a neighboring country. After that, fruit was stored at 8 °C for 0, 3, 6 or 9 days, following treatment with 0 or 100 $\mu L\ L^{-1}$ ethylene in 90 L hermetically sealed buckets at 20 °C. Subsequently, fruit remained at 20 °C for shelf-life evaluations.

Days to ripe

Number of days to ripe (DTR) was determined from the day fruit was transferred from 8 °C to 20 °C until fruit was fully ripened, after reaching 4-7 N of pulp firmness.

Peel color

The peel color was determined using a Konica Minolta colorimeter (Chroma meter, CR-32). The color was expressed by the rectangular coordinate system L, a* b* according to the Commission internationale de l'éclairage (CIE), where L is expressed as a percentage of brightness values (0% = black and 100% = white), a* represents the colors red (+) or green (-) and b* the colors yellow (+) or blue (-). Based on the a* and b* coordinates, the hue angle and chromaticity parameters were calculated (MINOLTA CORP, 1994). The reading was done at 3 different points of the fruit's peel.

Pulp firmness

Pulp firmness was evaluated through its resistance to penetration using an analog penetrometer model FT-327, with a cylindrical tip of 8 mm in diameter. Measurements were performed at two opposite points in the equatorial region of the fruit after removing the peel. The results were expressed in N.

Weight loss

The weight loss was estimated by weighing the same fruits individually on an analytical balance throughout the shelf life. Weight loss was calculated (fresh weight basis) using the following equation: Weight Loss (%) = Initial weight - Final weight/ Initial weight × 100

Total soluble sugars

The amount of total soluble sugars (TSS) was estimated by phenol sulfuric acid reagent method (DUBOIS et al., 1956). To prepare the alcoholic extract, 5 g of avocado pulp was homogenized at 14,000 g with pre-heated EtOH 80% then centrifuged at 2,000 g for 10 minutes. Centrifugation was repeated twice, and the final volume of the alcoholic extract filled to 25 ml. Later, 40 µL of the alcoholic extract, 210 µL of deionized water and 250 µL of 5% phenol was pipetted into a test tube, followed by vortexing. TSS was quantified via colorimetric assay by adding 1.25 mL of concentrated sulfuric acid to the test tube, following heating for 20 minutes at 30 °C. TSS was estimated against a standard curve of 1% sucrose measured at 540 nm. The results were expressed in percentage of fresh mass.

Reducing sugars

The reducing sugar content (RS) was quantified using the dinitrosalicylic acid (DNS) methodology (GONÇALVES et al., 2010) with modifications. The first step was to determine the standard curve, using a 0.2% fructose solution. For quantification, 500 µL of DNS, 200 µL of alcoholic extract and 300 µL of deionized water were pipetted into a test tube following heating for 5 minutes at 100 °C. The tubes were cooled in an ice water bath and 4 mL of deionized water was added to each tube. The readings were carried out in a spectrophotometer at 540 nm and the results expressed in percentage of fresh mass.

Total phenolic content

The content of total phenolic compounds (TPC) was determined by the Folin-Ciocalteu method (FU et al., 2010), with modifications. An aliquot of 200 µL of the alcoholic extract and 1,000 µL of the Folin-Ciocaltieu reagent (1:10) was added to a test tube. After 10 minutes, 800 µL of 7.5% sodium carbonate was added to the mixture, then the tubes were vortexed and kept in the dark for 30 minutes at room temperature. At the end of this step, the absorbance of the samples were quantified in a spectrophotometer at 760 nm, using gallic acid as a standard, and the results expressed in mg per 100 g of fresh mass.

Statistics

The experiment was conducted in a completely randomized design, with 3 replications, with each experimental unit consisting of 2 fruits. The data obtained was subjected to analysis of variance and the means compared by F test at 5% probability. Statistical analyses were performed using the R Core Team program.

RESULTS AND DISCUSSION

Days to ripe

The number of days to ripe was significantly affected by ethylene only for 0 and 3 days of cold storage. Control fruit took 9.3 and 6.7 days at 20 °C to ripen, after 0 and 3 days at 8 °C, respectively, while ethylene-treated fruit required 1.7 and 2.0 fewer days to ripen once transferred to 20 °C (Table 1). As can be observed in Table 1, the longer the fruit was stored at 8 °C, the shorter the time to final ripening at 20 °C.

For fruit cold stored for 0 and 3 d, ethylene treatment significantly reduced ripening time. These findings indicate that the application of 100 µL L^{-1} ethylene at 20 °C after 4 days (transport simulation) plus 0 or 3 days at 8 °C accelerates the ripening of 'Hass' avocados up to 2 days. This result is in accordance with previous works reporting ethylene effects on avocado ripening (EAKS, 1966; GAZIT & BLUMENFELD, 1970; EAKS, 1980; ZAUBERMAN et al., 1988; ARPAIA et al., 2018; FLORES et al., 2020). According to Arpaia et al. (2018), non-stored ‘Hass’ avocados treated with 40 µL L^{-1} ethylene took 7.9 days to ripe while control took 13.5 days. Flores et al. (2020) found that ethephon - an ethylene releaser, reduced ripening time of ‘Hass’ avocado by two days compared to untreated fruit, maintaining acceptable and homogenous firmness and color.

Table 1. Influence of ethylene applied at 20 °C on days to ripeness of early harvested ‘Hass’ avocados cold stored at 8 °C (CS) for 0, 3, 6 or 9 days prior to final ripening at 20 °C.

	Days to ripe			
	0 d CS	3 d CS	6 d CS	9 d CS
Control	9.3a	6.7a	4.7^{ns}	3.0^{ns}
Ethylene	7.6b	4.7b	4.0^{ns}	3.3^{ns}

Mean separation by F-test (P≤0.05), means with different letters within a storage duration are significantly different from each other.

The reduction in the number of days to ripe observed in ethylene-treated fruit can be attributed to the hormone’s role in promoting fruit ripening. In avocado, as in other climacteric fruit, the onset of ripening is marked by a large rise in ethylene production (SEYMOUR, TAYLOR & TUCKER, 2012). The rapid rise in ethylene production and the resultant rise in internal ethylene concentrations within the commodity stimulates the many changes associated with ripening - such as in color, texture, flavor and aroma (SALTVEIT, 2019). The exogenous applications of ethylene in physiologically mature fruit after harvest cause an earlier climacteric with consequent ripening (EAKS, 1978).

Fruit age after harvest influences how the fruit responds to ethylene exposure. YAHIA (2011) states that there may be relatively little benefit in ethylene treatment in terms of advance ripening if it is applied after three weeks of storage. Other authors indicate that even shorter storage times might diminish its benefits. ‘Hass’ avocados that were stored for 14 d at 5 °C and then ripened at 20 °C to eating firmness with 40 µL L^{-1} ethylene, took 5.3 days to ripe, similar to the control that took 5.7 days (ARPAIA et al., 2018). In our study, no significant difference in days to ripe was found for 6 and 9 d CS - considering the transport simulation, the total amount of storage time before final ripening was 10 and 13 days, respectively. We noticed that the longer the fruit was stored, the shorter the time to final ripening once transferred to 20 °C. While exogenous ethylene might be a tool to reduce the number of days to ripe if the fruit are treated shortly after harvest - up to one week, it can be pointless after a sustained period of storage. Still, commercial operators often use ethylene to provide a more uniform ripening regardless of its effect on hastening the ripening time (YAHIA, 2011).

Peel color

The peel color parameters a*, chroma (C*), Hue angle (H°), and lightness (L*) were significantly affected by ethylene for fruit stored for 3 days at 8 °C (3 d CS) prior to ripening at 20 °C (Figure 1). Peel color for 0, 6 and 9 d CS were not significantly altered (data not shown).

The parameter a* increased during final ripening at 20 °C, indicating a shift from green (lower a* values) towards magenta colors (higher a* values), in agreement with the ripening color changes in 'Hass' avocados. Skin of 'Hass' avocados changes color from green to purple/black as fruit ripen (COX et al., 2004) and this color change is one of the main ripeness indicators for both industry and consumers. Although the color change pattern was similar for both control and ethylene treated fruit, at 3 and 6 days of shelf life ethylene presented significantly higher a* values (less green, more purple), suggesting a more advanced ripeness stage at this point.

Hue angle (H°), chroma (C*), and lightness (L*) values all decreased with ripening for both control and treated fruit (Figure 1). Similar to the findings of this study, COX et al. (2004) report a decrease in H°, C* and L* of ethylene treated 'Hass' avocado fruit skin during ripening at 15, 20 and 25 °C.

Hue angle, C* and L* values were significantly lower in ethylene treated fruit at 6 days of shelf life. The decrease in L* values represents a shift from lighter to darker colors and in H° values express a transit towards reddish colors (red = hue angle of 0°) (MCGUIRE, 1992). Chroma (C*) is the amount of saturation of a color, and a decrease in C* represents a shift from clear, bright or brilliant colors to dull, less vivid colors (CIE, 2020).

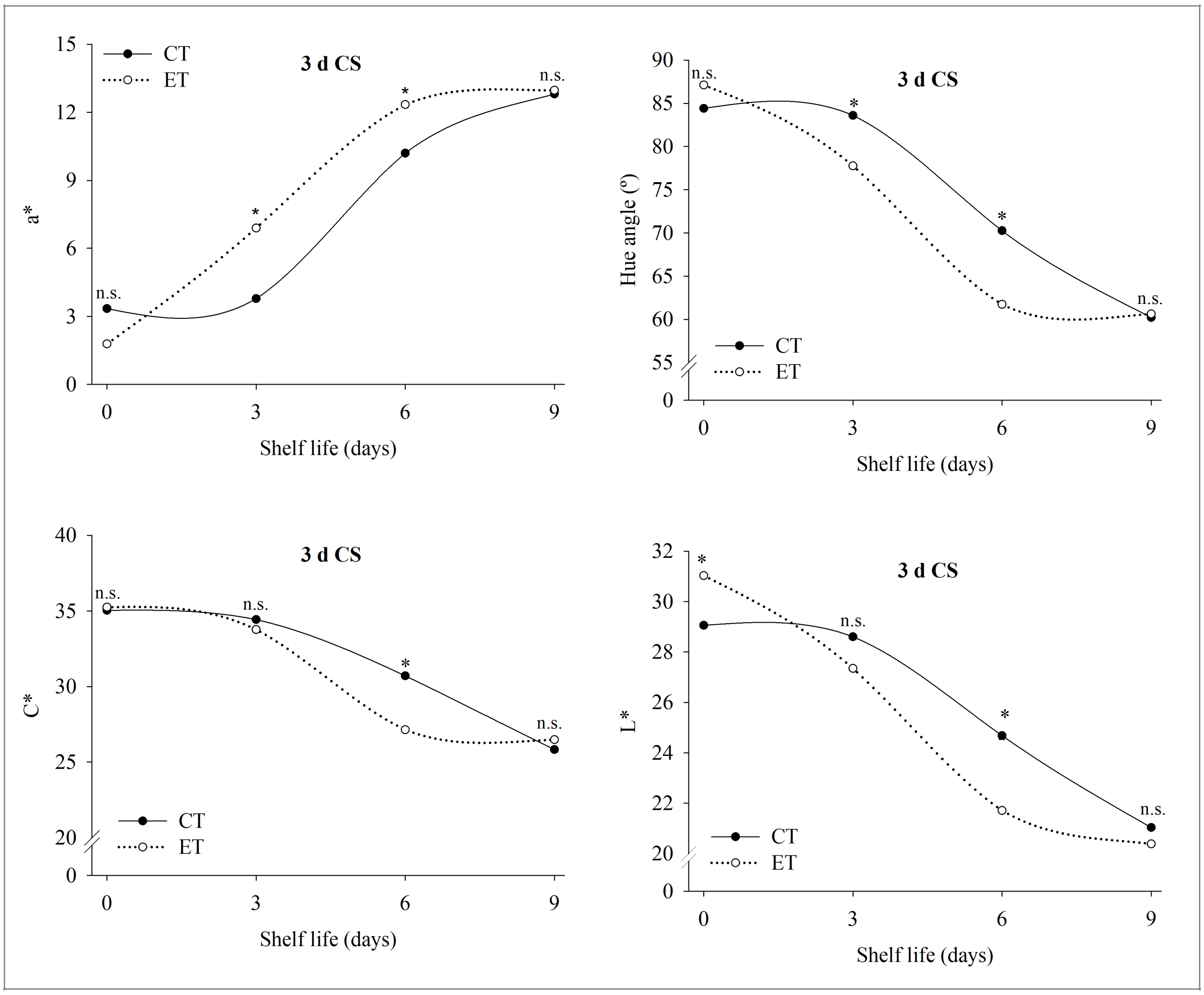

Figure 1. Influence of ethylene applied at 20 °C on peel color parameters (a*, C*, L* and Hue°) of early harvested 'Hass' avocados stored for 3 days at 8 °C prior to ripening at 20 °C.

Skin color can be used as an indicator of ripeness for dark-skinned avocados such as 'Hass' (MAGWAZA & TESDAY, 2015). As seen previously (Table 1), for 3 d CS fruits, at 6 days of shelf life, ethylene treated fruit had already reached the full ripeness stage whilst control not, which is in accordance with the marked color differences between treated and untreated fruit at this time point. The exogenous application of ethylene in climacteric fruits provides a considerable reduction in the period between harvest and the complete ripening of the fruits (BRAZ et al. 2008). Under certain conditions of this study, ethylene effectively hastened ripening in 'Hass' avocados through the promotion of a faster color change, corroborating with previous works that credits ethylene as an efficient tool in accelerating ripening of 'Hass' avocados.

Pulp firmness

Pulp firmness was significantly affected by ethylene for 0 and 3 CS. For 0 d CS, a significant difference was found at 6 days of shelf life and for 3 d CS, at 0 and 3 days (Figure 2). No differences between control and ethylene were found for 6 and 9 d CS (data not shown).

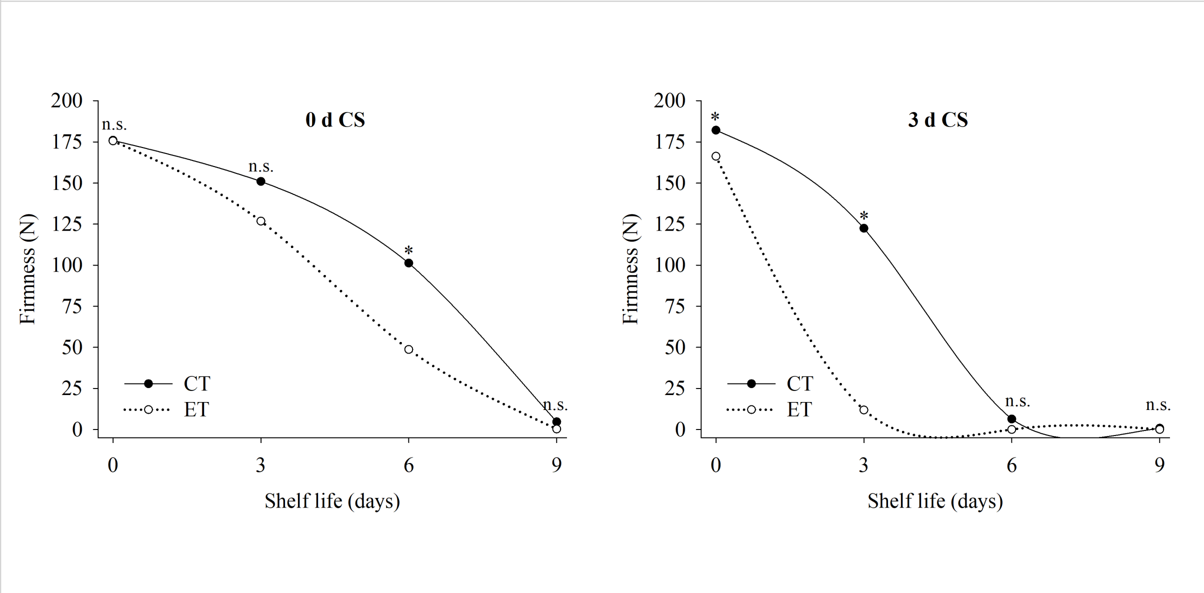

Figure 2. Influence of ethylene applied at 20 °C on pulp firmness of early harvested 'Hass' avocados stored for 0 and 3 days at 8 °C prior to ripening at 20 °C.

Non-stored (0 d CS) ethylene-treated avocados softened from an initial firmness of 175.5 N to 48.6 N within 6 days of shelf life, while control fruit softened from 175.9 N to 101.2 N in the same period of time. As for avocados stored for 3 days (3 d CS), ethylene-treated fruit softened from an initial pulp firmness of 166.2 N to 11.9 N within only 3 days of shelf life while control avocados remained firm hard, starting at 182 N and dropping to 122.4 N within 3 days at 20 °C. Regardless of the treatment, as the fruit ages from harvest (i.e., longer storage time), it takes fewer days to soften when transferred to 20 °C (data not shown).

Fruit softening is a major aspect of the ripening process in avocados (DEFILIPPI et al., 2018). According to Yahia and Woolf (2011), ethylene exposure during storage accelerates softening of avocados. The faster softening of ethylene-treated avocados during shelf life, evident at 6 and 3 days of shelf life, for 0 d and 3 d CS, respectively, highlights the impact of ethylene in hastening the ripening process. Climacteric fruit softening is associated with ethylene-induced cell wall degrading enzymes (e.g., cellulase, polygalacturonase, and pectinmethylesterase). According to Bower and Cutting (1988), the early stages of softening in the avocado appear to be due to cellulase, controlled at least in part by ethylene, with polygalacturonase (PG) responsible for final softening. The influence of ethylene on accelerating fruit softening has been reported for various climacteric fruits (HIWASA et al., 2003; HERTOG et al., 2016; TUCKER et al., 2017; FAN et al., 2018).

The lack of significant differences in pulp firmness between ethylene-treated and control avocados for 6 and 9 days CS might be due to inefficient treatment and/or to the somewhat longer storage time. Fruit age after harvest influences how the fruit responds to ripening management. According to ARPAIA et al. (2018), pre-conditioning can be an advantage if fruit are immediately treated aiming to reduce ripening time. According to YAHIA & WOOLF (2011), after about three weeks of storage there may be relatively little benefit in ethylene treatment. Thus, if there is a sustained period of time after harvest, fruit will be at a higher stage of ripeness, and simply rewarming the fruit with temperature management may be sufficient. However, commercial operators in general use ethylene at all times, since it provides a more uniform result (YAHIA & WOOLF, 2011).

Weight loss

Fruit water loss is often used interchangeably as weight loss, mass loss and moisture loss (LUFU, R.; AMBAW A.; OPARA, U. L., 2020). In this current work, weight loss is adopted. Ethylene exhibited a noteworthy impact on weight loss across all cold storage durations throughout the shelf life (Figure 3). Control fruit consistently demonstrated a significantly higher level of weight loss in comparison to ethylene-treated fruit across all assessed cold storage periods (0, 3, 6, and 9 days of cold storage). Irrespective of the treatment applied, a progressive increase in weight loss was observed over each day of the shelf life. The fresh weight loss percentages in control fruit ranged from 2.9% to 8.7%, whereas ethylene-treated fruit exhibited a narrower range of 1.4% to 3.8%. Notably, by the conclusion of the experiment, visual assessment indicated that shriveling was more pronounced in control fruit.

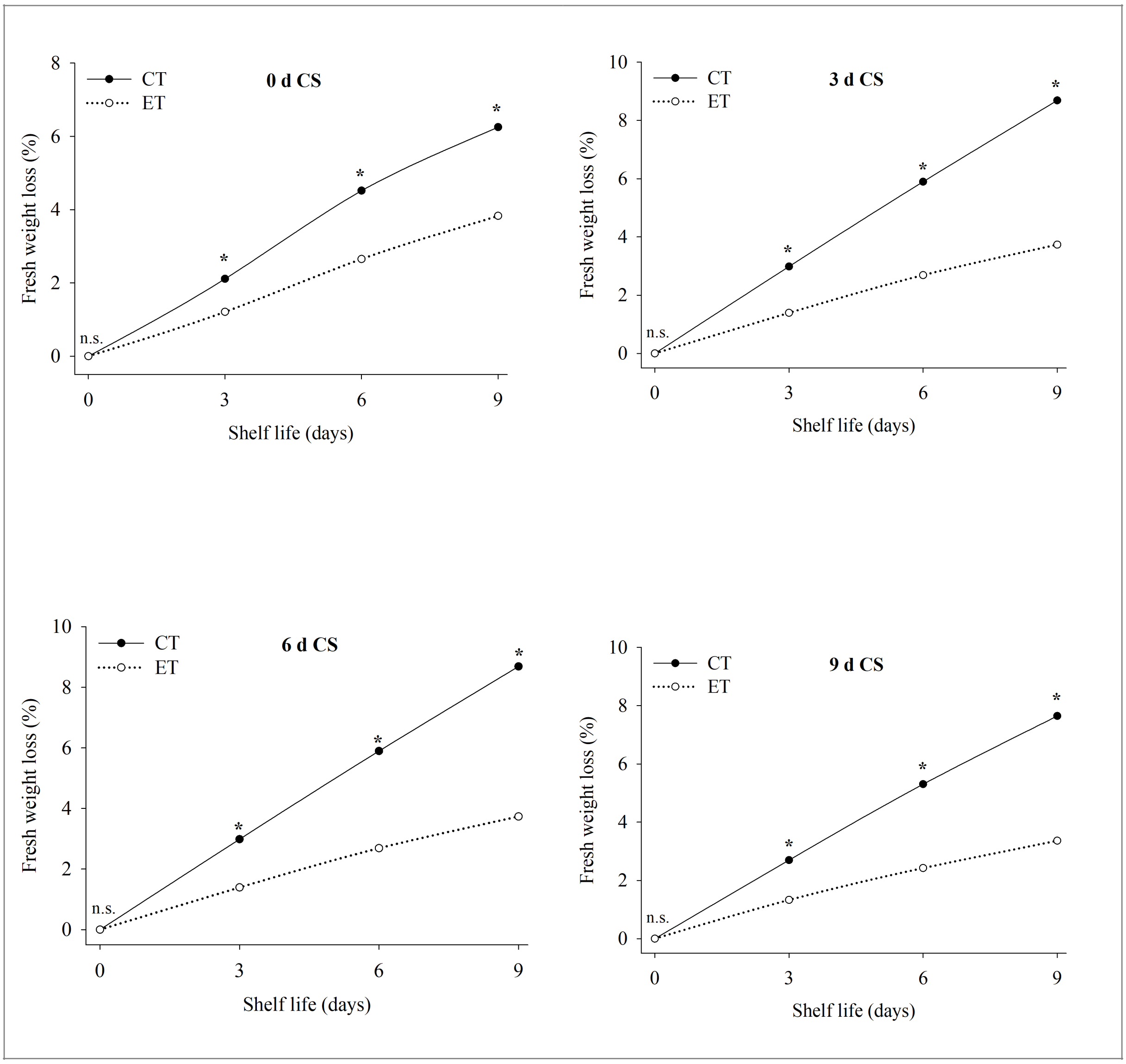

Figure 3. Influence of ethylene applied at 20 °C on fresh weight loss of early harvested ‘Hass’ avocados stored for 0, 3, 6 and 9 days at 8 °C prior to ripening at 20 °C.

Flores et al. (2018) reports that ‘Hass’ avocado weight loss increased over each day at 20 ± 2 °C and corresponded to 5.28%, 6.05%, and 6.74% for the control, ethephon 500 mg/l, and ethephon 1000 mg/l, respectively, after 9 days. Contrarily, Arpaia et al. (2018), when studying the influence of temperature and ethylene on ‘Hass’ avocado quality, reports that 40 μL L^{-1} ethylene slightly reduced weight loss - 5.9% control versus 5.5% ethylene.

As for most crops, weight loss is a significant avocado commercial quality factor. Bower & Jackson (2003) and Bower & Papli (2006) found in avocado fruits that weight loss constitutes one of the main factors of quality deterioration, mainly when above 6%. It is assumed that the majority of mass loss is due to water loss, thus high relative humidity during storage (85 to 95%) and use of wax coatings are aimed at reducing weight loss (YAHIA & WOOLF, 2011). Considering that both control and ethylene treated fruit went through the very same waxing processing line soon after harvest and remained in high humidity environments throughout shelf life, ethylene might have played a role in reducing weight loss.

Fruit and vegetables continue to undergo respiration and transpiration after harvest, utilising sugar and water reserves yet without replacement and therefore continue to lose turgidity and weight, resulting into shrivelling and shrinkage (LUFU et al., 2020). Climacteric fruits are characterized by a dramatic increase in autocatalytic ethylene production that is accompanied by a burst of respiration at the onset of ripening (HEWITT; DHINGRA, 2020). As fruit matures, changes in fruit texture, color, flavor, and other organoleptic qualities, are associated with increased metabolic activities, all of which contribute to higher water loss. In this study, ethylene-treated fruit, which ripened faster than control only for 0 and 3d CS, had lower weight loss percentages than the control group in all cold storage durations. These results suggest that applying ethylene, regardless of its effect as a ripening enhancer, can potentially reduce water loss.

Whether or not ethylene had an effect on weight loss in the present study, previous authors have linked fruit water loss - often used interchangeably as weight loss, with avocado ripening rates (BURDON et al., 2022). Also, according to Yahia (2011), some weight loss is important in promoting the ripening of avocado. The author found that storing fruit in a 100% RH environment - leading to negligible weight loss, resulted in very significant delays in ripening.

Water loss is a complex attribute involving multiple morphological, physiological and environmental factors. While ethylene is known to influence the ripening process, the specific effects on weight loss can be complex and depend on various factors. Additional experiments with different concentrations of ethylene and exposure durations, as well as environmental factors, are necessary to gain a comprehensive understanding of the effects of ethylene on avocado ripening rates and weight loss.

Total soluble sugars, Reducing sugars, Total phenolic content

Total soluble sugars, reducing sugars and total phenolic content were not significantly altered by the treatments in any of the cold storage times, thus are not presented.

CONCLUSIONS

The present study verifies that exogenous ethylene accelerates the ripening of early-harvested 'Hass' avocados. Pre-conditioning with 100 ppm ethylene at 20 °C after 4 days (transport simulation) plus 0 or 3 days at 8 °C hastens the ripening up to 2 days. Ethylene ripening can be a useful tool to anticipate ripening during transit or at the terminal market before going to the retailer and, thus, provide consumers with 'ready-to-eat' fruit. This is especially pertinent to the avocado industry in the beginning of the season, when fruits tend to take longer to ripe. While pre-conditioning can be convenient for freshly harvested fruit, after more than a week of cold storage there may be relatively little benefit in ethylene treatment and simply rewarming the fruit with careful temperature management may be sufficient. However, using ethylene at all times, regardless of time spent in storage, might provide a more predictable result.

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GENERAL CONCLUSION

Based on the findings from the two experiments conducted in this study, it is evident that the application of ethylene gas significantly influences the ripening process of early-harvested 'Hass' avocados. In the first experiment, where ethylene was applied at 8 °C before cold storage, substantial acceleration in ripening was observed. Avocados treated with ethylene reached optimum firmness for consumption in significantly fewer days compared to untreated fruits, demonstrating the efficacy of ethylene even at lower temperatures. Moreover, treated fruits exhibited reduced weight loss during storage, further highlighting the positive impact of ethylene treatment on maintaining fruit quality.

Similarly, in the second experiment, where ethylene was applied at 20 °C after cold storage, a notable acceleration in ripening was observed, although to a slightly lesser extent compared to the first experiment. Nonetheless, ethylene-treated fruits still exhibited faster ripening and lower weight loss compared to untreated fruits, indicating the effectiveness of ethylene application in enhancing the post-harvest ripening process of 'Hass' avocados. Overall, these findings provide valuable insights for the avocado industry, suggesting that ethylene treatment can be a practical and efficient method for reducing the ripening period of early-harvested avocados, thereby potentially improving sales and marketability. Further research could explore optimal ethylene concentrations and application methods to maximize the efficiency of this approach in Brazilian commercial avocado production.