

ORIGINAL RESEARCH ARTICLE

Postharvest ripening of Hass and Méndez avocado fruit cultivars treated with ethephon

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ABSTRACT

A problem in post-harvest of avocado (*Persea americana* Mill.) is the heterogeneity in fruit ripening, due to differences in the time of fruit set and the inability to ripen on the tree, a situation that causes inconsistencies in quality and differences in the response to preservation and processing technologies. In postharvest, the application of ethylene gas in hermetic chambers has been used to advance ripening; however, the use of ethylene releasers in liquid form (ethephon) has been proposed as an alternative, mainly for the treatment of low volumes of fruit. The present work was carried out in the production zone of Salvador Escalante (Michoacán, Mexico) with the objective of evaluating the effect of the application of two concentrations of ethephon on the time and homogenization of fruit ripening of avocado cultivars (cv.) Hass and Méndez. Fruits with 23.4% (cv. Hass) and 24% (cv. Méndez) of dry matter were harvested; one group was immersed in a solution of ethephon 500 mg/L and the other in 1,000 mg/L, both for 5 minutes; the treated fruits plus a control were stored at 20 °C for 11 days. Changes in respiration, ethylene production, weight loss, firmness, epicarp and pulp color, total phenol, chlorophyll and total carotenoid concentrations were evaluated. The results showed that ethephon doses of 1,000 mg/L in cv. Hass and 500 mg/L in cv. Méndez presented a ripening process 2 days earlier than the control.

Keywords: 2-Chloroethyl Phosphonic Acid; Quality; Color Index; Ethylene; *Persea americana* Mill

ARTICLE INFO

Received: 4 July 2021
Accepted: 8 August 2021
Available online: 19 August 2021

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1. Introduction

In post-harvest, avocado (*Persea americana* Mill.) cultivar (cv.) Hass fruits present a climacteric behavior in which four physiological states are identified^[1] in response to ethylene treatment: (1) inhibition, which constitutes a period where the fruit is insensitive to exogenous ethylene^[2]; (2) pre-climacteric, exogenous ethylene favors autocatalytic ethylene production in the fruit; (3) climacteric, with autocatalytic ethylene production associated with the stimulus of changes inherent to ripening; and (4) post-climacteric, with a decrease in the production of this phytohormone. This behavior allows assuming the physiological possibility of advancing the ripening process by stimulating ethylene synthesis in fruits in pre-climacteric state^[2,3]. One of the post-harvest problems in avocado cv. Hass and cv. Méndez, is the heterogeneity in fruit ripening due to differences in the time of tie-up and the inability of fruits to ripen on the tree, which results in inconsistencies in fruit quality, as well as differences in the response to the application of preservation and processing technologies^[4,5].

According to several studies^[6,7] the application of exogenous ethylene in pre-climacteric phase promotes and homogenizes ripening, a situation that in avocado fruits is important to have fruits in edible maturity stage before time and with lots with homogeneous quality for fresh consumption and processing purposes. 2-chloroethyl phosphonic acid (Ethephon) is a water-soluble liquid organic acid that hydrolyzes at physiological pH (≥ 5) to form ethylene ($\text{CH}_2=\text{CH}_2$), phosphate (H_2PO_4^-) and chlorine ions (Cl^-), therefore, in postharvest fruit it constitutes an alternative to the use of ethylene gas to advance and homogenize ripening, which allows having fruit ready for fresh consumption or for processing purposes. The amount of ethylene released depends on the pH of the solution and the relative humidity of the environment; moreover, the response effectiveness varies according to the species, cultivar, production technology, dosage, application method and temperature^[8,9]. Therefore, the objective of the present study was to evaluate the effect of the application of two concentrations of 2-chloroethyl phosphonic acid on the timing and homogenization of fruit ripening of avocado cultivars cvs. Hass and Méndez.

2. Materials and methods

2.1 Plant material and treatments

Avocado fruits of Hass and Méndez cultivars were harvested with $23.4 \pm 0.5\%$ and $24.0 \pm 0.3\%$ dry matter (DM), respectively, developed in the production area of Salvador Escalante (Michoacán, Mexico). Prior to the establishment of the experiment, those fruits that showed external damage were eliminated and subsequently separated into three lots of 38 fruits each: one of them remained as a control, another was treated for 5 min by immersion with a solution of 500 mg/L of ethephon, and the third received 1,000 mg/L of the previous product for an equal time of 5 min. The two ethephon solutions were prepared with BAYER's Ethrel 240[®] compound at 21.70% by weight of 2-chloroethyl phosphonic acid, both solutions were adjusted to pH 5 with NaOH (10 N). The treated fruits were stored at 20 ± 2 °C and relative humidity of $60 \pm 5\%$ for

11 days to evaluate ripening based on physiological, biophysical and biochemical variables.

2.2 Respiratory intensity and ethylene production

They were determined daily by gas chromatography according to the headspace method^[10]; for this, a random sample of eight fruits was taken from each treatment, and four experimental units (replicates) of two fruits each were established, which were placed in hermetically sealed containers of 2.12 L capacity for 1 hour. A 1 ml sample of the headspace gas was taken and injected into a gas chromatograph (Hewlett Packard, model 5890 series II), with an open type column, porous silica gel layer packing that was simultaneously connected to a flame ionization detector (FID) and a thermal conductivity detector (TCD). The operating conditions were: column temperature 150 °C, FID 180 °C and TCD 180 °C; use 500 $\mu\text{L/L}$ of CO_2 standard and 20 $\mu\text{L/L}$ of ethylene, both from INFRA[®]. Respiration data were reported as mL/L per hour of CO_2 and ethylene concentration in $\mu\text{L/kg}$ per hour.

2.3 Weight loss and fruit firmness

Weight loss was determined by measuring every 2 days the weight of five fruits per treatment individually with an ALSEP EY-2200 digital balance, equation 1 was used to obtain the data. Firmness was determined with a texturometer (Wagner Force Five model FDV-30) with a conical tip of 7 mm diameter; measurements were taken every 2 days on a sample of five fruits per treatment. Values were reported in Newton (N).

$$\text{Weight loss (\%)} = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} * 100 \quad (1)$$

2.4 Color change in the epicarp

It was determined individually, in a sample of five fruits per treatment, using a HunterLab reflection colorimeter (Reston Virginia model D25-PC2) with a D65 illumination system and an observer angle of 2°. With the values obtained (L^* , a^* and b^*), lightness (L^*), saturation index or chroma (C^*), hue angle or hue (H^*), color change and color index (CI) were calculated according to Equations 2,

3, 4 and 5^[11-13].

$$C^* = \sqrt{a^2 + b^2} \quad (2)$$

$$H^* = \tan^{-1}\left(\frac{b}{a}\right) \quad (3)$$

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2} \quad (4)$$

$$IC = -10(ab)/L \quad (5)$$

2.5 Quantification of total phenols (TP)

TP extraction was performed from 1 g of pulp and the addition of 10 ml of a methanol-water solution (8:2 v/v), followed by stirring until a homogeneous macerate was achieved. Quantification was carried out according to the method proposed by Pío-León *et al.*^[14] with some modifications, among them, to an aliquot of 200 of extract was added, in the following order: 2.5 ml of distilled water, 100 µl of Folin Ciocalteu-water reagent (1:1 v/v) and 200 µl of Na₂CO₃-water (2:8 w/v); then they were kept in darkness for 30 min and finally, optical density (OD) readings were performed at 765 nm in a digital spectrophotometer (GENESYS 10V Thermo Electron Corporation), the results were reported as gallic acid content in mg/100 g of fresh pulp (mg AG 100/g pf).

2.6 Extraction and quantification of chlorophyll and total carotenoids

For the extraction of chlorophyll (a, b and total), 1 g of pulp was used to which 10 ml of acetone-water (8:2 v/v) was added, macerated and left to stand for 24 h in the dark at 4 °C. The determination was performed following the methodology of Pompelli *et al.*^[15], for which 3 ml of the extract was used and OD readings were taken at 645 and 663 nm, the results were reported as µg of chlorophyll a, b and total/g fresh pulp. The extraction of total carotenoids was done from the method proposed by Acacio-Chirino *et al.*^[16] with some modifications, to 10 g of pulp 20 ml of acetone was added, macerated, shaken and decanted. Acetone was added until the pigments were completely extracted. Then, 20 ml of petroleum ether and 20 ml of distilled water were added to the extract, mixed gently and left to stand

for 10 min, after which the lower layer was discarded and the process was repeated twice more. Once the acetone-free extract was obtained, 10 ml of sodium hydroxide-water (4:6 w/v) were added to saponify the sample. Finally, a wash was made with 10 ml of anhydrous Na₂SO₄-water (1:9 w/v) and the reading was made at 454 nm. The results were expressed as µg of β-carotene/g in fresh pulp. Chlorophyll and carotenoid extraction and quantification was performed after 11 days of storage at 20 °C.

2.7 Statistical analysis of data

The Kruskal-Wallis test was used as a non-parametric alternative to analysis of variance (ANOVA) to determine the statistically significant difference between the medians at a 95% confidence level. For chlorophyll and carotenoids, a completely randomized experimental design with a 2 × 3 factorial arrangement [cultivar Hass and Méndez) and ethephon doses (500 and 1,000 mg/L)] was used, and a Tukey mean comparison was performed with a 95% confidence level. Data were analyzed with the statistical package SAS software version 9.0^[17].

3. Results and discussion

In avocado fruits, the onset of the ripening process is related to changes in ethylene sensitivity in the preclimacteric phase, which means the occurrence of autocatalytic production of this phytohormone up to a physiological stimulus concentration that favors changes related to ripening^[1,18]; moreover, exogenous ethylene accelerates respiration and advances climacteric^[5,18,19]. In this sense, fruits presented the climacteric pattern of respiration during ripening (**Figure 1**). In cv. Hass, the respiratory climacteric resulted higher with a maximum of 122.93 mL/kg per hour of CO₂ after 8 days of storage at 20 °C in the treatment with 1,000 mg/L ethephon; in the treatment with 500 mg/L it resulted on day nine, and in the control, the maximum occurred at day 10 under the same conditions.

In cv. Méndez fruits, the treatment with 500 mg/L of ethephon stimulated the highest respiration rate with a maximum of 157.34 mL/kg per hour of

CO₂ at 10 days of storage, followed by the control and treatment with 1,000 mg/L, after 11 days at 20 °C (**Figure 1A, C**). In the case of ethylene, in cv. Hass, autocatalytic production occurred at 5 days of storage in fruits treated with 1000 mg/L and at 7 days in the other two treatments (**Figure 1B**). With respect to cv. Méndez, the treatments with 500 and 1,000 mg/L presented autocatalytic production at 7 days, while in the control it occurred at the 8 days at 20 °C (**Figure 1D**). Similarly, Blakey *et*

al.^[20] found in avocado cv. Hass treated with ethephon (50 mL/L) a significant increase in respiration and ethylene production after six hours of storage at 21 °C. Sañudo-Barajas *et al.*^[21] achieved in Maradol papaya fruits an earlier onset of maximum climacteric on day 3 after the application of 2.5 g/L of ethephon, compared to the control that occurred on day 7, without significant changes in respiration.

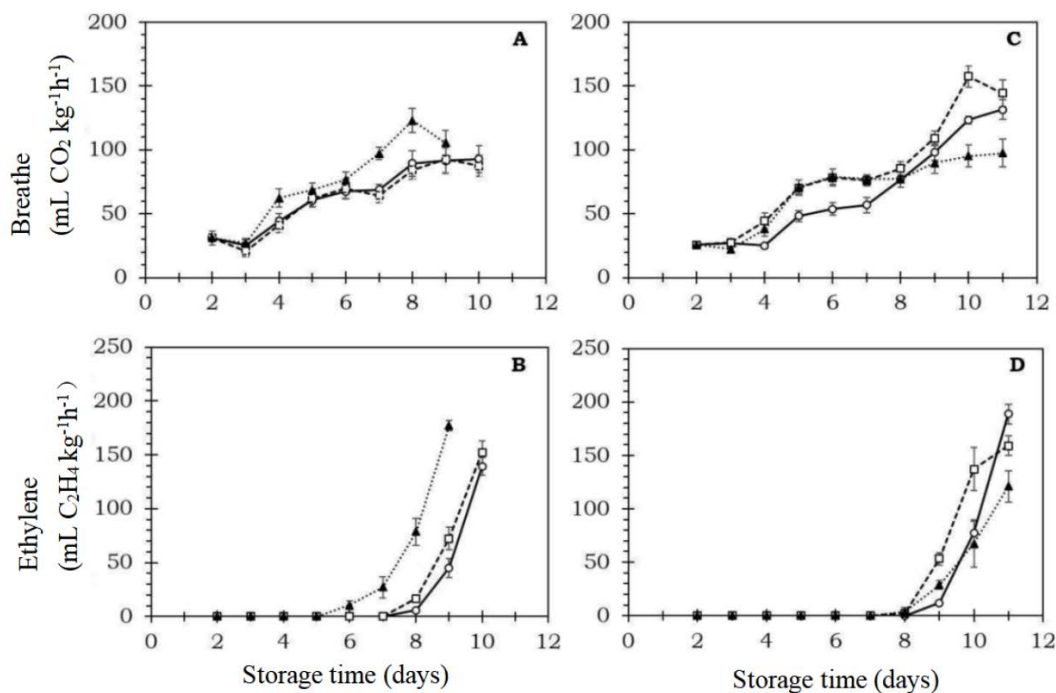


Figure 1. Respiratory intensity and ethylene production in avocado fruits cv. Hass (A, B) and cv. Méndez (C, D). Treated with: 0 (—○—), 500 (—□—) and 1000 (—▲—) mg/L of ethephon and stored at 20 ± 2 °C. Mean value ± SD, n = 4.

During ripening, weight losses increased ($P < 0.05$) as storage time progressed (**Figure 2, Table 1**). In fruits of cv. Hass the loss corresponded to 5.28%, 6.05% and 6.74% for the control, ethephon 500 mg/L and ethephon 1,000 mg/L, respectively, after 9 days of exposure at 20 °C (**Figure 2A**). Fruits of this cultivar treated with ethephon showed severe deterioration after 11 days of storage. In cv. Méndez, after the same storage period, weight losses were lower in the control (4.08%) compared to ethephon 500 mg/L (5.32%) and ethephon 1,000 mg/L (5.73%) (**Figure 2C**). Bower and Jackson^[22] and Bower and Papli^[23] found in avocado fruits that weight losses are one of the main factors of quality deterioration, mainly when they exceed 6%, a con-

dition that is maintained of cv. Méndez store for 9 days. Martin and Rose^[24] indicated that the content and composition of cuticular, epicuticular and intracuticular waxes, accumulated during fruit development strongly influence the sensitivity to water losses during postharvest handling, which means that, between both cultivars evaluated, there are differences in the accumulation of these compounds, with the Hass cv. being more sensitive (**Figure 2**).

On the other hand, in both cultivars, flesh firmness decreased ($P < 0.05$) as the ripening process progressed (**Figure 2, Table 1**).

Untreated fruit (control) of both cultivars showed a greater firmness than fruit treated with ethephon, suggesting a greater advance in ripening

of the latter (**Figure 2**). After 11 days of treatment, advanced deterioration due to senescence occurred in the fruits of cv. Hass (**Figure 2B**). In the case of cv. Méndez, the reduction in firmness after 11 days of storage was greater in fruit treated with 1,000 mg/L ethephon than in the control (**Figure 2D**). Firmness reduction in fruits decreases as a result of physiological processes characteristic of ripening.

According to Prasanna *et al.*^[25] and Pedreschi *et al.*^[26] this decrease occurs due to changes in the metabolism of cell wall components and an increase in the activity of enzymes such as pectylmethylesterase, polygalacturonase and pectate lyase. In tomato fruits treated with ethephon (500, 1,000 and 1,500 mg/L) a significant decrease in firmness was also recorded^[27].

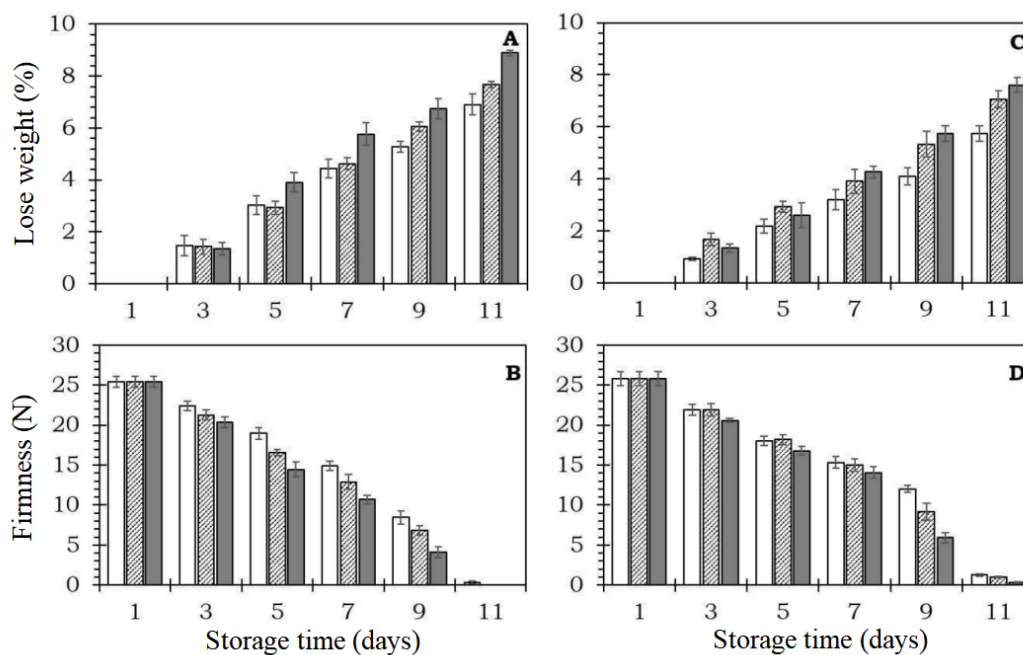


Figure 2. Firmness and weight loss behavior of avocado fruits cv. Hass (A, B) and cv. Méndez (C, D).

Treated with: 0 (□), 500 (▒) and 1,000 (■) mg/L of ethephon and stored at 20 ± 2 °C. Mean value \pm SD, n = 5.

Table 1. Results of the *p*-value of the main effects and interactions of ethephon dosage and storage time in the Kruskal-Wallis test on postharvest quality variables for Hass and Méndez avocado cultivars

Cultivate	Effect	Respiration (mL CO ₂ :kg/h)	Ethylene (μL C ₂ H ₄ , kg/h)	Weight loss (%)	Firmness (N)	Total phenols (mg, GA·100g)
Hass	Dosage	<0.0001	0.0897	<0.0001	<0.0001	<0.0001
	Storage day	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Interaction	0.8941	0.1354	0.5259	0.9455	0.7795
Méndez	Dosage	<0.0001	<0.0001	<0.0001	<0.0001	0.0021
	Day of storage	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Interaction	0.0489	<0.0001	0.1051	0.4933	0.5746

p-value in bold indicates significant effect ($p < 0.05$, test X^2).

The external color of avocado fruits changed significantly ($P < 0.05$) (**Table 2**). Changes in decreased lightness (L^*) and saturation index (C^*), as well as an increase in hue angle (H^*) were observed during the ripening process after 11 days at 20 °C (**Figure 3**). During the ripening stage, the change in color (ΔE) increased significantly ($P \leq 0.05$) with respect to the initial value (**Figure 4**); while the CI

decreased during ripening in both cultivars, being lower at 11 days ($P \leq 0.05$) (**Figure 5, Table 2**). Color is undoubtedly an important ripening indicator for the industrial sector and consumers; Henao-Rojas and Rodriguez mention that CI decreases during ripening and that significant decreases in CI correlate with an increased speed of the ripening process. Cox *et al.*^[28] found during ripening chang-

es in epicarp color in avocado fruits from green to dark violet as a result of cyanidin-3-glucoside accumulation.

Table 2. Results of the P-value of the main effects and interactions of ethephon dosage and storage time in the Kruskal-Wallis test, in external color for Hass and Méndez avocado cultivars

Cultivate	Effect	Photometry (L*)	Saturation index (C*)	Pitch angle (H*)	Color change (ΔE)	Color index (CI)
Hass	Dosage	0.0352	<0.0001	<0.0001	<0.0001	<0.0001
	Day of storage	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Interaction	0.8819	<0.0001	0.7845	0.7863	<0.0001
Méndez	Dosage	0.0010	<0.0001	<0.0001	<0.0001	<0.0001
	Storage day	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Interaction	0.9703	0.1307	0.1748	0.1190	0.0015

P-value in bold indicates significant effect ($P < 0.05$, test λ^2).

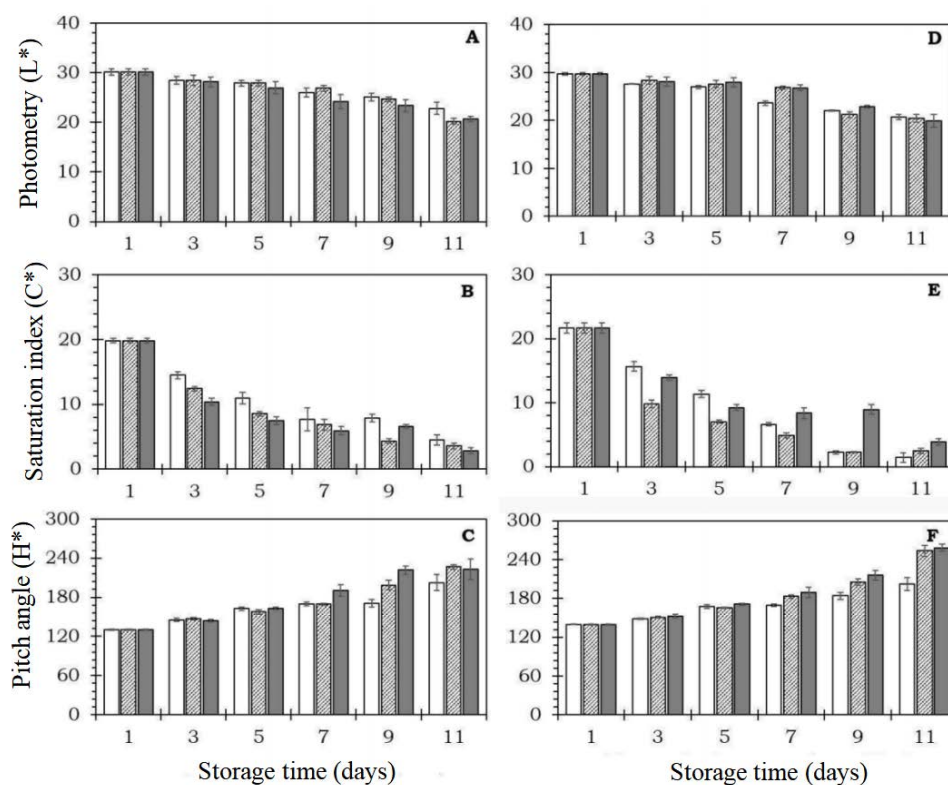


Figure 3. Behavior of color components in avocado fruits cv. Hass (A, B, C) and cv. Méndez (D, E, F). Treated with: 0 (□), 500 (▨) and 1,000 (■) mg/L of ethephon and stored at 20 ± 2 °C. Mean value \pm SD, n = 5.

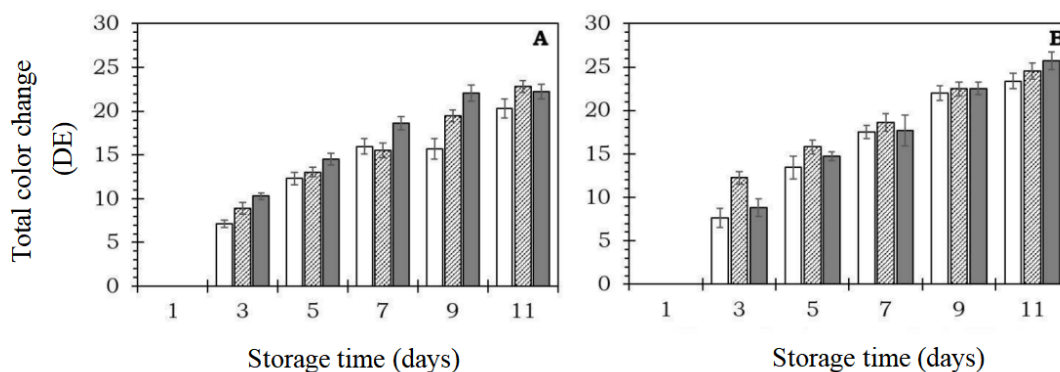


Figure 4. Color change in avocado fruits cv. Hass (A) and cv. Méndez (B). Treated with: 0 (□), 500 (▨) and 1,000 (■) mg/L of ethephon and stored at 20 ± 2 °C. Mean value \pm SD, n = 5.

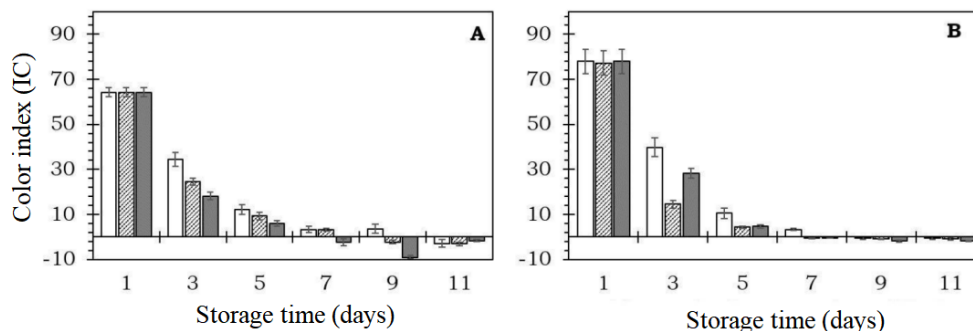


Figure 5. Color index in avocado fruits cv. Hass (A, B, C) and cv. Méndez (D, E, F).

Treated with: 0 (□), 500 (▨) and 1,000 (■) mg/L of ethephon and stored.

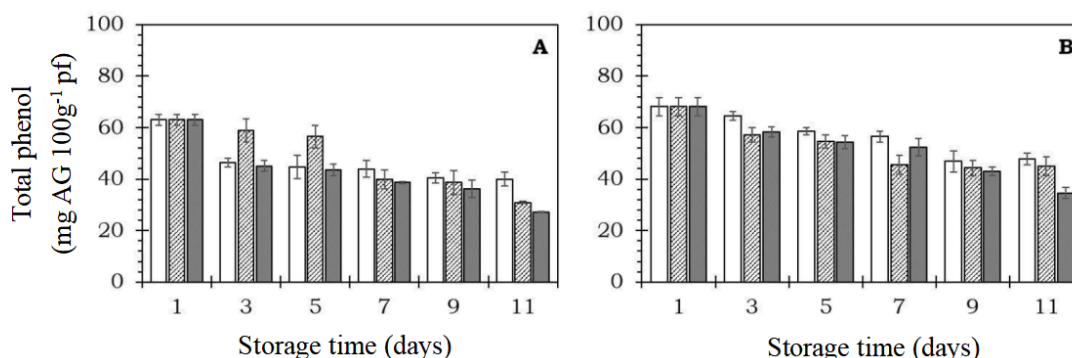


Figure 6. Change in total phenol content in avocado fruits cv. Hass (A) and cv. Méndez (B).

Treated with: 0 (□), 500 (▨) and 1,000 (■) mg/L of ethephon and stored at 20 ± 2 °C. Mean value \pm SD, n = 5.

The concentration of total phenols in the fruits of both cultivars determined at harvest resulted in 60 mg/100 g pf GA and around 30–40 mg/100 g pf GA at the stage of consumption maturity. For the treatment with 1000 mg/L ethephon, in both cultivars the TP concentration decreased ($P \leq 0.05$) during the ripening process at 20 °C (**Figure 6**, **Table 1**). On the other hand, in the fruits of cv. Hass, after 11 days of storage, the TP content decreased 56.89% in those treated with 1,000 mg/L, 51.03% in those treated with 500 mg/l and, in the control, the loss of TP resulted in 36.57%. On the other hand, the TP content in the pulp of avocado cv.

Méndez fruits decreased by 49.12% in those treated with 1,000 mg/L, 33.89% in those treated with 500 mg/L, while in the control fruits the loss of these metabolites was 29.85%. Villa-Rodriguez *et al.*^[29] reported a TP content of 10 mg/100 g pf FA at physiological maturity and 35 mg/100 g pf FA at consumption maturity. According to Villa-Rodriguez *et al.*^[30] and Sousa *et al.*^[31] the content of phenolic compounds is significantly affected by climate, cultivar, production technique, ripening and storage conditions, among others, therefore the phenol content found in this study can be partially explained by these factors.

Table 3. The contents of chlorophyll and carotenoid in the pulp of Hass and Méndez avocado cultivars at the consumption maturity stage

Cultivate	Dose ethephon (mg/L)	Chlorophyll a (µg/g).	Chlorophyll b (µg/g).	Total chlorophyll (µg/g).	Total carotenoids (µg/g).
Hass	0	40.27 \pm 16.68 ab	16.62 \pm 3.04 ab	56.85 \pm 19.41 ab	17.60 \pm 1.61 d
	500	22.16 \pm 0.69 bc	7.59 \pm 0.55 c	29.76 \pm 0.98 c	21.28 \pm 1.12 cd
	1000	9.02 \pm 1.07 c	4.07 \pm 1.93 c	13.08 \pm 1.61 c	22.56 \pm 0.85 bc
Méndez	0	48.27 \pm 8.35 a	19.13 \pm 1.14 a	67.40 \pm 9.42 a	26.56 \pm 2.05 ab
	500	23.38 \pm 0.52 bc	12.51 \pm 1.16 b	35.90 \pm 1.33 bc	28.21 \pm 0.51 a
	1000	9.00 \pm 1.17 c	7.28 \pm 0.91 c	20.22 \pm 1.58 c	23.52 \pm 2.39 bc

Mean value \pm SD, n = 3. Values with different letters within the same column are statistically different at a significance level of $\alpha = 0.05$.

Chlorophyll contents showed differences ($P \leq 0.05$) due to the effect of cultivar and ethephon dose (**Table 3**). In both cultivars, the highest total chlorophyll content occurred in the treatment at the stage of consumption maturity. Similarly, the concentration of total carotenoids varied ($P \leq 0.05$) by cultivar effect and the dose of ethephon applied to induce ripening; however, the pulp of the fruits of cv. Méndez presented higher total carotenoid content compared to cv. Hass. With the 500 mg/L treatment in cv. Méndez, the highest retention of this pigment occurred at the consumption maturity stage ($28.21 \pm 0.51 \mu\text{g/g}$). Likewise, it was observed that the dose of ethephon favored the presence of carotenoids (Table 3). Associated with the changes in flesh color from green-yellow to yellow shade during ripening, the lower chlorophyll content (a, b and total) and higher concentration of carotenoids, results significantly important in terms of flesh shade for fresh consumption and processing purposes^[32]. Cox *et al.*^[28] and Ashton *et al.*^[33] found a reduction in chlorophyll concentration in avocado cv. Hass fruits due to the effect of the ripening process, as occurred in the present study (**Table 3**). Lu *et al.*^[34] found differences in carotenoid contents in avocado cv. Hass pulp at consumption maturity stage by effect of development conditions and harvest time, with variations from 5 $\mu\text{g/g}$ to 40 $\mu\text{g/g}$ in total carotenoids. The values found in the present work vary between 17 and 28 $\mu\text{g/g}$, which allows assuming that cultivar, dry matter content at harvest, and postharvest handling also influence the content of these pigments. With respect to these pigments, the treatment with 500 mg/L favored a greater accumulation that is important for the benefits they confer to human health, for their antioxidant capacity and the conservation of avocado pulp functionality.

4. Conclusions

Treatment with the ethylene releasing agent ethephon at a dose of 1,000 mg/L in avocado fruit cv. Hass and 500 mg/L in avocado fruit cv. Méndez, stored at $20 \pm 2 \text{ }^\circ\text{C}$, brings forward the onset of autocatalytic production and, therefore, reduces the ripening process by 2 days compared to untreated

fruit, maintaining the firmness and color of the epicarp in an acceptable and homogeneous manner. The treatment of avocado fruit with ethephon, as opposed to treatment with ethylene gas, is more economical as it does not require complex installations, which allows its application for the ripening of low volumes of product, both for growers and marketers. It also leads to homogenization in the ripening of avocado fruits and to the acceleration of losses of chlorophyll content and increased carotenoids in the pulp, which makes it possible to offer a product with uniform quality in terms of organoleptic and functional characteristics, achieving greater acceptance by consumers.

Conflict of interest

The authors declared no conflict of interest.

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