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Firmness and antioxidant compound changes in avocado (*Persea americana* Mill.) pulp during ripening at different storage temperatures

Nallely Rosas-Flores[1]; Saucedo-Veloz, Crescenciano[2]; López-Jiménez, Alfredo[2*]; Saucedo-Reyes, Daniela[2]; Valle-Guadarrama, Salvador[3]; Loera-Alvarado, Gerardo[4]

[1] Tecnológico de Estudios Superiores de Chicoloapan. División de Ingeniería en Desarrollo Comunitario. Camino al Monte s/n col. Loma de Guadalupe, Chicoloapan de Juárez, Estado de México, México. C.P. 56380.

[2] Colegio de Postgraduados. Campus Montecillo. Recursos Genéticos y Productividad-Fruticultura. Carretera México-Texcoco km 36.5, Estado de México, México. C.P. 56264.

[3] Universidad Autónoma Chapingo. Ingeniería Agroindustrial. Carretera México-Texcoco km 38.5, Chapingo, Texcoco, Estado de México, México. C.P. 56264.

[4] Colegio de Postgraduados, Campus San Luis Potosí. Iturbide 73, Salinas de Hidalgo., San Luis Potosí, México. C.P. 78600.

* Correspondence: lopezja@colpos.mx

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ABSTRACT

Objective: To evaluate changes in firmness, total phenol content, chlorophylls, carotenoid profile and antioxidant capacity in the pulp of two avocado cultivars and one genotype at different storage temperatures.

Design/methodology/approach: Avocado fruits were divided into two groups. One group of avocado fruit was stored at 20 °C for eight days, while the second group was stored at 2 °C for 21 days, followed by two additional days at 20 °C to allow ripening. Firmness, total phenols, antioxidant capacity, pigments, and carotenoid profile were determined in the pulp after the storage period.

Results: Avocado fruit on cold storage continued the normal ripening process; however, quality deterioration occurred more rapidly than in fruit stored at 20 °C.

Findings/conclusions: During cold storage, flesh firmness of the cultivars (Colín V33 and Hass) and genotype (Oaxaca-7) was not affected. However, once they were transferred to ripening temperature (20 °C), their shelf life was reduced to two days, leading to a rapid deterioration of pulp quality. This deterioration occurred because the contents of phenols, chlorophylls, and carotenoids decreased significantly, thereby affecting the antioxidant activity of the avocado fruits under study, as well as the nutraceutical value provided by chlorophylls and carotenoids, specifically lutein.

Keywords: *Persea americana* Mill., carotenoids, antioxidant capacity, firmness, total phenol content.

INTRODUCTION

Chilling injury in avocado fruits is one of the main problems associated with storage under refrigeration conditions. It is characterized by epicarp spotting, pulp browning, and abnormalities in the ripening process when fruits are exposed to critical temperatures that

induce these physiological alterations (Wang *et al*., 2012). The response to refrigeration temperatures may differ among cultivars and genotypes due to antioxidant capacity, which is determined by the content of phenolic compounds and carotenoids (Bertiling *et al*., 2007; Lu *et al*., 2009).

Phenolic compounds are produced during the secondary metabolism of plants and are associated with the color, flavor, and antioxidant capacity of horticultural products (Sultana and Anwar, 2008). They occur in a wide range of structures, either as free phenolic acids or bound to other molecules such as sugars, or they may be polymerized into more complex structures, including flavonoids, anthocyanins, tannins, and lignins. Furthermore, due to their antioxidant capacity and their relationship with human health, phenolic compounds are considered nutraceutical compounds (Naczk and Shahidi, 2006). Carotenoids are natural pigments that perform essential functions and provide important nutritional benefits to human health, especially α- and β-carotenes and β-cryptoxanthin, which are precursors of vitamin A, retinol, and retinoic acid (Fraser and Bramley, 2004; Krinsky *et al*., 1994). In plants, carotenoids regulate photosynthesis and prevent photooxidative damage by scavenging free radicals and quenching harmful triplet chlorophyll molecules, thereby inhibiting the generation of singlet oxygen, which limits damage to membranes and proteins (DellaPenna and Pogson, 2006). The presence of carotenoids in fruits provides protection against adverse environmental conditions caused by temperature (high or low), by conferring stability to membrane lipids. In addition, their antioxidant capacity exerts a protective effect on fruit tissues against different types of stress (Lado *et al*., 2016a). In various fruits, during the ripening process, the content and composition of carotenoids in the pulp and peel are modified, depending on the species, cultivar, and postharvest handling conditions (Alquézar *et al*., 2008; Lado *et al*., 2016b).

In postharvest conditions, during cold storage, avocado fruit may exhibit sensitivity to chilling injury due to its tropical and subtropical origin. Such damage is mainly manifested by different types of pulp discoloration, including internal browning and darkening of vascular bundles (Whiley *et al*., 2002). However, susceptibility to chilling injury depends on the cultivar, tissue type, structural components, bioactive compound content, production technology, and postharvest handling, among other factors (Wang *et al*., 2010). In avocado pulp, the carotenoids present in the highest proportion include lutein, neoxanthin, and β-carotene, which contribute to the protective system by scavenging reactive oxygen species (ROS) generated as a secondary response to chilling injury (Lado *et al*., 2016a). The objective of this research was to determine changes in firmness, total phenolic content, chlorophylls, carotenoid profile, and antioxidant capacity in the pulp of two avocado cultivars and one genotype stored at different temperatures.

MATERIALS AND METHODS

Plant material

Fruits from two avocado cultivars (*Persea americana* Mill.), Hass ($35.24 \pm 0.62\%$ dry matter) and Colín V-33 ($34.10 \pm 0.46\%$ dry matter), as well as from the genotype identified as Oaxaca-7 ($26.89 \pm 0.44\%$ dry matter), were harvested at the “La Cruz” Experimental Center, belonging to the Salvador Sánchez Colín Foundation (CICTAMEX, S. C.), located

in the municipality of Coatepec Harinas, State of Mexico, Mexico, between 18° 48’ and 19° 05’ N latitude and 99° 43’ and 99° 54’ W longitude, at an altitude of 2,260 m.

Treatments

One group of fruits was stored at 20±2 °C to allow ripening, while another group was kept under cold storage (2±1 °C) for 21 days and subsequently transferred to 20±2 °C. Fruits directly exposed to ripening conditions were analyzed one day after harvest and after eight days of storage. Fruits stored under refrigeration temperature were analyzed at the end of the cold storage period and after two days of exposure to 20±2 °C.

Response variables

Pulp firmness, total phenolic content, total chlorophyll and carotenoid content, antioxidant capacity, and carotenoid profile were evaluated. Pulp firmness (N) was measured using a Chatillon texture analyzer equipped with a Wagner Force Five sensor model FDV-30, and a 7-mm conical probe. For this purpose, the peel was removed from the equatorial region of the fruit, and the force required to penetrate the pulp was determined.

For the quantification of total phenols, extraction was performed using 500 mg of freeze-dried pulp, to which 10 mL of a methanol-water solution (8:2, v/v) was added. Each sample was then allowed to stand for 24 h at 4 °C. Total phenolic content was determined according to the method proposed by Pío-León *et al.* (2012), with some modifications. Briefly, to a 200-μL aliquot of each extract, the following reagents were added in order: 2.5 mL of distilled water, 100 μL of Folin-Ciocalteu reagent diluted with water (1:1, v/v), and 200 μL of Na_2CO_3 solution in water (2:8, w/v). Subsequently, samples were incubated in the dark for 30 min, and absorbance was measured at 765 nm using a digital spectrophotometer (GENESYS 10V, Thermo Electron Corporation). Results were obtained using a calibration curve and expressed as mg of gallic acid per 100 g of dry weight (mg GA 100 g^{-1} DW).

For pigment extraction (chlorophylls and total carotenoids), the methodology proposed by Rodrigo *et al.* (2004) was followed with some modifications. Briefly, 600-700 mg of freeze-dried pulp was mixed with 3.0 mL of Milli-Q water and allowed to stand for 10 min. Subsequently, 4.0 mL of methanol and 8.0 mL of dichloromethane were added. The mixture was vortexed for a few seconds and then placed in an ultrasonic bath for 5 min at room temperature. Afterwards, samples were centrifuged at 4,500 rpm for 10 min at 4 °C to separate the liquid and solid phases. The lower phase (organic phase) was recovered, and the addition of dichloromethane was repeated until complete discoloration was achieved. Finally, dichloromethane was evaporated using a rotary evaporator (water bath at 30 °C), obtaining the chlorophyll and carotenoid concentrate (dry sample).

Chlorophyll content (a, b, and total) was quantified according to the method proposed by Rodrigo *et al.* (2004). For this purpose, an aliquot of the dried extract was dissolved in 0.5 mL of dichloromethane, 300 μL of acetone, and 4.2 mL of a petroleum ether-ethyl ether solution in a 9:1 (v/v) ratio (solution A). The mixture was then vortexed and brought to a known final volume. Absorbance measurements were subsequently performed using a spectrophotometer (Thermo Scientific Multiskan Spectrum) at 644, 662, and 760 nm,

respectively. Results were expressed as μg g^{-1} dry weight (μg g^{-1} DW). Subsequently, the extract was concentrated using a rotary evaporator at 30 °C to determine total carotenoid content, following the method proposed by Rodrigo *et al.* (2003) with some modifications. Briefly, 1.0 mL of dichloromethane and 5.2 mL of methanol were added to the dried sample, and the volume was adjusted to 8.0 mL. Then, 1.0 mL of potassium hydroxide solution (6:4, w/v) was added, the mixture was vigorously shaken, and allowed to stand for 12 h to promote the saponification reaction. Afterwards, 2.0 mL of Milli-Q water and 6.0 mL of solution A were added. The mixture was vortexed vigorously and left to stand. The upper phase was recovered, and the procedure was repeated until the phase became colorless. Finally, the extract was brought to a known final volume with solution A, and absorbance was measured using a spectrophotometer (Thermo Scientific Multiskan Spectrum) at 450 and 540 nm, using an extinction coefficient for β-carotene of E1%=2500. Data were expressed as μg β-carotene g^{-1} dry weight (μg β-carotene g^{-1} DW).

For carotenoid profiling, samples were dried under a nitrogen stream, 15 mL of acetone was added, and they were stored at −80 °C for 24 h. Subsequently, samples were centrifuged at 4,500 rpm for 10 min at 4 °C, and the supernatant was recovered. The extract was then evaporated to dryness under an N atmosphere and stored at −20 °C until analysis.

Individual carotenoid composition was analyzed by high-performance liquid chromatography (HPLC) using a Waters liquid chromatography system equipped with a 600E pump, coupled to a photodiode array detector (model 2998) and Empower software (Waters).

A C30 carotenoid column (250×4.6 mm, 5 μm) coupled to a C30 guard column (20×4.0 mm, 5 μm) was used (YMC Europe GmbH). Samples were dissolved in a chloroform:methanol:acetone mixture (3:2:1, v/v/v), and carotenoid separation was performed using an elution gradient composed of methanol (MeOH), water, and methyl tert-butyl ether (MTBE). A chromatogram was obtained for each run, in which carotenoid peaks were identified by their maximum wavelength, and their concentrations were calculated using calibration curves for violaxanthin, neoxanthin, luteoxanthin, lutein, antheraxanthin, β-cryptoxanthin, and α- and β-carotene. Three replicates were performed for each analytical determination. The entire procedure was carried out under dim light conditions to prevent pigment degradation. Concentrations were expressed as μg g^{-1} dry weight (μg g^{-1} DW).

Total antioxidant capacity was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (Ferric Reducing Antioxidant Power) methods. For both assays, an extract was prepared from 50 mg of freeze-dried pulp by adding 4 mL of a methanol-water solution (8:2, v/v). The mixture was then centrifuged at 4,500 rpm for 15 min at 4 °C. Subsequently, the supernatant was collected in a graduated tube to quantify the volume and stored at −20 °C until analysis.

For quantification using the DPPH method, the procedure proposed by Brand-Williams *et al.* (1995) was followed with some modifications. Briefly, 3.9 mg of DPPH was dissolved in 100 mL of methanol-water (8:2, v/v). In a polyurethane microplate, 10 μL of the sample extract and 290 μL of the DPPH solution were added. After loading the plate, samples were incubated for 30 min in the dark at room temperature. Absorbance was measured at

512 nm using 300 μL of methanol-water (8:2, v/v) as the blank. A calibration curve was prepared using different concentrations of ascorbic acid, and results were expressed as μg of ascorbic acid per g of pulp on a dry weight basis (μg AAs g^{-1} DW).

The FRAP assay was based on the methodology developed by Benzie and Strain (1996), with modifications. A 10 mM TPTZ solution (2,4,6-tri(2-pyridyl)-1,3,5-triazine) was prepared according to the following procedure: a 40 mM HCl solution was prepared (1.46 mL of HCl in 1.0 L of water at room temperature), and 0.31 g of TPTZ was dissolved in 100 mL of this solution, heating in a water bath at 50 °C. On the other hand, a 300 mM acetate buffer solution at pH 3.6 was prepared by mixing 3.1 g of sodium acetate and 16 mL of glacial acetic acid in 100 mL of water. In addition, a 20 mM ferric chloride solution was prepared by dissolving 0.135 g in 25 mL of water.

The above solutions were mixed in a 1:10:1 (v/v/v) ratio to form the FRAP reagent, which was placed in a water bath at 37 °C for 10 min. Afterwards, the microplate was loaded by adding 40 μL of the sample extract and 260 μL of the FRAP reagent. Once the plate was prepared, it was incubated for 30 min in the dark at 37 °C. Absorbance was measured at 570 nm, using methanol-water (80:20, v/v) as the blank. Results were obtained from a calibration curve and expressed as μg of ascorbic acid per g of pulp on a dry weight basis (μg AAs g^{-1} DW).

Data analysis

Data were analyzed using analysis of variance (ANOVA) under a completely randomized design, and mean comparisons were performed using Tukey's test (α=0.05). The analysis was conducted using the statistical package SAS System for Windows version 9.0. Each fruit was considered an experimental unit, and three replicates were performed for all variables.

RESULTS AND DISCUSSION

After eight days of storage at 20±2 °C, a significant difference ($p<0.05$) was found in the pulp firmness of the avocado fruits studied compared with the initial condition (Table 1), with a firmness loss greater than 93% in the three evaluated materials. Likewise, a significant difference ($p<0.05$) was observed in the firmness of fruits from the Hass and Colín V33 cultivars stored under refrigeration conditions, showing a decrease of approximately 30% after removal from storage. However, after two days under ripening conditions, a firmness loss greater than 94% was observed in both cultivars. In contrast, firmness in the Oaxaca-7 genotype showed a reduction of 93.5% at the end of cold storage and 95.9% after two days of ripening (Table 2).

The loss of firmness reflected the typical softening of avocado pulp associated with the progression of fruit ripening. This process is related to modifications in the chemical structure of the polymers that make up the cell wall, mainly pectic substances and cellulose, which are degraded due to increased activity of the enzymes pectin methylesterase, polygalacturonase, and cellulases (Arévalo-Galarza *et al.*, 2002; Blakey *et al.*, 2014). The pattern of firmness loss during ripening suggests that this process in fruits exposed to refrigeration conditions proceeded normally with respect to this variable.

Table 1. Changes in firmness, total phenolic content, and antioxidant capacity (DPPH and FRAP) in the pulp of avocado fruits (*Persea americana* Mill.) stored at 20±1 °C for eight days.

Variable	Cultivar	0 days	8 days
Firmness (N)	Hass	29.12±2.07[a]	0.79±0.17[b]
	Colín V-33	23.85±2.66[a]	0.29±0.08[b]
	Oaxaca-7*	16.54±0.53[a]	1.00±0.28[b]
Total phenolic content (GA mg g^{-1} DW)	Hass	1040.72±125.15[b]	3313.33±171.59[a]
	Colín V-33	1238.86±58.87[a]	1098.73±63.08[b]
	Oaxaca-7	1099.08±61.82[b]	1331.44±126.35[a]
DPPH antioxidant capacity (AA μg g^{-1} DW)	Hass	1350.71±19.18[b]	2736.26±72.68[a]
	Colín V-33	694.39±70.35[a]	753.58±16.2[a]
	Oaxaca-7	1341.24±80.59[b]	1798.30±83.04[a]
Ferric Reducing Antioxidant Power (μg AAs g^{-1} DW)	Hass	375.25±6.22[b]	656.61±31.13[a]
	Colín V-33	698.61±19.98[a]	498.91±11.47[b]
	Oaxaca-7	876.97±12.47[a]	813.97±21.36[b]

*Genotype. DW: dry weight; DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: Ferric Reducing Antioxidant Power; AA: ascorbic acid. Means followed by the same letter within each row are not significantly different (α=0.05). Values are presented as mean±SD, n=3.

Table 2. Changes in firmness, total phenolic content, and antioxidant capacity (DPPH and FRAP) in the pulp of avocado fruits (*Persea americana* Mill.) stored at 2±1 °C for 21 days, followed by two days at 20±2 °C.

Variable	Cultivar	Storage/ripening time		
		0 days	21 days	23 days
Firmness (N)	Hass	29.12±2.07[a]	19.68±1.21[b]	1.65±0.49[c]
	Colín V-33	23.85±2.67[a]	16.85±1.99[b]	0.98±0.16[c]
	Oaxaca-7*	16.54±0.53[a]	1.06±0.51[b]	0.67±0.12[b]
Total phenolic content (GA mg g^{-1} DW)	Hass	1040.72±125.16[c]	1384.40±149.91[b]	1739.65±24.40[a]
	Colín V-33	1238.86±58.87[b]	775.66±92.99[c]	1937.43±23.84[a]
	Oaxaca-7	1099.08±61.83[a]	1074.92±49.10[a]	806.25±67.92[b]
DPPH antioxidant capacity (AA μg g^{-1} DW)	Hass	1267.00±149.82[c]	3423.40±203.92[a]	1900.20±171.92[b]
	Colín V-33	694.39±70.36[b]	981.94±74.05[a]	1066.10±20.73[a]
	Oaxaca-7	1560.40±322.74[a]	1251.20±203.78[a]	614.70±22.41[b]
Ferric Reducing Antioxidant Power (μg AAs g^{-1} DW)	Hass	656.62±31.14[c]	1776.46±59.23[a]	990.10±115.07[b]
	Colín V-33	698.61±19.98[a]	592.65±14.27[b]	665.92±4.88[a]
	Oaxaca-7	876.80±12.48[a]	687.52±23.18[b]	375.25±6.22[c]

*Genotype. DW: dry weight; DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: Ferric Reducing Antioxidant Power; AAs: ascorbic acid. Means followed by the same letter within each row are not significantly different (α=0.05). Values are presented as mean±SD, n=3.

A significant difference ($p<0.05$) was found in the content of phenolic compounds in fruits from the three evaluated materials between the initial condition and after eight days of storage. In the Hass cultivar, total phenolic content tripled, reaching a final concentration of 3313.33 mg GA g^{-1} DW, whereas in the Oaxaca-7 genotype, an increase of approximately 20% was observed, with a final content of 1331.44 mg GA g^{-1} DW. In

contrast, the Colín V33 cultivar showed an 11% reduction, ending with 1098.73 mg GA g^{-1} DW (Table 1).

In the case of fruits stored under refrigeration conditions (21 days at 2±1 °C), a significant difference ($p<0.05$) was found in the content of phenolic compounds compared with the initial condition. In the Hass cultivar, an increase of 33% was observed at the end of cold storage, followed by a further 67% increase during ripening (two days at 20±2 °C), reaching a final concentration of 1739.65 mg GA g^{-1} DW. In the Colín V33 cultivar, a 37% decrease was observed at the end of storage, followed by a subsequent 56% increase during ripening, with a final concentration of 1937.43 mg GA g^{-1} DW. Meanwhile, phenolic compound content in the Oaxaca-7 genotype remained unchanged during refrigerated storage; however, after ripening, a 26% reduction was observed, resulting in a final content of 806.25 mg GA g^{-1} DW (Table 2). Phenolic compounds are responsible for the highest antioxidant activity in horticultural products (Heim *et al.*, 2002). According to the results obtained using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method in fruits directly exposed to ripening temperature, a significant increase ($p<0.05$) was found only in the Hass cultivar and the Oaxaca-7 genotype, where an increase of slightly more than twofold and 34%, respectively, was observed. In contrast, no significant change was detected in the Colín V33 cultivar (Table 1).

On the other hand, in fruits stored under refrigeration conditions (21 days at 2.0±1.0 °C), a significant difference ($p<0.05$) was observed both at the end of storage and after ripening compared with the initial condition. In the Hass and Colín V33 cultivars, increases of 170% and 40% were observed at the end of cold storage, respectively; however, during ripening, the increase was close to 50% in both cultivars. Meanwhile, in the Oaxaca-7 genotype, a decrease of 20% was found at the end of storage and of 60% during ripening compared with the initial condition (Table 2).

For the Ferric Reducing Antioxidant Power (FRAP) assay, a significant difference ($p<0.05$) was found in fruits directly exposed to ripening conditions compared with the initial condition. In the Hass cultivar, an increase of approximately 75% was observed, whereas in the Colín V33 cultivar and the Oaxaca-7 genotype, reductions of 28.58% and 7.18%, respectively, were detected.

In fruits stored under refrigeration conditions (21 days at 2±1 °C) and subsequently ripened (two days at 20±2 °C), a significant difference ($p<0.05$) was found in FRAP values. Notably, the Hass cultivar showed an increase of 170% at the end of cold storage and a further 50% increase during ripening. In contrast, the Oaxaca-7 genotype exhibited reductions of 21.6% and 57% in FRAP at the end of storage and during ripening, respectively. In the case of the Colín V33 cultivar, a 15% reduction in FRAP was observed at the end of refrigeration storage and remained unchanged during ripening (Table 2). An increase in antioxidant capacity has been reported by Daiuto *et al.* (2011), who stated that antioxidant capacity measured by the DPPH method increased in 'Fuerte' avocado fruits during the ripening process. In this regard, the results obtained in the present study suggest that the differences observed in antioxidant capacity among the fruits evaluated are related to variations in their

phenolic compound profiles. A similar response, in terms of a significant increase in antioxidant capacity, was observed in fruits of the Hass cultivar when measured using the FRAP method.

The results obtained in this study are consistent with those reported by Tomás-Barberán and Espín (2001) regarding the effect of cultivar and/or genetic material on the antioxidant activity of avocado fruits. In addition, no clear relationship was observed between total phenolic content and antioxidant capacity in the cultivars and genotype evaluated. This response agrees with the findings of Daiuto *et al.* (2011), who reported no significant correlation between total phenolic content and antioxidant capacity in 'Fuerte' avocado, suggesting that variations in the content and profile of phenolic compounds present in each genetic material may influence antioxidant behavior.

It is important to note that the higher antioxidant capacity values obtained using the DPPH method, compared with the FRAP method, support the hypothesis that this may be due to the presence of lipophilic compounds, as well as other constituents such as α-tocopherol, carotenoids, chlorophylls, and flavonoids in avocado extracts, which contribute to a greater stabilization of the DPPH free radical (Moreno *et al.*, 2014).

In fruits directly exposed to ripening conditions, a significant difference ($p<0.05$) was observed in pigment content compared with the initial condition. In the 'Hass' cultivar, a decrease of 43, 50, 29, and 53% was recorded in total chlorophyll, chlorophyll *a*, chlorophyll *b*, and total carotenoids, respectively.

A similar pattern was found in the Colín V33 cultivar, with a 37% reduction in total chlorophyll and a 54% decrease in chlorophyll a, whereas chlorophyll b and total carotenoids remained unchanged, showing no significant differences.

Table 3. Changes in chlorophyll content (total, *a*, and *b*) and total carotenoids in the pulp of avocado fruits (*Persea americana* Mill.) stored at 20±1 °C for eight days.

Variable	**Cultivar**	**Rippening time**	
		0 days	**8 days**
Total clorophyll (μg g^{-1} DW)	Hass	103.48 ± 2.50^{a}	58.55 ± 6.30^{b}
	Colín V-33	41.48 ± 2.53^{a}	25.87 ± 0.97^{b}
	Oaxaca-7*	184.02 ± 50.39^{a}	116.77 ± 30.66^{a}
Chlorophyll a (μg g^{-1} DW)	Hass	70.75 ± 1.56^{a}	35.32 ± 2.43^{b}
	Colín V-33	33.95 ± 2.28^{a}	15.64 ± 0.36^{b}
	Oaxaca-7	119.25 ± 26.49^{a}	69.54 ± 13.09^{b}
Chlorophyll b (μg g^{-1} DW)	Hass	32.72 ± 3.66^{a}	23.23 ± 3.87^{b}
	Colín V33	7.53 ± 1.92^{a}	10.22 ± 1.99^{a}
	Oaxaca-7	64.77 ± 23.91^{a}	47.22 ± 17.65^{a}
Total Carotenoid Content (β-carotene μg g^{-1} DW)	Hass	20.41 ± 2.37^{a}	9.63 ± 1.66^{b}
	Colín V-33	16.88 ± 1.39^{a}	14.17 ± 1.37^{a}
	Oaxaca-7	34.70 ± 5.3^{a}	16.09 ± 2.07^{b}

*Genotype. DW: dry weight (g). Means followed by the same letter within each row are not significantly different (α=0.05). Values are expressed as mean±SD, n=3.

In contrast, in the Oaxaca-7 genotype, only chlorophyll a showed a loss of 40%, while total chlorophyll and chlorophyll *b* remained without significant changes. Additionally, a 54% increase in total carotenoids was observed (Table 3).

In fruits stored under refrigerated conditions, significant differences ($p<0.05$) were observed in certain cultivars and pigments, either immediately after cold storage or during ripening. In the cultivar *Hass*, at the end of refrigeration, decreases of 15%, 28%, and 48% were recorded in total chlorophyll, chlorophyll *a*, and total carotenoids, respectively, whereas chlorophyll *b* remained constant. However, during ripening, total chlorophyll as well as chlorophyll *a* and *b* declined by approximately 44%, while total carotenoid content showed no significant changes.

In the cultivar Colín V33, a similar behavior was observed at the end of storage; however, the reductions in total chlorophyll, chlorophyll *a*, and total carotenoids were 56%, 74%, and 37%, respectively. During ripening, chlorophyll degradation continued, reaching reductions of up to 75% in total chlorophyll, 81% in chlorophyll a, and 46% in chlorophyll *b*, whereas total carotenoids remained unchanged. In the case of the genotype Oaxaca-7, only a 38% loss of chlorophyll *a* and a 55% reduction in total carotenoid content were observed up to the ripening stage. Total chlorophyll and chlorophyll *b* remained unchanged (Table 4).

Pigment content is determined by the plant material and the stage of maturity (Soong and Barlow, 2004), and the metabolism of these compounds directly affects their concentration. In the case of the cultivar *Hass*, Wang *et al.* (2012) reported an increase in phenolic compounds after cold storage for 21 and 35 days, demonstrating that in this cultivar exposure to refrigeration temperatures promotes the accumulation of total phenols. However, according to Di Stefano *et al.* (2017), during avocado fruit ripening

Table 4. Changes in chlorophyll content (total, *a* and *b*) and total carotenoids in the pulp of avocado fruits (*Persea americana* Mill.) stored at 2±1 °C for 21 days, followed by two days at 20±1 °C.

Variable	Cultivar	Storage time + ripening time		
		0 days	**21 days**	**23 days**
Total clorophyll (μg g^{-1} DW)	Hass	103.48 ± 2.51^{a}	87.44 ± 5.07^{b}	59.03 ± 3.51^{c}
	Colín V-33	41.48 ± 2.54^{a}	18.23 ± 4.34^{b}	10.44 ± 0.94^{c}
	Oaxaca-7*	184.03 ± 50.40^{a}	150.66 ± 2.19^{a}	124.32 ± 2.88^{a}
Chlorophyll *a* (μg g^{-1} DW)	Hass	70.75 ± 1.57^{a}	50.52 ± 7.96^{b}	39.08 ± 1.60^{c}
	Colín V-33	33.95 ± 2.29^{a}	8.87 ± 1.34^{b}	6.34 ± 0.94^{b}
	Oaxaca-7	119.25 ± 26.50^{a}	83.01 ± 3.06^{ab}	72.81 ± 0.58^{b}
Chlorophyll *b* (μg g^{-1} DW)	Hass	32.72 ± 3.66^{a}	36.92 ± 2.90^{a}	18.75 ± 0.70^{b}
	Colín V-33	7.53 ± 1.92^{ab}	9.35 ± 3.04^{a}	4.09 ± 0.23^{b}
	Oaxaca-7	64.77 ± 23.91^{a}	67.65 ± 0.98^{a}	31.51 ± 3.21^{a}
Total carotenoid content (β-carotene μg g^{-1} DW)	Hass	20.41 ± 2.38^{a}	10.55 ± 1.77^{b}	11.35 ± 3.99^{b}
	Colín V-33	16.88 ± 1.39^{ab}	10.65 ± 0.84^{b}	19.34 ± 5.36^{a}
	Oaxaca-7	34.70 ± 5.36^{a}	27.38 ± 6.11^{ab}	15.54 ± 5.30^{b}

*Genotype. DW: dry weight (g). Means followed by the same letter within each row are not significantly different ($\alpha=0.05$). Values are expressed as mean±SD, n=3.

there are differences among cultivars in both the profile and type of phenolic compounds, which explains the contrasting patterns observed in the total phenol content of the fruits evaluated in this study. In other studies (Ashton *et al.*, 2006; Cox *et al.*, 2004), a decrease in chlorophyll concentration has been observed in 'Hass' avocado fruits as a result of the ripening process. These authors also reported high concentrations of chlorophyll *a* and low levels of chlorophyll *b*, as was likewise found in the present study. Regarding total carotenoids, exposure to refrigeration conditions did not affect the normal carotenoid metabolism associated with ripening in the genotype and the cultivar *Hass*. It has been noted that factors such as harvest season, agroclimatic conditions, and production technology significantly influence carotenoid synthesis in avocado fruits. Furthermore, during the ripening process, these pigments tend to decrease, as has been reported by several researchers (Ashton *et al.*, 2006; Lu *et al.*, 2009).

With regard to the carotenoid profile, lutein was the most abundant carotenoid in the pulp of avocado fruits from both cultivars and the genotype. At harvest, lutein concentrations of 1.19 $\mu g\ g^{-1}$ DW were detected in the cultivar Colín V33, 12.61 $\mu g\ g^{-1}$ DW in the genotype, and 2.65 $\mu g\ g^{-1}$ DW in the cultivar *Hass*. At the edible ripeness stage, the content of this carotenoid decreased to 0.59 $\mu g\ g^{-1}$ DW in Colín V33, 5.00 $\mu g\ g^{-1}$ DW in the genotype, and 1.81 $\mu g\ g^{-1}$ DW in *Hass* (Figure 1), representing reductions of 50.4%, 60.3%, and 31.7%, respectively.

Other carotenoids were also detected in relevant amounts, including neoxanthin, trans-violaxanthin, 9 -cis-neoxanthin, luteoxanthin, cis-violaxanthin, antheraxanthin, β-cryptoxanthin, α-carotene, and β-carotene; however, all were present at concentrations below 1.00 $\mu g\ g^{-1}$ DW.

After 21 days of storage at 2 ± 1 °C, carotenoid concentrations decreased, even after transfer to ripening temperature. In this regard, lutein declined to 0.65 $\mu g\ g^{-1}$ DW in the cultivar Colín V33, 5.56 $\mu g\ g^{-1}$ DW in the genotype, and 2.33 $\mu g\ g-1$ DW in the cultivar Hass, corresponding to reductions of 45.8%, 55.9%, and 12.1%, respectively, relative to the values at harvest.

It is noteworthy that changes were also observed in the relative abundance of the other detected carotenoids. Thus, in the genotype, after 8 days of ripening at 20 ± 2 °C, the concentration order from highest to lowest was lutein, neoxanthin, and cis-violaxanthin. However, after 21 days at 2 ± 1 °C followed by 2 days at 20 ± 2 °C, the order shifted to neoxanthin, lutein, and cis-violaxanthin.

Lutein is a carotenoid typically found in green tissues and was detected in the highest abundance in the pulp of avocado fruits; its concentration decreases during the ripening process (Ashton *et al.*, 2006; Lado *et al.*, 2017), showing a pattern similar to that observed in the present study. Other researchers have also reported the presence of zeaxanthin, β-cryptoxanthin, α-carotene, and β-carotene in avocado pulp (Lu *et al.*, 2009). Lutein and zeaxanthin have been associated with a lower incidence of ocular diseases such as cataracts and age-related macular degeneration. They also function as antioxidants, protecting against the formation of reactive oxygen species. On the other hand, β-cryptoxanthin, α-carotene, and β-carotene exhibit provitamin A activity. Additionally, these compounds may exert other health-related functions of importance in humans, such as enhancing the

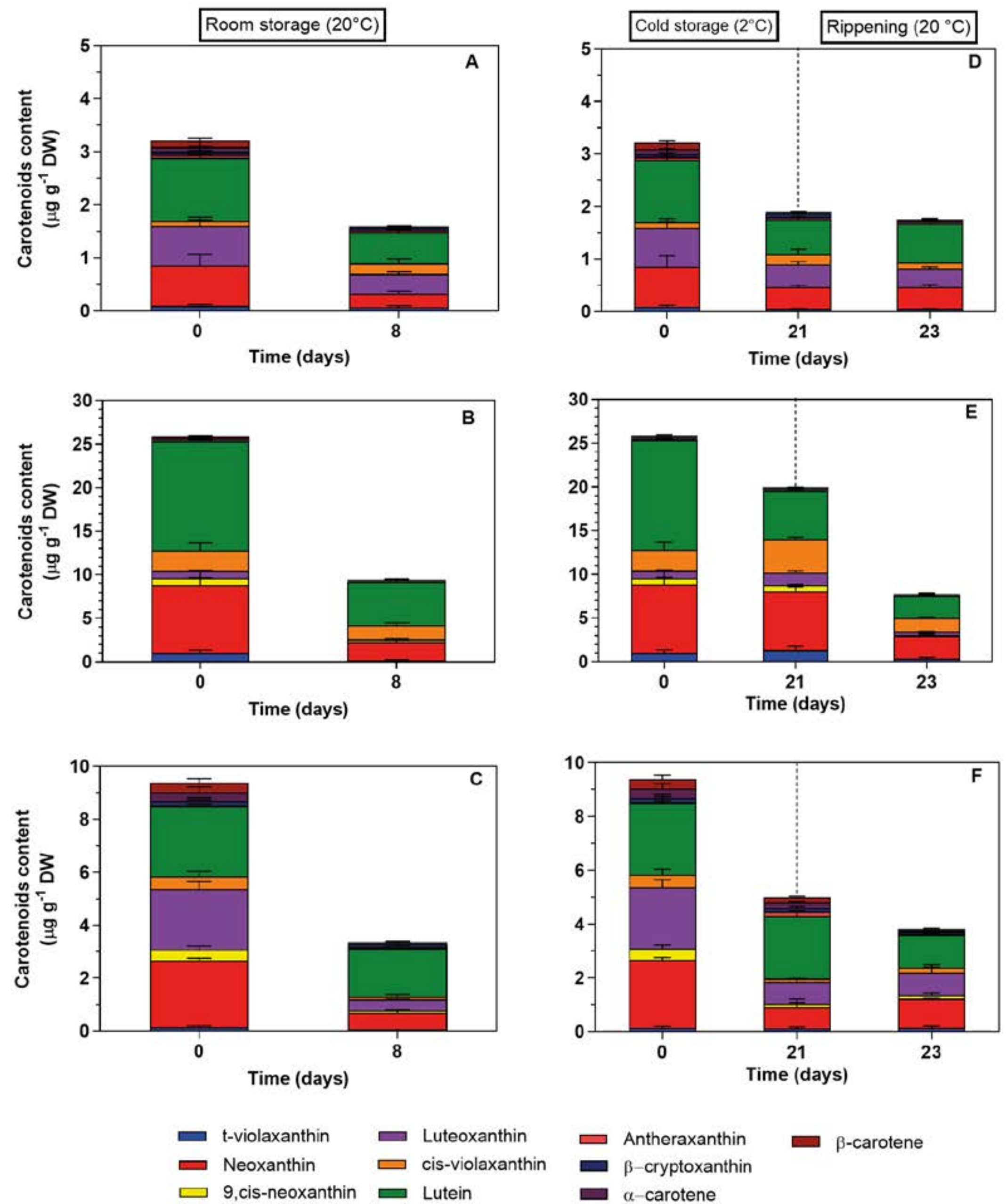

Figure 1. Carotenoid behavior in the pulp of avocado fruits from *Hass* (C, F), Colín V-33 (A, D), and Oaxaca-7 (B, E) stored at 20±1 °C, and stored at 2±1 °C for 21 days followed by 2 days at 20±1 °C. Carotenoid data are presented as mean ± SD. Source: Author's own elaboration based on experimental data.

immune system and providing photoprotection to tissues, including epithelial and ocular tissues (Beltrán *et al.*, 2012; Olmedilla-Alonso and Estévez-Santiago, 2017; Rodriguez-Concepcion *et al.*, 2018). It has also been reported that the carotenoid profile is modified in response to developmental stage, variety or cultivar, harvest date, production technologies, environmental conditions, and postharvest handling. Under normal light conditions, higher concentrations of lutein, β-carotene, violaxanthin, and neoxanthin are typically found in

green plant tissues, whereas zeaxanthin, α-carotene, β-cryptoxanthin, and antheraxanthin occur at lower levels.

The results obtained in the present study highlight the effect of cultivar and genotype on the carotenoid content, concentration dynamics, and profile, as well as the influence of maturity stage and storage conditions. These factors ultimately translate into differences in pulp color and the nutraceutical value of avocado fruits (Rodriguez-Concepcion *et al.*, 2018).

CONCLUSIONS

During cold storage, fruit quality was not affected in the cultivars (Colín V33 and *Hass*) and the genotype (Oaxaca-7). However, once the fruits were transferred to ripening temperature (20 °C), their shelf life was reduced to only two days, and pulp quality deteriorated rapidly. This decline was associated with a significant decrease in phenols, chlorophylls, and carotenoids, which negatively affected the antioxidant activity of the avocado fruits under study, as well as the nutraceutical value provided by chlorophyll and carotenoid contents, particularly lutein.

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