Article

Extracts of *Persea americana* Mill. that delay ripening in avocado fruits

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Abstract

The majority of postharvest research on the fruits of *Persea americana* Mill. is focused on delaying maturation. In this work, we studied the effect of the application of non-polar extracts obtained from branches, peduncle and avocado leaves 'Hass' variety on the delay of fruit ripening with and without peduncle. In addition, the maturation of *P. americana* fruits collected with their branches of original size with leaves (ROH) and without leaves (ROSH), and branches at half of their original size with leaves (RMH) and without leaves (RMSH) was evaluated. The variables measured in fruits treated with non-polar extracts were firmness, respiration, equatorial diameter and color. In the fruits with branches, firmness, equatorial diameter, length and color were determined. The firmness was determined with a hedonic scale of 5 to 1, the breathing speed (mL CO_2 $kg^{-1}h^{-1}$) with a CO_2 meter, the color variables (L, a, b) with the android application 'Color Grab', the equatorial diameter and length, were measured with a digital vernier. According to the results, the presence of the peduncle in fruits treated with hexane extracts delayed their maturation; with an average firmness of 4.3 and green coloration at 10 d post-harvest. The peduncle extracts at 100 and 200 ppm had a significant effect in maintaining the green coloration of the fruits until 10 d after harvest. The evaluation of maturity in fruits collected with branches, showed that the presence of leaves retards its maturation.

Keywords: color, firmness, postharvest, respiration.

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Introduction

At the national level avocado plays a very important role in the economy, although its consumption is sensitive to changes in the price. Of the national production, 69% of the fruits are used for fresh consumption, 19% for industry and 12% for export. An annual per capita consumption of 10 kg is reported (BANCOMEXT, 2010).

Avocado producing areas in the world are far from the commercial zones, so it is important to delay ripening between the harvest and the arrival of the fruit at the point of consumption (Roman and Yahia, 2002). Over the years, various methodologies have been used to delay ripening and preserve the fruit or pulp. Examples of these include refrigeration, controlled atmospheres, application of waxes and pressure reduction (Trejo *et al*., 1992).

It is known that some fruits mature more quickly when they are detached from the tree than when they remain attached to it. Avocado is a classic example, since its fruits do not mature or show climacteric levels of ethylene production while they are attached to the tree. Abeles (1973) coined the term 'tree factor' to describe an alleged inhibitor of the production of ethylene in the tissue of the fleshy fruit attached to the tree.

Peter and Roy (1975) carried out studies on the inhibition of maturation in the fruit of avocado attached to the tree of *Persea americana* Mill., where they found that, when removing leaves on branches detached from the tree, the abscission of the fruit was accelerated and later matured. Consequently, the separated fruits of the branch with the attached peduncle, took longer to mature than when it was removed. With this, they came to the conclusion that the peduncle and the stem can provide a fruit ripening inhibitor, in addition that an auxin is at least one factor involved in the inhibition of ripening.

The objectives of this research were to evaluate the effect of the application of non-polar extracts of leaves, branches and avocado peduncle on the maturation of fruits of avocado with peduncle (FP) and without peduncle (FSP), to determine if its use allows post-harvest conservation. In addition, the maturation of *P. americana* fruits collected with its branches with and without leaves was evaluated.

Materials and methods

Vegetal material

The branches and fruit of avocado 'Hass' with peduncle, whose harvest index was 25.9% of dry matter on average, were collected from homogeneous trees in November 2015, in the 'Huerto La Labor' of the Salvador Sanchez Colín Foundation CICTAMEX, SC. in Temascaltepec of Gonzalez, State of Mexico (19° 02' 39.4" north latitude 099° 58' 35.62" west longitude). The transfer of the fruits and branches was done at room temperature, to the Postharvest Physiology laboratory of the Montecillos Campus Graduate School. Mexico-Texcoco Highway km 36.5, Montecillos, Texcoco, State of Mexico.

Preparation of extracts

The branches, peduncles and avocado leaves 'Hass' were macerated separately in hexane for 48 h. After said time, the mixtures were filtered. The filtrate obtained was evaporated under vacuum in a rotary evaporator (Buchi® Rotavapor® R-3), to obtain the extract and hexane. The recovered hexane was used to make a second extraction of the plant material and the same procedures of maceration, extraction, filtration and evaporation were followed. The process was repeated once more and in the end the extracts were combined.

Each of the extracts of branches, peduncles and leaves were suspended in water using Tween 20 (8×10^{-5} mL mL^{-1}) as dispersant to obtain suspensions at concentrations of 100 and 200 ppm.

Treatments (application of non-polar extracts)

The FP and FSP were treated with the different suspensions with non-polar extracts at concentrations of 100 and 200 ppm. Each of the suspensions of the extracts were placed in plastic cups, in which the avocados were fixed (Figure 1). Four repetitions were made of each treatment, as a control, acetylsalicylic acid was used at 1 $mgmL^{-1}$ and as a white aqueous solution of Tween 20 (8×10^{-5} mL mL^{-1}). The variables of firmness, color and equatorial diameter were measured every day, while the respiration rate was measured every third day until the fruits reached a firmness of 1.

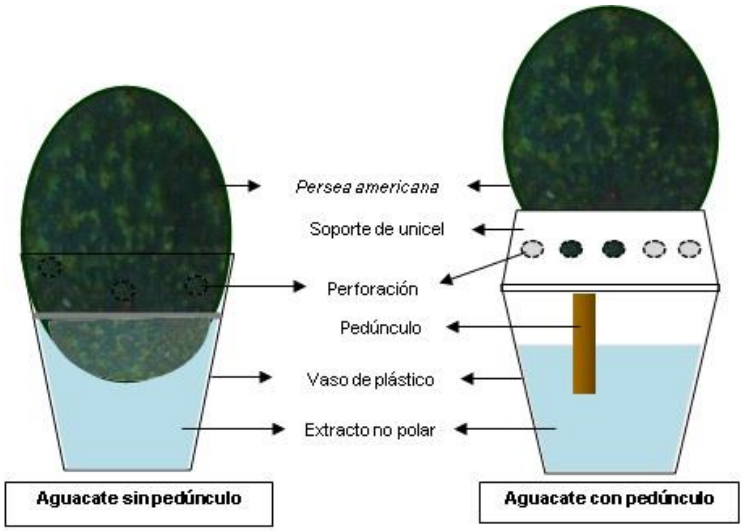

Figure 1. 'Hass' avocado fruits in non-polar extracts.

Evaluation of the maturation of avocado fruits in branches

The branches of *Persea americana* containing at least 4 avocado fruits were cut from the tree and immediately wet blanket cloth was placed on the cutting end. Subsequently four treatments were prepared: branches of original size conserving their leaves (ROH), branches of original size without leaves (ROSH), branches at half their size with leaves (RMH) and branches at half their size without leaves (RMSH). Measurements of color, firmness, equatorial and longitudinal diameter were made every third day.

The firmness was determined manually, using a hedonic scale of 5 to 1, where levels of 5= hard, 4= starts softening, 3= slightly soft, 2= soft and 1= soft and very soft. The application for android 'Color Grab' was used to determine the angle Hue and the saturation index, using the scale L, a, b, and the following equations respectively: $°h^{*}$= degrees (Arc tan (b/a)) and IS=$\sqrt{a^2+b^2}$. The CO_2 measurement was carried out with a carbon dioxide and temperature monitor (Telaire® 7001), based on the methodology of (Saltveit and Sharaf, 1992). The equatorial diameter and length were determined with a Truper digital vernier, in millimeters, for this the ends of the equatorial zone and the longitudinal part of the fruit were labeled, in order to keep track of the measurements.

Experimental design and statistical analysis

In the fruits treated with non-polar extracts, a transversal analysis was carried out through an experimental design of 2x2 factorial blocks, considering as blocks the trees from which the fruits were collected and as treatments the hexanic extracts to which they were subjected the avocados. Additionally, a longitudinal analysis was carried out in relation to the equatorial firmness and diameter.

In the case of branches with fruits, a completely randomized experimental design was used for firmness, equatorial diameter and length, where the experimental unit was avocado and the treatments were branch size and the presence or absence of leaves.

A comparison of vectors of means was used using a multivariate analysis for color measurements.

Results and discussion

Avocado fruits with extracts

The initial firmness of the fruits, once harvested and placed in the extracts, was 5. As the days passed, it decreased until reaching 1. The longitudinal analysis indicates that the extracts studied did not show significantly different effects on the firmness of the fruit (Pr> F= 0.9978, Table1). However, the presence of peduncle in the fruits did show significant effects on the firmness, fulfilling the criterion of convergence (Pr> F= 0.0002, Table 2).

Table 1. Type 3 test of fixed effects on the effect of non-polar extracts on the firmness of 'Hass' avocado fruits.

Effect	Degrees of freedom of the numerator	Degrees of freedom of the denominator	F-Value	Pr> F
Extracts	9	47	0.15	0.9978
Time	7	47	135.65	<0.0001
Extracts∗time	63	47	0.83	0.7625

Table 2. Type trhee test of fixed effects on the effect of the presence of peduncle on the firmness of 'Hass' avocado fruits.

Effect	Degrees of freedom of the numerator	Degrees of freedom of the denominator	F-Value	Pr> F
Peduncle	1	55	15.71	0.0002
Time	7	55	178.17	<0.0001
Peduncle∗time	7	55	2.31	0.0384

In the cross-sectional analysis, the presence or absence of peduncles in avocados induced significant differences in firmness as the days passed (Table 3).

Table 3. Firmness of 'Hass' avocado with and without peduncle treated with non-polar extracts at 10, 12 and 14 d post-harvest.

N	Test of Tukey's Studentized Range (HSD) for Y		
	Mean [z]	Peduncle	Post-harvest days
30	4.3 a	FP	10
33	3.8 b	FSP	
30	3.3 a	FP	12
33	2.9 b	FSP	
30	2.1 a	FP	14
33	1.4 b	FSP	

[z]= means with the same letter are not significantly different (Tukey, 0.05).

The transversal analysis from day 10 after harvest showed that the presence of peduncle keeps the fruit more firm with respect to fruits without peduncle. This agrees with Proctor and Miesle (1991) who mention that the maturation of a fruit is due to the loss of firmness, which is closely related to the enzymatic alteration of the median lamella and cell wall of the fruits, which are constituted mainly by pectic substances, cellulose and hemicellulose. Said the previous thing, can make reference to that the presence of the peduncle avoids a fast maduration in the fruit, acting like a retarder of this.

Colour

There was a decrease in the values of the 'L' coordinate (brightness), a change from negative to positive values in the case of the coordinate 'a' and a decrease in the coordinate 'b'. From eight to 14 d after the harvest, significant differences were found in the color coordinates (L, a, b) with respect to the presence of peduncle in the fruits, this with a comparison of vectors of means using a multivariate analysis with a Pr> F= <0.0001 (Figure 2).

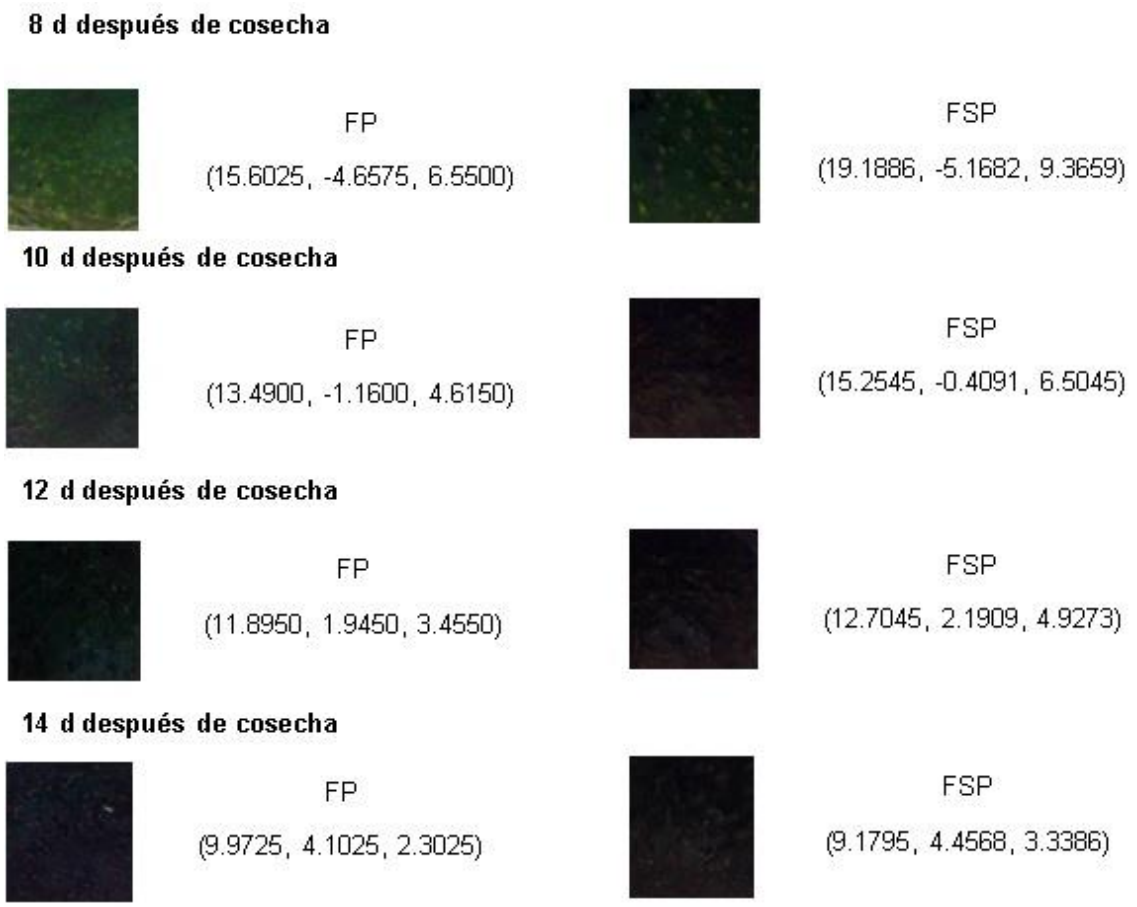

Figure 2. Color changes (coordinates L, a, b) in the skin of 'Hass' fruit treated with non-polar extracts at 8, 10, 12 and 14 d postharvest.

Some extracts applied to the fruits allowed to see significant differences in skin color at 8, 10 and 14 d postharvest; through, the color coordinates (L, a, b). Acetylsalicylic acid (1 mg mL^{-1}) and distilled water showed a significant difference at 8 d post-harvest with a value of Pr> F= 0.0059 (Figure 3).

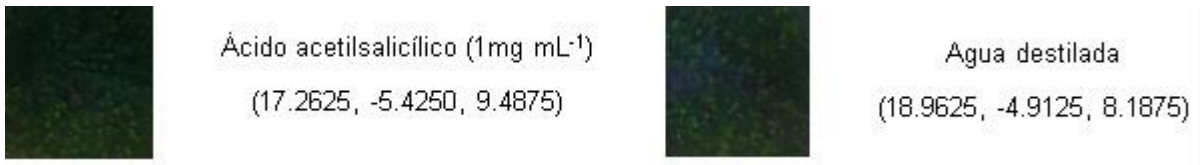

Figure 3. Color (coordinates L, a, b) in the skin of 'Hass' fruit treated with acetylsalicylic acid (1 mg mL^{-1}) and distilled water.

At 10 d postharvest the fruits of the treatments with acetylsalicylic acid (1 mg mL^{-1}) and peduncle extract (200 ppm) had a significant difference with a value of Pr> F= 0.0083 (Figure 4). Similar behavior occurred between acetylsalicylic (1 mg mL^{-1}) and the peduncle extract (100 ppm) with a Pr> F= 0.0074 (Figure 5).

Figure 4. Color (coordinates L, a, b) on the skin of 'Hass' fruits treated with acetylsalicylic acid (1 mg mL^{-1}) and peduncle extract (200 ppm).

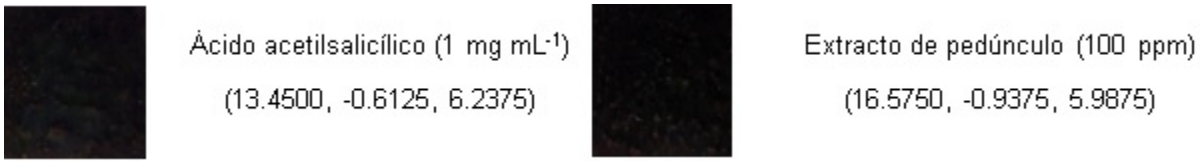

Figure 5. Color (coordinates L, a, b) in the skin of 'Hass' fruits treated with acetylsalicylic acid (1 mg mL^{-1}) and peduncle extract (100 ppm).

The treatment of extract of peduncle (200 ppm) and branch (200 ppm) showed significant differences at 14 d post-harvest with a value of Pr> F= 0.0061 (Figure 6).

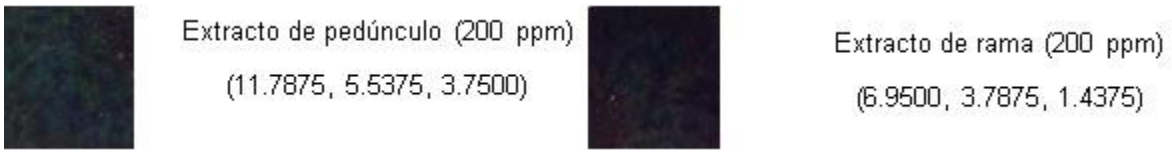

Figure 6. Color (coordinates L, a, b) in the skin of 'Hass' fruits treated with extract of peduncle (200 ppm) and branch (200 ppm).

The presence of peduncle in the avocado during its evaluation with the different extracts allowed the fruit to maintain a greater green color, unlike the fruits without peduncle that acquired the dark tonality more quickly (Figure 2).

Initially acetylsalicylic acid (1 mg mL^{-1}) showed the greatest effectiveness to maintain the green coloration of the fruit (Figure 3), but as the days (10 and 14 d postharvest) the hexanic extract of peduncle at 100 and 200 ppm were passing, as well as the branch extract at 200 ppm showed a greater effect on the coloration. Considering the relation that exists between the color of the peel of the fruit and its degree of maturity, it is observed that acetylsalicylic acid and hexanic extracts of peduncle and branch, as well as the presence of peduncle delayed ripening. These results agree with previous works (Biale, 1960; Peter and Roy, 1975), which showed that the peduncle and avocado stem can act as a source of maturation inhibiting hormone.

On the other hand, acetylsalicylic acid, derived from salicylic acid, reduces the synthesis of ethylene and in some species, causes a delay in senescence (Martínez *et al*., 2004).

Respiratory rate

The fruits treated with non-polar extracts presented a typical climacteric pattern, reaching a climacteric maximum at day nine after harvest with a value of 85.57 mL of CO_2 kg^{-1} h^{-1} (Figure 7), which agrees with Lopez and Cajuste (1999).

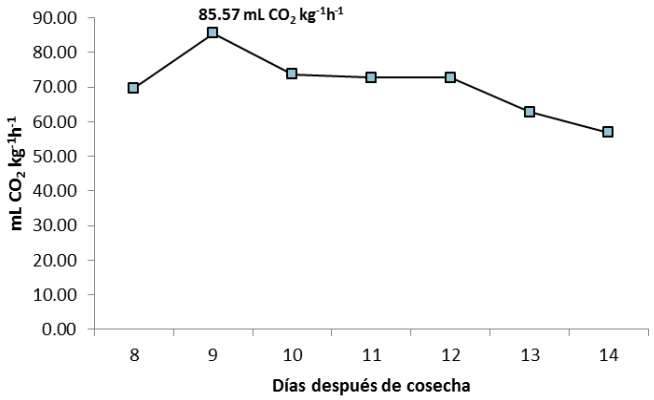

Figure 7. Behavior of 'Hass' fruit respiration under ambient temperature conditions.

Equatorial diameter

The equatorial diameter decreased on average 3 mm at 14 d post-harvest (Figure 8), which is attributed to the loss of water in the fruits, caused by the transpiration process, where internal factors; morphological, anatomical characteristics, the relationship between surface and volume, surface damage and the state of maturity of the fruit influence (Kader, 2002).

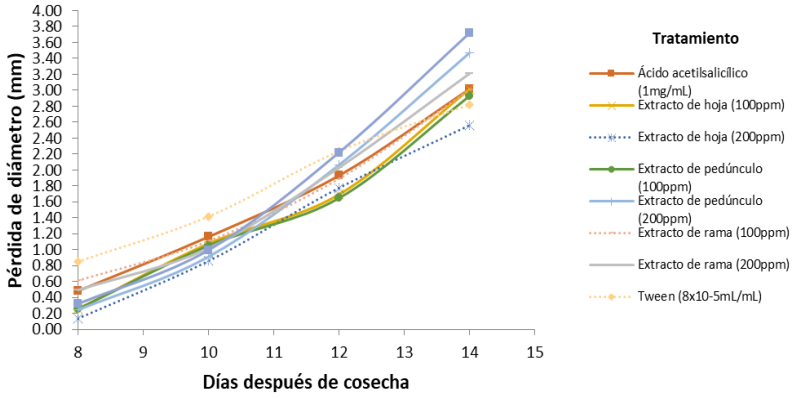

Figure 8. Equatorial diameter loss in 'Hass' fruits treated with non-polar extracts. Fruits of avocado with branches.

It was found that the presence or absence of leaves in the branches did not show significant differences in relation to the firmness at 4, 6 and 8 d after the collection, this with a value of Pr> F= 0.8782, Pr> F= 0.9769 and Pr> F= 0.5624, respectively (Table 4).

Table 4. Firmness of 'Hass' avocado fruits on branches with and without leaves at four, six and eight days after collection.

N	Test of Tukey's Studentized Range (HSD) for Y		
	Mean[z]	Leaves	Days after collection
8	4.1 a	Without leaves	4
8	4 a	With leaves	
8	3.5 a	Without leaves	6
8	3.5 a	With leaves	
8	2.8 a	Without leaves	8
8	3.1 a	With leaves	

[z]= means with the same letter are not significantly different (Tukey, 0.05).

In the size of the branches; whole and half, no significant effect was found in relation to the firmness, through the transversal analyzes at 4, 6 and 8 d after the collection, this with a value of Pr> F= 0.5073, Pr> F= 0.5201 and Pr> F= 0.2619, respectively (Table 5).

Table 5. Firmness of 'Hass' avocado fruits in whole branches and in half at four, six and eight days after collection.

N	Test of Tukey's Studentized Range (HSD) for Y		
	Means[z]	Branch size	Days after collection
8	3.9 a	Whole	4
8	4.2 a	Half	
8	3.3 a	Whole	6
8	3.6 a	Half	
8	2.6 a	Whole	8
8	3.3 a	Half	

[z]= means with the same letter are not significantly different (Tukey, 0.05).

Peter and Roy (1975), mention that the branch either in full size or in half, is not involved with the firmness of the fruit while it is attached to it, because the abscission of the avocado occurs before any softening, in addition that the subsequent ripening of the avocado fruits is not delayed by the presence or proximity of the leaves in the branches and therefore, they do not support the hypothesis that the leaves are a source of inhibitor of maturation described by (Burg and Burg, 1964).

Colour

The presence of leaves in the branches showed significant differences in the multivariate analysis at 4, 6 and 8 d after collection, with respect to the skin color of the fruit (Figure 9), with values of Pr> F= 0.0045 at the 4 d, Pr> F= 0.0020 at 6 d and Pr> F= 0.0022 at 8 d.

Figure 9. Color changes (coordinates L, a, b) in the skin of 'Hass' fruits in branches with and without leaves at 8, 10, 12 and 14 d after their collection.

The size of the branches; whole or half of its original size, had significant differences in the multivariate analysis at 4, 6 and 8 d after its collection, with values of Pr> F= 0.0577, Pr> F= 0.0432 and Pr> F= 0.0248 respectively, this in relation to the color coordinates (L, a, b) present in the skin of the fruit (Figure 10).

Figure 10. Color changes (coordinates L, a, b) in the skin of 'Hass' fruits in whole branches and in half at 4, 6, and 8 d after their collection.

The presence of leaves in the branches delayed the loss of the green color of the fruits (Figure 9), according to Burg and Burg (1964), the leaves provide substances inhibiting maturation.

In relation to the size of the branch, in Figure 10 it is shown that the coordinates L, a, b, which are related to a greater green coloration, correspond to fruits present in branches at half of their original size.

The results obtained in the equations of saturation index (IS) and pitch angle (°h*) during the analysis of the fruits, had an expected behavior during its maturation, that is to say, decreases were reported in the IS, with initial values of 20.2 and at the end of 0.9, which agrees with previous studies by López-López and Cajuste-Bontemps (1999), who found initial IS values in the 'Hass' avocado peel of 50, until reaching 0 as maturation progressed. The angle of tone (°h*) showed an increase, having initial values of -88.4° reaching during the ripening of the fruit at 81.7°.

Equatorial diameter and length

These parameters had an average decrease of 3 mm in both the equatorial and longitudinal diameter of the fruits, at 8 d after the collection of branches (Figures 11 and 12).

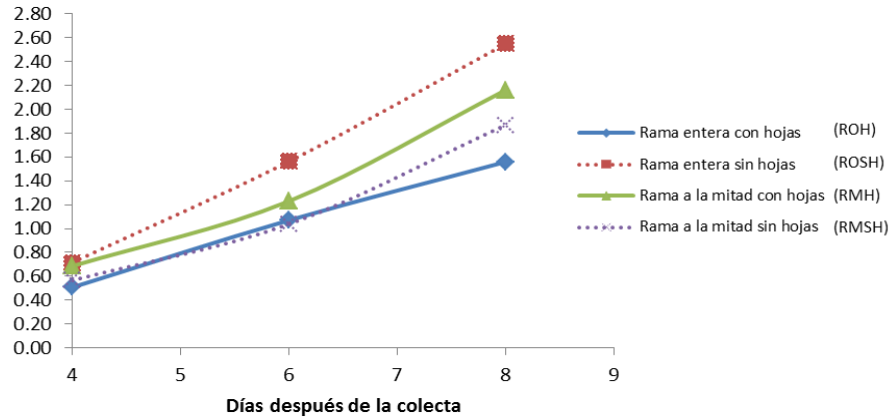

Figure 11. Equatorial diameter loss in 'Hass' fruits with ROH, ROSH, RMH and RMSH.

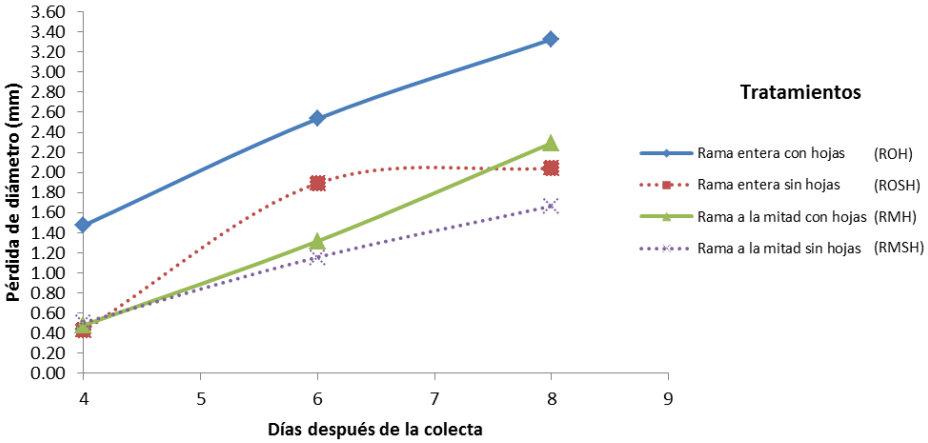

Figure 12. Loss of length in 'Hass' fruits with ROH, ROSH, RMH and RMSH.

Conclusions

The presence of peduncle in the avocado fruits 'Hass' allowed to delay its maturation, having these greater firmness and green coloration unlike fruits without peduncle. The avocado leaves could be the source of a substance (hormone) that delays maturation, since the fruits that were found in branches with leaves had the greenest skin, unlike the branches without leaves. The acetylsalicylic acid, the peduncle and branch extracts allowed the green coloration of the skin of the avocados to be maintained two days longer than the control. The loss of water in the fruits caused a decrease in its equatorial diameter and length.

Acknowledgments

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