



## Identification of pre-harvest factors that affect fatty acid profiles of avocado fruit (*Persea americana* Mill) cv. 'Hass' at harvest



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### ABSTRACT

'Hass' avocado is the most important avocado variety cultivated worldwide. In Chile alone, there are nearly 40,000 ha, distributed between the IV and VI regions, with production areas located close to both the coast and to the hills. Given the increasing competitiveness of fruit export markets, the quality of organoleptic attributes is a key issue in consumer acceptance. The quality of avocados is related to many attributes, especially oil content and firmness (among others), and these attributes are influenced by storage, growing and environmental conditions, as well as the stage of maturity/ripening.

This study measured the fatty acid profiles of avocado fruits during two seasons from 12 localities cultivated with the variety 'Hass.' Fifty additional variables were measured, including climate, nutrition, vegetative development, and agricultural management (called pre-harvest variables). The data obtained were analyzed with a partial mean squares multivariate regression (PLS). The analysis showed that the contents of oleic, palmitic, and palmitoleic acids were influenced by climatic and nutritional factors, with mean annual maximum temperature proving most important. In localities with lower temperatures, the 18-carbon fatty acid content increased, and the 16-carbon fatty acid content decreased. Moreover, the N and Mg contents in the mesocarp at harvest were related to the contents of palmitic and palmitoleic acids, and when the levels of N and Mg increased in the mesocarp, the 16-carbon fatty acid content decreased.

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### 1. Introduction

Avocado (*Persea americana* Mill.) is a tropical-subtropical fruit that is highly appreciated worldwide. The main cultivar consumed is 'Hass,' which is produced by a large number of countries, with Mexico, Chile, USA, and Peru being the main producers. These countries have very different climatic conditions and management systems, which leads to great variation in the chemical composition of the commercialized fruit and in its postharvest duration.

Avocado fruit is unique in its nutritional value due to its high content of mostly unsaturated oils (Ozdemir and Topuz, 2004; Takenaga et al., 2008; Ariza et al., 2011; Donetti and Terry, 2014) that may reach approximately 79% of the fatty acids present in the mesocarp. Of these, 13.6% are polyunsaturated (Takenaga et al., 2008). The main fatty acids present in the mesocarp include monounsaturated oleic acid (50–60% fatty acid content), saturated palmitic acid (15–20%),

unsaturated palmitoleic acid (6–10%), unsaturated linoleic acid (11–15%), and linolenic acid (near 1%).

A number of reports have indicated that the oil content and composition vary according to the location of the orchard (Landahl et al., 2009; Lu et al., 2009; Donetti and Terry, 2014), the variety (Ozdemir and Topuz, 2004; Takenaga et al., 2008), the number of days between flowering and harvest (Ozdemir and Topuz, 2004; Donetti and Terry, 2014), the dry matter contents (Requejo-Tapia et al., 1999), and even to the part of the fruit measured (Landahl et al., 2009). Additionally, Ozdemir and Topuz (2004) found that postharvest management affects the acid content, although the effect is small.

Donetti and Terry (2014) showed that Chilean avocados in the United Kingdom market arrive with oleic acid contents between 57% and 61% (those from Spain have 54–60% and those from Peru have 40–47%) and suggested that oleic acid content may serve as a marker of the place of origin of the fruit. Ratovohery et al. (1988) indicated that the fatty acid content of avocados depends on geography and climate. Similar results were reported in olive by Ranalli et al. (1999), who showed that the fatty acid composition depends on climate and soil factors. Requejo-Tapia et al. (1999) compared avocados grown in

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two areas, finding that the zone with the lower mean temperature had higher contents of monounsaturated fatty acid (oleic acid) and lower levels of saturated fatty acid (palmitic) than the zone with the higher mean temperature. Similarly, [Canvin \(1965\)](#) showed that oleic content increases with lower temperature in some seeded fruits. However, [Requejo-Tapia et al. \(1999\)](#) also indicated that temperature cannot be the only factor that determines the rate of lipid synthesis. To our knowledge, there are no studies that analyze by integrating different pre-harvest factors such as climate, soil and plantation management on the composition of fatty acids in fruit.

A strategy for differentiation in the world market is to sell healthy and efficacious products. This is only possible if the chemical characteristics of the product are well known. Thus, it is critical to understand the relationships between climate, soil, and management and the lipid composition of avocados to stimulate the development of these desirable fatty acids. The objectives of this study were to determine the pre-harvest factors that affect the bio-active fatty acids of avocado fruit var. 'Hass' in order to obtain homogeneous prime material for the industry and to add value to exported fresh fruit.

## 2. Materials and methods

### 2.1. Study locations

In central Chile, avocados are predominately grown in the valleys of the 'El Maipo,' 'Aconcagua,' 'La Ligua,' and 'Petorca' rivers. These rivers originate in the Andes mountain range and generally flow west to the Pacific Ocean. There are plantations in localities close to the sea (elevation 112 m) and others close to the mountains (elevation 1103 m); thus the climatic, topographic, and soil conditions of the plantations are widely variable. The mean annual maximum temperature may reach 23.3 °C in the highest zones and 18.6 °C near the coast ([Table 1](#)). We studied 12 localities growing 'Hass' avocados grafted on 'Mexicola' rootstock. Seven localities were in the 'Aconcagua' Valley, two were in the 'El Maipo' Valley, and two were in the 'La Ligua' and 'Petorca' valleys. These localities have different conditions of climate, topography, and soil and agricultural management, and thus account for different pre-harvest conditions on the fatty acid composition of the fruit at harvest. [Table 1](#) indicates the climatic conditions of the areas in which the experimental sites are located.

### 2.2. Characterization of experimental sites and variables measured

Two homogeneous trees were used as a unit with three repetitions in each locality over two growing seasons (2012–2013 and 2013–2014). Each year in each repetition, the following were measured: a) nutrient content in leaves: N, P, K, Ca, Mg, Zn, Mn, B, and Cl; b) nutrient content of the mesocarp of harvested fruit: N, K, Ca, Mg; N/Ca, Ca/K, and K/Mg ratios; c) agro-climatic characteristics of each locality: annual solar radiation, relative humidity, reference evapotranspiration (Eto), mean annual temperature, absolute January maximum, absolute June minimum, maximum mean annual temperature,

minimum mean annual temperature, and mean annual thermal amplitude; d) water applied to plants: total water applied, water applied in spring, and water applied in autumn and winter; e) vegetative tree development: trunk diameter and relative chlorophyll content in leaves (SPAD), index of leaf area, tree age, number of fruits per tree, number of sylleptic shoots, and days from 50% flowering to harvest; f) soil characteristics: percentage of sand, percentage of silt, percentage of clay and percentage of soil macropores; g) topographic characteristics: Universal Transverse Mercator (UTM) east, UTM north (UTMN), elevation, slope of plantation rows and orientation of plantation rows; h) agronomic management: level of tree pruning, application of growth regulators and level of tree ringing; and i) dry matter content of fruits at harvest.

Leaf analyses were performed in March and fruit analyses at harvest. To analyze leaf nutrients, samples of 60–80 fully mature and expanded leaves were obtained from each repetition from summer growth branches which had stopped development, thus we collected 3- to 4-month-old leaves at the end of autumn. For analyses of fruit nutrients, we sampled five fruits per repetition. Leaves and fruits were dried at 60 °C and ground. N content was analyzed using the Kjeldahl method; B by colorimetry; Ca, Mg, and Zn by atomic absorption spectrophotometry; and K by atomic emission spectrophotometry.

Temperature, relative humidity, wind velocity, Eto, and radiation were obtained from meteorological stations present in the orchards where the experimental sites were located. The amount of water applied was obtained from volumetric meters installed in the irrigation equipment and the amount of precipitation was obtained from the meteorological stations. The diameter of the tree trunks was measured with a caliper in March. The SPAD value was obtained from a mean of 50 leaves per repetition measured with a Minolta Model 502 plus instrument. The leaf area index was estimated from the photosynthetically active radiation (PAR) intercepted by the plant foliage at midday. The PAR measurements were measured once per year between August and September in a quadrant consisting of the trees of each repetition using a (Decagon model Sunfleck) ceptometer. The contents of sand, silt, clay, and calcium carbonate and the macroporosity were determined in each repetition in the first soil horizon. The sand, silt, and clay were measured by the method of [Bouyoucos \(1962\)](#) and calcium carbonate by the potentiometric titration method with acid. The macroporosity was calculated using the relationship proposed by [Ball and Smith \(1991\)](#). The topographic characteristics UTM, UTMN, elevation, slope, and row orientation were measured with a Garmin GPS model eTrex Vista HCx.

### 2.3. Fruit quality analyses

We sampled fruit from the three repetitions at the 12 localities when the fruit dry matter reached values close to  $25.3 \pm 1.5\%$ . Oil was extracted from the fruit pulp, based on the methodology of [Bligh and Dyer \(1959\)](#), to obtain the fat or oil of the mesocarp by direct extraction with solvents under cold conditions. This process used 5 fruits per repetition (15 per locality) that were cut in half, peeled, and the pulp

**Table 1**  
Climate characteristics of five experimental sites located in the low, middle, and high elevations.

Zones	Altitude	UTME	RH	ETO	Temperature		
	m.a.s.l	Km	%	Mm year <sup>-1</sup>	Average annual °C	Absolute maximum January °C	Average maximum annual °C
Low	112 ± 5	283 ± 3	85 ± 0.0	796 ± 38	13.5 ± 0.6	25.7 ± 2.0	18.6 ± 0.7
Lower middle	161 ± 5	261 ± 3	85 ± 0.1	882 ± 2	12.8 ± 0.3	28.5 ± 1.8	20.7 ± 1.2
Middle	342 ± 5	308 ± 3	74 ± 3.1	1119 ± 25	14.8 ± 0.7	32.7 ± 0.1	23.2 ± 0.5
High middle	489 ± 5	322 ± 3	75 ± 0.0	1069 ± 9	15.6 ± 0.7	34.3 ± 0.3	24.4 ± 0.7
High	1103 ± 5	354 ± 3	55 ± 0.1	1931 ± 96	16.6 ± 0.7	35.1 ± 0.1	23.3 ± 0.7

separated from the seed and skin. Each pulp sample was crushed and homogenized separately. Samples of 50 g were combined with 70 ml distilled water, 200 ml methanol, and 100 ml chloroform and were mixed for 2 min in a blender. Then, 100 ml chloroform and 100 ml distilled water were added and the solution was mixed for an additional 30 s. The mixture was vacuum filtered using a Büchner funnel and no. 1 filter paper over a 500 ml Kitasato flask to separate the solid residues. Finally, the solution was vacuum distilled in a Buchi model R215 Advanced rotary evaporator to rapidly evaporate the sample and obtain the oil extract.

The composition of fatty acids was obtained from the oil by gas chromatography using the official A.O.C.S. Ce 1–62 (1993) method. Gas chromatography was performed using a Hewlett–Packard model 5890 series II chromatograph (using H<sub>2</sub> as the carrier gas) connected to a Hewlett–Packard 3397 integrator. The capillary column used was of BPX-70 fused silica (length 100 cm and internal width 0.25 mm with a film thickness of 0.2 µm). The oven temperature was programmed from 160 °C to 220 °C with a heating rate of 2 °C/min. The temperature of the injector and detector was fixed at 250 °C.

#### 2.4. Data management and statistical analyses

The data collected over the 2 sampling years were first analyzed by exploratory descriptive analysis and simple regressions. Following this, a principal components analysis (PCA) was performed and predictive models were developed using partial mean squares multivariate regression (PLS). These analyses were performed with SIMCA-P + 12.01 software (Umetrics, Umeå, Sweden).

Algebraically, principal components (PC) are linear combinations of  $p$ -random variables ( $\times 1, \times 2, \dots, \times p$ ) that allow the PCA to condense information in two ways, by identifying the relationships between different observations that comprise the scores matrix and by determining the relationships between different variables of the data set, known as the loadings matrix (Saavedra et al., 2011). The axes derived from this analysis represent the maximum variance directions, wherein the first principal component (PC1) is located along the direction of maximum variance of the data set, the second component (PC2) is located along the direction of the second greatest variance, and so on. All of the PCs are simultaneously orthogonal to each other, and there is no co-variance among them (Saavedra et al., 2013).

PLS is a linear regression method whereby the multivariate variables correspond to the observations. This modeling technique establishes the relationship between two sets of predictor and response variables. PLS is a correlation analysis that estimates the values of one variable from a set of controllable independent variables (Yañez et al., 2012).

### 3. Results and discussion

#### 3.1. Lipid analysis

The analysis of lipids showed that, in the studied localities, the fatty acids formed were mainly C16 and C18 carbon chains (Table 2). The mean C18 acid content in the fruit mesocarp was 81.5%, of which 69.2% ± 4.2 was oleic acid (C18:1) and 11.6% ± 1.7 was linoleic acid

(C18:2); therefore, oleic acid was predominant in 'Hass' avocados. This concurs with the reports of Olaeta et al. (1999), Ozdemir and Topuz (2004), Meyer and Terry (2008), and Landahl et al. (2009), who indicated that oleic acid is 50–60% of the total fatty acids in this fruit.

#### 3.2. Pre-harvest factors that affect oleic acid concentration in the avocado mesocarp

The PLS analysis of the C18 fatty acids (oleic and linoleic acids) showed that oleic content was related to pre-harvest values (Fig. 1); however, linoleic acid was not. Oleic acid concentration increased from 66.6 ± 0.8% in the higher elevation areas of cultivation to 75.4 ± 1.4% in the lower areas (Table 2). We applied a variance importance to the projection (VIP) as part of the PLS (Eriksson et al., 2006). This analysis creates a hierarchy of the explanatory capacity of the independent variables on the variable(s) to be predicted. The VIP is based on the weighted sum of squares of the weights of the model factors ( $w^*$ ), allowing a hierarchical ordering of the independent variables (Wold et al., 1993). Of the 50 independent variables studied, according to the VIP, the variables that showed the greatest effect on oleic acid in the mesocarp are the UTM coordinates altitude of the site, maximum mean annual temperature, Mn content in leaves, reference evapotranspiration, Mg content in leaves, percentage of macropores in the soil, and relative humidity (Fig. 1).

From our results, the independent variables affecting oleic concentration in avocado fruits in this study can be grouped into three categories: 1) those related to the climate (UTM coordinates, site altitude, mean annual maximum temperature, January absolute maximum temperature, reference evapotranspiration and relative humidity; 2) those related to plant nutrition (Mn and Mg content in leaves) and; 3) those related to soil properties (macroporosity). This agrees in part with the reports of Ratovohery et al. (1988) and Ranalli et al. (1999), who indicated that the composition of fatty acids in the fruit depends on the geography and the climate. Requejo-Tapia et al. (1999) suggested that temperature may influence the synthesis and composition of lipids in the fruit, and Kaiser and Wolstenholme (1994) reported that with lower temperatures, plant membranes must be composed of higher levels of unsaturated fatty acids in order to function adequately.

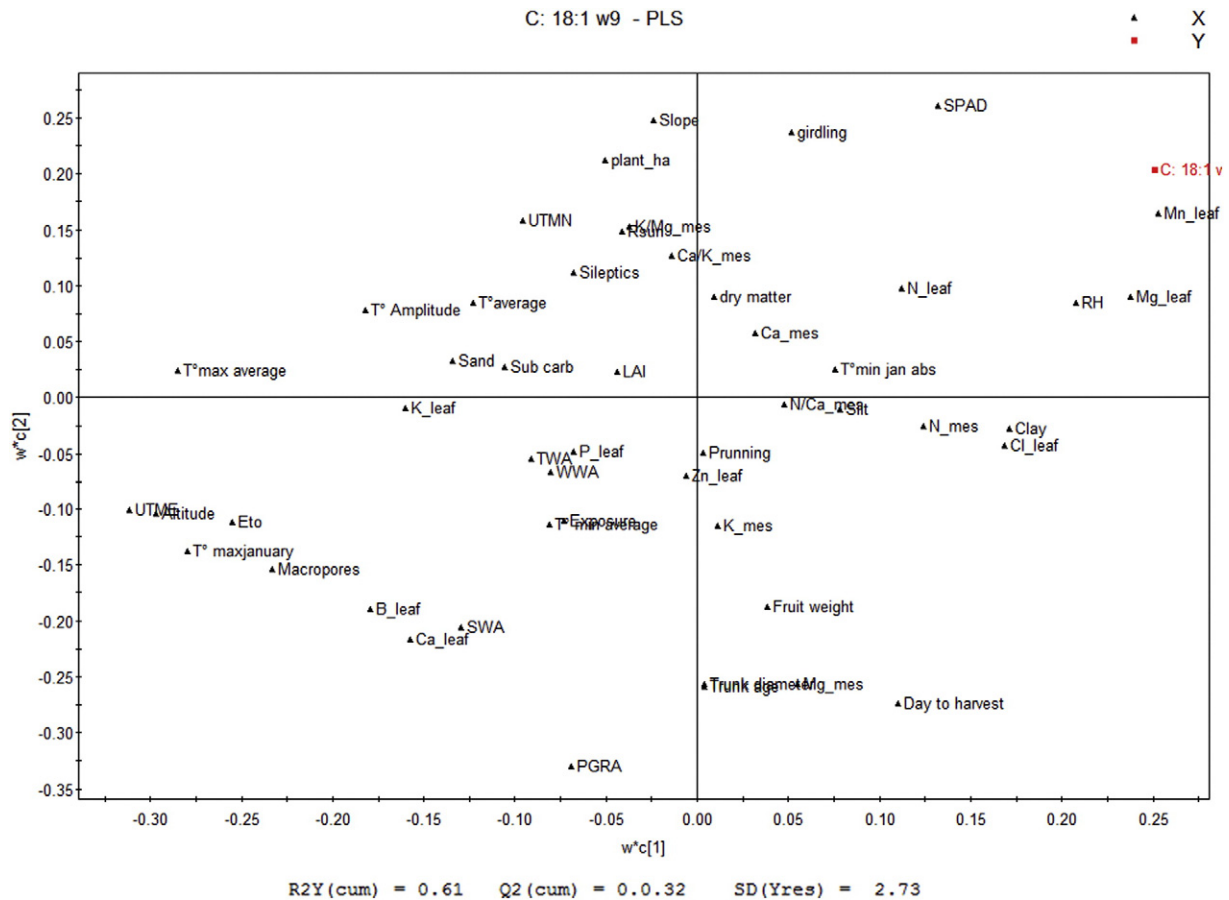
The variables related to climate (temperature) are in the opposite quadrant from the dependent variable oleic acid (Fig. 1a). This implies that with increases in the independent variables related to climate (UTM coordinates, altitude, mean maximum annual temperature, and January absolute maximum temperature), the concentration of oleic acid in the mesocarp decreases (Table 2). This agrees with the results of Requejo-Tapia et al. (1999) and Canvin (1965), who reported that fruits in areas with lower temperatures had higher oleic acid contents. Kaiser and Wolstenholme (1994) found approximately 20% less oleic acid in the warmest site compared with the coldest.

Increases in the leaf Mn and Mg contents were associated with an increase of oleic acid in the mesocarp (Fig. 1, Tables 2 and 3). These variables were located in the same quadrant in the PBS, indicating that they have direct proportionality. Climate variables had a greater effect on the concentration of oleic acid than the nutritional variables (Fig. 1). Requejo-Tapia et al. (1999) suggested that temperature

**Table 2**

Composition of fatty acids in five experimental sites located at low, middle, and high elevations high elevations.

Zones	Monoenoic (%)	C16: 1w9 (%)	C18: 1w9 (%)	C20.1 (%)	Polyenoic (%)	C18:2 (%)	C20 (%)	C18:3 (%)	Saturated acid (%)	C16 (%)	C17 (%)	C18 (%)
Low	78 ± 1.2	1.8 ± 0.2	75.4 ± 1.4	0.73 ± 0.15	10.7 ± 0.8	9.9 ± 0.6	0.22 ± 0.10	0.65 ± 0.28	11.4 ± 1.6	10.7 ± 1.5	0.12 ± 0.11	0.63 ± 0.22
Lower middle	75 ± 1.9	2.2 ± 0.2	71.1 ± 1.5	0.97 ± 0.59	14.4 ± 1.7	13.4 ± 1.2	0.10 ± 0.11	0.90 ± 0.55	10.7 ± 0.6	10.2 ± 0.5	0.05 ± 0.05	0.48 ± 0.16
Middle	71 ± 1.6	4.4 ± 0.9	66.3 ± 2.2	0.70 ± 0.55	13.9 ± 0.4	13.3 ± 0.6	0.10 ± 0.11	0.50 ± 0.35	14.7 ± 1.4	14.2 ± 1.3	0.10 ± 0.08	0.50 ± 0.08
High middle	71 ± 1.9	4.3 ± 1.3	66.0 ± 2.6	0.50 ± 0.14	10.9 ± 2.4	10.2 ± 2.0	0.15 ± 0.05	0.62 ± 0.31	17.3 ± 2.4	16.6 ± 2.3	0.08 ± 0.04	0.65 ± 0.19
High	72 ± 1.0	4.3 ± 1.2	66.6 ± 0.8	0.72 ± 0.15	12.2 ± 2.0	11.4 ± 1.4	0.10 ± 0.00	0.73 ± 0.48	14.9 ± 2.0	14.2 ± 1.5	0.03 ± 0.06	0.67 ± 0.14



**Fig. 1.** Partial minimum squares multivariate regression (PLS) between the monounsaturated oleic acid and the nutritional content of plant and fruit, climate characteristics, plant vegetative development, and physical characteristics of the soil.

influences the synthesis and composition of lipids in the fruit. Our results indicate that oleic acid content may be strongly determined by the locality in which the fruit is grown.

### 3.3. Pre-harvest factors that affect 16-carbon fatty acid concentration in avocado mesocarp

In this study, the C16 fatty acids had a mean concentration of 16.6% in the mesocarp of the fruit; palmitic acid (C16:0) had a mean of  $13.2 \pm 2.7\%$  and palmitoleic (C16:1)  $3.4 \pm 1.3\%$  (Table 2). The VIP analysis indicated that, of the 50 independent variables studied, those that most affected 16-carbon fatty acids were the N content in the mesocarp at harvest, mean maximum annual temperature, the UTM coordinates, and site altitude (Fig. 2a and b).

The independent variables that affected the concentration of palmitoleic acid in the fruits may be separated into two groups: those related to climate (mean maximum annual temperature, UTM

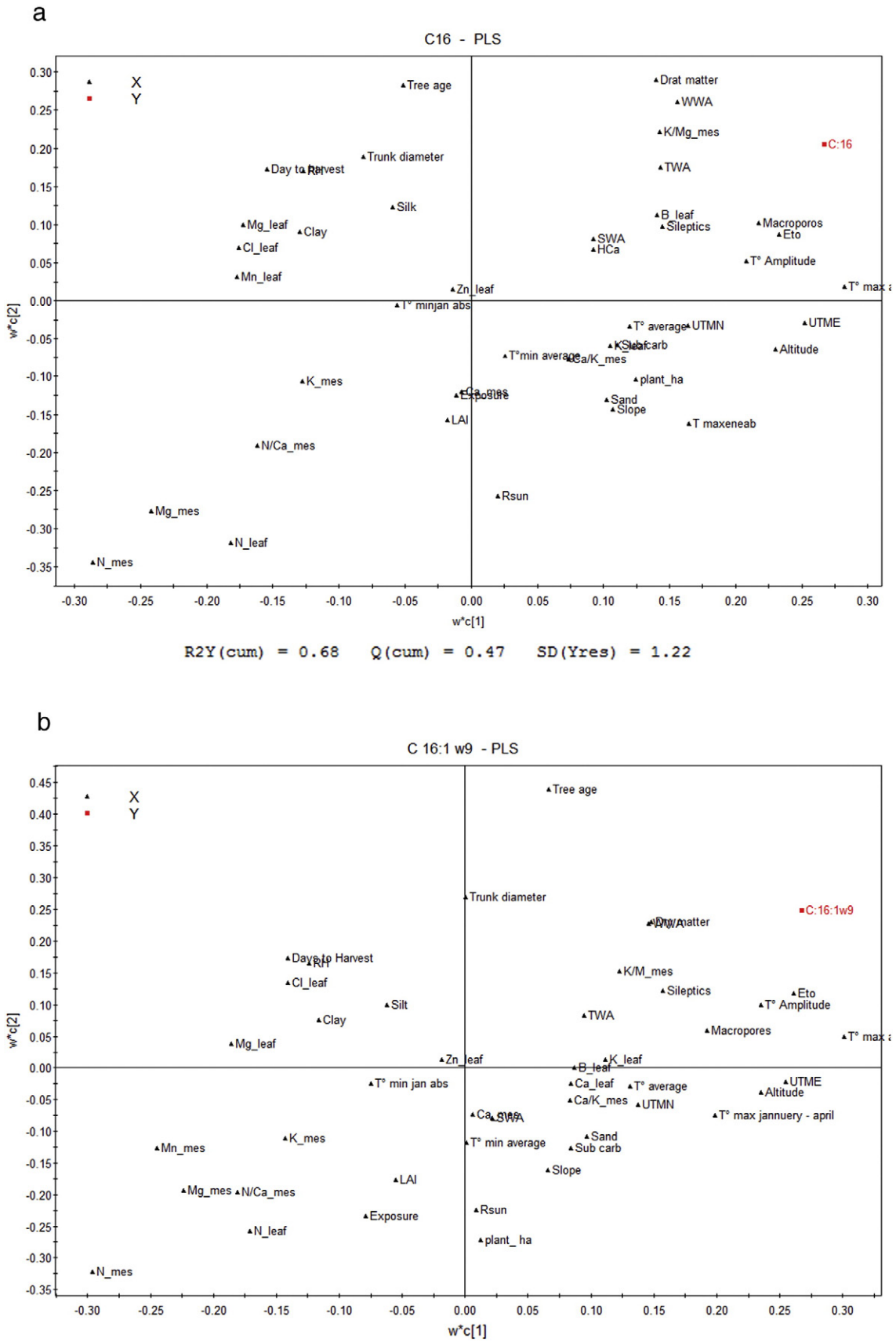
coordinates, reference evapotranspiration, and site altitude) and those related to plant nutrition (N and Mg concentrations in the mesocarp at harvest). The independent variables related to climate were positively associated with the concentrations of palmitic and palmitoleic acids in the mesocarp (Fig. 2b), while those related to plant nutrition were negatively correlated with the concentration of palmitoleic acid in the fruits. The variables that affected the concentration of palmitic and palmitoleic acids in the mesocarp were similar to those that affected the concentration of oleic acid, but inverse (Figs. 1, 2a and b); in the lower and cooler localities, palmitic and palmitoleic acid concentrations decreased and oleic acid increased, while the opposite was true in the higher and warmer localities (Fig. 3).

The maximum mean annual temperature is largely responsible for the contents of oleic and palmitic acids in the fruit mesocarp (Fig. 3). In the warmest locality, the maximum mean annual temperature is  $23.3^\circ\text{C}$  and the palmitic acid concentration was 25% greater than in the coldest locality (where the maximum mean annual temperature

**Table 3**  
Physical soil characteristics (macroporosity) and nutrients (N, Mg, Mn in leaves; and N, Mg in mesocarp) content of avocado from five experimental sites located in the low, middle, and high elevations, that according to partial minimum squares multivariate regression (PLS) are related to the concentration of fatty acids.

Zones	Leaf N (%)	Leaf Mg (%)	Leaf Mn (ppm)	Mesocarp N (%)	Mesocarp Mg (%)	Macroporosity (%)	Dry matter at harvest (%)
Low	$2.2 \pm 0.11$	$0.70 \pm 0.15$	$700 \pm 156$	$1.4 \pm 0.1$	$0.11 \pm 0.0$	$15.2 \pm 0.0$	$25.6 \pm 1.3$
Lower middle	$2.2 \pm 0.10$	$0.53 \pm 0.06$	$458 \pm 71$	$1.7 \pm 0.2$	$0.11 \pm 0.0$	$16.1 \pm 0.0$	$23.1 \pm 1.2$
Middle	$2.1 \pm 0.07$	$0.49 \pm 0.07$	$93 \pm 6$	$1.3 \pm 0.2$	$0.10 \pm 0.0$	$11.9 \pm 0.0$	$26.9 \pm 2.6$
High middle	$1.5 \pm 0.05$	$0.46 \pm 0.03$	$241 \pm 43$	$0.9 \pm 0.5$	$0.08 \pm 0.0$	$40.3 \pm 0.0$	$26.5 \pm 1.6$
High	$2.1 \pm 0.13$	$0.44 \pm 0.10$	$275 \pm 54$	$1.1 \pm 0.1$	$0.09 \pm 0.0$	$22.1 \pm 0.0$	$24.5 \pm 1.3$





**Fig. 2.** Partial minimum squares multivariate regression (PLS) of palmitic (a) and palmitoleic (b) acids and the nutritional content of plant and fruit, climate characteristics, plant vegetative development, and physical characteristics of the soil.

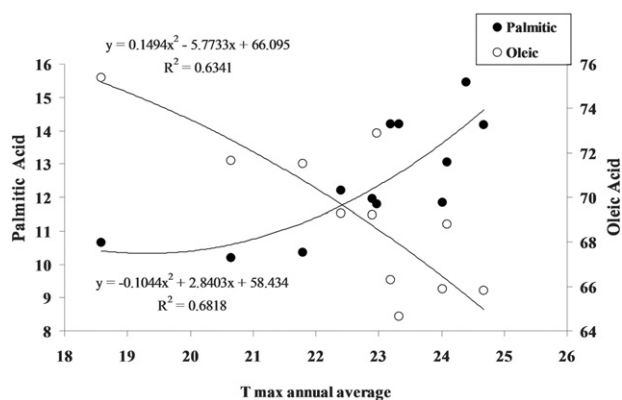


Fig. 3. Relationship between maximum mean temperature and the content of oleic and palmitic acids.

was 4.7 °C less). This agrees with Requejo-Tapia et al. (1999), who compared orchards in two climatic zones of New Zealand and found that on plantations with lower mean temperatures, the fruit has less palmitoleic acid and more oleic acid than in warmer areas.

#### 4. Conclusions

In this study, we showed that concentration of the main fatty acids of the 'Hass' avocado mesocarp are influenced by climatic and nutritional factors. The maximum mean annual temperature was found to be the most important variable affecting the concentrations of oleic, palmitic, and palmitoleic acids. The mesocarp of avocado fruits had lower amounts of 18-carbon fatty acids and greater concentrations of 16-carbon fatty acids in localities with higher temperatures. The N and Mg contents of fruit mesocarps at harvest were related to the contents of palmitic and palmitoleic acids and increases in the levels of N and Mg decreased the content of 16-carbon fatty acids.

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