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Influence of plant density on fruit and foliar nutritional composition for Hass avocado in Colombia

Influencia de la densidad de plantío del aguacate Hass sobre el contenido de nutrientes en frutos y hojas en Colombia

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ABSTRACT

This study aimed to determine the effect of plant density on avocado fruit and leaf nutritional composition in commercial orchard *cv.* Hass in the department of Antioquia, Colombia. The experimental design was a randomized complete block with three replicates. The treatments consisted of six plant densities (204, 278, 333, 400, 625, and 816 tree ha^{-1}), and each experimental unit consisted of six 9-year-old trees. The 333 and 400 trees ha^{-1} plant densities presented the highest leaf concentrations of N, P, Ca, Mg, S, Zn, and B and fruits' lowest saturated fatty acid contents. The nutritional balance index for N, Ca, Mg, S, Fe, Mn, Zn, and B was significantly affected by plant densities. The plant density significantly does not affect K, Ca, S, and Fe concentration in fruits, and the percentage of avocado fruits by size showed no significant differences due to plant distances.

Keywords:
Fruit quality
Harvest season
Main harvest
Planting system
Secondary harvest
Tree spacing

RESUMEN

El presente estudio tuvo como objetivo determinar el efecto de la densidad de plantío sobre la composición nutricional de frutos y hojas de aguacate en un huerto comercial del *cv.* Hass en el departamento de Antioquia, Colombia. Se utilizó un diseño experimental bloques completos al azar con tres repeticiones. Los tratamientos consistieron en seis densidades de plantío (204, 278, 333, 400, 625 y 816 árboles ha^{-1}) y la unidad experimental estuvo compuesta por seis árboles de 9 años de edad. Las densidades de plantas de 333 y 400 árboles ha^{-1} presentaron las mayores concentraciones foliares de N, P, Ca, Mg, S, Zn y B y los menores contenidos de ácidos grasos saturados en frutos. El índice de balance nutricional para N, Ca, Mg, S, Fe, Mn, Zn y B se vio afectado significativamente por las densidades de las plantas. La densidad de plantas no afectó significativamente la concentración de K, Ca, S y Fe en los frutos, y el porcentaje de frutos de aguacate por calibre no mostró diferencias significativas debido a las distancias de las plantas.

Palabras clave:
Calidad del fruto
Época de cosecha
Cosecha principal
Sistemas de siembra
Cosecha secundaria
Espaciamiento entre árboles

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World avocado production in 2021 was 8,865,672 tons, and Colombia achieved 11.28% of the total avocado production. Mexico had 26.40%, Peru 8.95%, and the Dominican Republic 7.30%. Regarding the harvested area, Colombia ranked second (10.97%), behind Mexico (26.40%) (FAO 2023). In the last decade, Colombia increased the harvested area by 335%, going from 21,592 ha (2010) to 94,111 ha (2021). In the same period, production went from 205,443 tons to 976,617 tons, representing an increase of 381% (FAO 2023). However, even though the potential yield of the avocado tree is 32.5 t ha^{-1}, the average world yield was 10.12 t ha^{-1}, presenting a 70% gap to the potential yield of the species for the year 2020 (Gazit and Degani 2013; FAO 2023).

Avocado commercial orchards are traditionally established at wide spacing, between 7x7 m (204 trees per ha) and 10x10 m (100 trees per hectare). The yield per unit area is low during the first few years after planting, which increases until the trees begin to shade each other. The yields decrease after 5 to 10 years; nonetheless, there is great interest in using high-density plantations to increase productivity and yields, especially in the early life of orchards (Menzel and Lagadec 2014). In avocados, the challenge is to reduce the time between planting and full canopy development and maintain orchard productivity and fruit quality once the tree reaches total growth. When establishing an orchard, the choice of planting rate determines the time it takes for the canopy to develop fully, achieving maximum light interception (Whiley et al. 2013). Thus, optimal plant density improves the photosynthetic canopy yield by increasing the light penetration to lower leaves in the canopy, which is beneficial for yield and fruit quality (Ding et al. 2022). There are many options for plant density and orchard design for a given environmental, social, and economic condition. The choice must aim at an appropriate balance between simplicity and complexity that corresponds to the ability to manage and maintain the orchard (Whiley et al. 2013).

Recently, different plant densities, especially the high ones, have been used without evaluating them under Colombian conditions, where temperature and relative humidity limit the continuous development of trees. The topography, cultivar, growing systems pruning, and driving have become critical factors in determining the optimal tree spacing and orchard design (Bernal-Estrada and Díaz-Díaz 2020). So, there has been a lot of interest in using high-density plantations to increase yields, fruit quality, and productivity, especially in the early life of orchards (Menzel and Lagadec 2014). Therefore, the improvements in the fruit quality caused by changing cultivation strategies have been little studied in recent years (Salazar-García et al. 2019). The avocado cultivar and the management practices and conditions where it is cultivated influence the fruit's nutrient concentration (Salazar-García et al. 2019). This is important since the leaf concentration and balance of nutrients at the time of harvest of the avocado fruit is fundamental to ensure the yield and quality of avocado (Arpaia et al. 2015; Salazar-García et al. 2019).

Proper plant density not only helps obtain a high yield and increases fruit quality but also saves labor and production materials (Jovicich et al. 2004); determining the appropriate plant density could provide important economic benefits for growers (Ding et al. 2022). Numerous studies have proven that changing plant density impacts yield (Calori et al. 2017; Haque and Sakimin 2022; Cano-Gallego et al. 2023). Colombia has high genetic and agroecological variability in which avocados are grown, which generates both heterogeneous production systems and management agricultural practices heterogeneity influencing their yield and fruit quality (Carvalho et al. 2014; Tamayo et al. 2018). Although planting high densities has the potential to obtain avocado higher yields than traditional plantations, especially in the first years of production (Menzel and Lagadec 2014), very little is known about plant density effects elements in the canopy. For the above, the present study aims to determine the effect of plant density on fruit and leaf nutritional composition in commercial orchards of Hass avocado in the department of Antioquia, Colombia.

MATERIALS AND METHODS

Location

This research was carried out in two 9-year-old commercial orchards of avocado *cv.* Hass in Antioquia, Colombia, for three consecutive years (2019-2021). The avocado trees planted were grafted on creole rootstock with a scion bud of *cv.* Hass and a minimum of five growth points (standard procedure). The first orchard was located in the Rionegro municipality at 06°5'56.8"LN and 075° 26' 21.9" LW (2,200 meters above sea level (masl)). The second one was in El Peñol municipality at 06°11'28.4"LN and 075° 14' 34.4" LW (2,100 masl). The WatchdogTM 2000 portable weather stations (Spectrum Technologies, 3600 Thayer Court, 107 Aurora, IL 60504) recorded climatic variables in each location. Rionegro presented a mean temperature of 17.2 °C, a maximum of 23.8 °C, and a minimum of 13.0 °C, with an annual rainfall of 1,800 mm. In El Peñol, a mean temperature of 18.5 °C, a maximum of 23.0 °C and a minimum of 14.9 °C was recorded, with an accumulated annual rainfall of 1,921 mm. According to Belda et al. (2014), the region's climate is Cw subtropical dry-winter, according to Köppen's classification. The soil of the experimental area is representative of the region, being classified as an Andosol according to FAO World Reference Base classification (Delmelle et al. 2015).

Experimental design

A randomized complete block experimental design with three replications was used. The experiment unit consisted of 15 *cv*. Hass avocado trees and the treatments of six plant densities. The plant densities (trees ha^{-1}) evaluated were: 204 (7x7 m), 278 (6x6 m), 333 (6x5 m), 400 (5x5 m), 625 (4x4 m) and 816 (3.5x3.5 m).

Five harvest seasons were carried out between 2019 and 2021. Three main harvests (2019M, 2020M, and 2021M) and two secondary harvests (2020S and 2021S). The main harvest (February-March flowering period) was carried out between December and January (next year), and the secondary (mitaca) harvest (August-September flowering period) was carried out in June-July (next year).

Soil analysis

The soil nutrient availability was carried out after each harvest (2019M, 2020S, 2020M, 2021S, 2021M). Subsamples below the tree canopy and at a depth of 0-30 cm were taken for chemical analysis. The results of the soil analysis are presented in Table 1.

Table 1. Chemical soil characteristics.

Treatment	pH	EC	OM	Al	P	S	CEC	Ca	Mg	K	Fe	Cu	Mn	Zn	B
		(Ds m^{-1})	(%)	(mg kg^{-1})			(cmol kg^{-1})				(mg kg^{-1})				
204	5.17	2.27	20.93	0.39	44.25	98.14	18.56	13.90	2.29	1.52	96.73	4.94	11.51	78.62	2.31
278	5.68	0.83	21.87	*	33.96	51.55	16.42	11.65	3.01	1.62	138.53	4.79	4.91	202.12	3.61
333	5.15	1.55	21.88	0.58	43.10	71.05	14.94	10.86	2.11	0.90	133.38	5.23	12.77	78.09	1.98
400	5.82	1.49	11.18	*	73.76	43.63	19.17	14.89	2.78	1.33	152.71	6.09	7.60	64.26	1.77
625	4.94	2.04	23.86	1.94	99.28	174.86	12.38	5.94	1.14	2.67	815.03	9.77	7.69	78.78	4.93
816	5.14	1.06	19.56	0.85	46.65	69.05	7.78	4.61	1.05	0.82	143.77	7.01	5.34	32.67	2.31

*Not available

Mineral leaf content

Four leaves per tree were selected for the analysis of leaf tissue, one at each cardinal point. The fifth leaf of the last growth flow was collected after each harvest (2019M, 2020S, 2020M, 2021S, 2021M). The leaf was mature but not senescent, without fruiting, healthy (without physical or chemical damage or affected by pests or diseases), and older than three months, according to Maldonado (2002). The leaves were washed with distilled water and dried at 60 °C for 48 h in an oven with forced air circulation, Memmert UL 80 (Memmert GmbH + Co. KG, Büchenbach, Germany), or until a constant weight was reached. Next, they were milled, and placed in paper bags, and the total contents of N (EPA method 351.3) where determinated (USEPA 1993), P, K, Ca, Mg, Cl, S, Fe, Cu, Mn, Zn (inductively coupled plasma atomic emission spectrometer iCAP 7000 Plus (Thermo Scientific, Waltham, MA)), and B according to modified NTC 5404 (ICONTEC 2011).

Nutritional balance index

To determine the nutritional balance index (NBI), Salazar-García and Lazcano-Ferrat (2001) adjusted the Kenworthy methodology for the *cv*. Hass avocado was used. The nutrient leaf analysis results were used to determine the balance index (B) for each mineral element, according to Equations (1) and (2).

Equation 1. Suppose the value reported in the laboratory (X) was less than the standard value:

$$P = (X/S)100 \quad I = (100-P)\ (CV/100) \quad B = P + I \qquad (1)$$

Equation 2. If the value reported in the laboratory (X) was greater than the standard value:

$$P = (X/S)100 \quad I = (P-100)\ (CV/100) \quad B = P + I \qquad (2)$$

where, S = standard value, I = influence of the variation, P = percentage of the standard, CV = coefficient of variation and B = balance index. Standard values (S) and coefficients of variation (CV) were used according to Salazar-García and Lazcano-Ferrat (2001).

Mineral fruit composition

To evaluate the concentration of nutrients, 75 fruits were randomly taken per plant distance, and each harvest with approximately 24% of dry material. Subsequently, the fruit structures were individualized, removing the peel, pulp, seed coat, and seed. All the fruit components were dried until a constant weight was reached in forced air ovens at 60 °C. After drying, all fruit components were milled to determine N (EPA method 351.3) (USEPA 1993), P, K, Ca, Mg, Cl, S, Fe, Cu, Mn, Zn (inductively coupled plasma atomic emission spectrometer iCAP 7000 Plus (Thermo Scientific, Waltham, MA)), and B according to modified NTC 5404 (ICONTEC 2011), by treatment and harvest in each location.

Fruit fatty acid and vitamin E profile

Five avocado fruits were taken per tree (180 per treatment) for each harvest season (2019M and 2020S) to quantify the fatty acid content. For each fruit, the mesocarp (pulp) was removed, homogenized, and lyophilized to determine the content (grams of fatty acid per 100 grams of fresh pulp) of oleic, palmitoleic, palmitic, linoleic, eladic, stearic, and fatty saturated acid. A gas chromatograph coupled to a mass spectrometer (Agilent 7890/MSD 5975C) was used, equipped with a capillary column (ZB-5 Zebron of low polarity) to separate the compounds of interest.

Vitamin E content (g α-tocopherol/100 g fresh pulp) was determined from five avocado fruits per tree (180 fruit per treatment) for each harvest season (2019M and 2020S). The mesocarp (pulp) was removed, homogenized, and frozen for each fruit. A gas chromatograph coupled to a mass spectrometer (Agilent 7890/MSD 5975C) was used before derivatization using BSTFA.

Fruit caliber

A random selection of 75 fruits per tree was each harvested. It was weighted and individually characterized by caliber according to established by the FAO in the CODEX STAN 197-1995 Revision (Table 2) for export sizes (FAO 2011).

Table 2. Fruit quality of avocado cv. Hass is characterized by weight and size for export according to CODEX STAN 197-1995.

Caliber	Fruit weight (g)		Caliber	Fruit weight (g)	
	Minimum	Maximum		Minimum	Maximum
Discard	0	80	24	170.1	181
Industrial	80.1	94	22	181.1	200
32	94.1	135	20	200.1	217
30	135.1	149	18	217.1	249
28	149.1	160	16	249.1	284
26	160.1	170	14	284.1	600

(FAO 2011).

Statistical analysis

The statistical analysis was performed using the agricolae package in the R project statistical program (R Core Team 2021). A two-way ANOVA was carried out for the plant density factor (204, 278, 333, 400, 625, and 816 trees ha^{-1}) and the harvest season factor (2019M, 2020S, 2020M, 2021S, and 2021M). Differences between means were evaluated through analysis of variance, followed by Tukey's HSD mean comparison test, with a probability greater than 95%. A Pearson correlation analysis was used to examine the relationship among the content of all nutrients in the fruit and leaves of avocado.

RESULTS AND DISCUSSION

Leaf mineral composition

Table 3 shows the nutritional composition of avocado tree leaves, where there were significant differences due to plant density (*P*<0.001) and harvest time (*P*<0.001). The 333 and 400 trees ha^{-1} plant densities presented the highest leaf concentrations of N, P, Ca, Mg, S, Zn, and B. For K and Fe, higher concentrations were observed in the density of 625 trees ha^{-1}, and Mn was present in higher concentrations in the density of 204 trees ha^{-1}. On the contrary, the highest and lowest densities (204 and 816 trees ha^{-1}) reached the lowest concentrations of the evaluated nutrients. The 2019M harvest presented the highest levels of nutrients for N, Ca, Mg, S, Fe, Mn, and Zn; the other nutrients do not show marked significant differences at a particular plant density (Table 3).

Table 3. Effect of plant density and harvest season on *cv.* Hass avocado leaf mineral composition.

Treatment	N	P	K	Ca	Mg	S	Fe	Mn	Zn	B
	(%)						(mg kg^{-1})			
Plant density*/*P* values	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
204	2.18^{b}	0.11^{b}	0.97^{c}	1.42^{b}	0.35^{b}	0.19^{c}	60.82^{e}	166.31^{a}	37.82^{b}	45.1^{2b}
278	2.09^{cd}	0.09^{c}	0.95^{cd}	1.36^{bc}	0.33^{b}	0.16^{e}	104.75^{b}	105.92^{bc}	22.86^{c}	24.53^{e}
333	2.25^{b}	0.12^{b}	0.87^{d}	2.01^{a}	0.40^{a}	0.24^{b}	74.98^{d}	124.97^{b}	28.95^{c}	30.90^{d}
400	2.36^{a}	0.14^{a}	1.06^{b}	2.03^{a}	0.40^{a}	0.28^{a}	72.02^{d}	118.17^{bc}	49.91^{a}	50.76^{a}
625	2.17^{b}	0.09^{c}	1.14^{a}	1.28^{c}	0.33^{b}	0.19^{cd}	117.11^{a}	103.77^{c}	25.30^{c}	36.93^{c}
816	2.03^{c}	0.11^{b}	1.01^{bc}	1.45^{b}	0.39^{a}	0.18^{de}	85.43^{c}	38.75^{d}	22.69^{c}	26.78^{e}
Year/*P* values**	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
2019M	2.23^{ab}	0.10^{b}	1.01^{b}	2.01^{a}	0.43^{a}	0.28^{a}	122.34^{a}	164.96^{a}	39.09^{a}	30.56^{c}
2020S	2.08^{d}	0.10^{b}	1.00^{b}	1.40^{d}	0.35^{b}	0.19^{b}	78.87^{c}	92.19^{c}	27.27^{bc}	46.71^{a}
2020M	2.28^{a}	0.12^{a}	0.90^{c}	1.50^{c}	0.33^{b}	0.18^{b}	87.22^{b}	92.94^{c}	26.77^{c}	36.87^{b}
2021S	2.16^{bc}	0.11^{a}	1.08^{a}	1.34^{d}	0.34^{b}	0.19^{b}	58.04^{d}	72.22^{d}	30.33^{bc}	37.31^{b}
2021M	2.15^{cd}	0.12^{a}	1.02^{ab}	1.71^{b}	0.37^{b}	0.19^{b}	82.79^{bc}	125.94^{b}	32.81^{b}	27.72^{d}
Mean	2.18	0.11	1.00	1.59	0.36	0.21	85.85	109.65	31.25	35.83

*Trees ha^{-1} **Main harvesting (M). Secondary harvesting (S). Values with similar letters for each nutrient within location or rootstock/scion relation are significantly different (HSD, *P*<0.05).

In addition to the foliar nutrient diagnosis status and the amount of nutrients removed, the assimilation dynamics of the different nutrients during fruit development are important to precise fertilization management decisions (Silber et al. 2018; Salazar-García et al. 2019). Maldonado-Torres et al. (2007) set the nutritional standards for the *cv.* Hass avocado for a yield greater than 20 t ha^{-1}. Therefore, according to the results, the nutritional concentrations of N, K, Ca, Fe, Mn, and Zn were between optimum and high levels, while P was at low levels, and Mg and B were at low concentrations. Maldonado-Torres et al. (2007) stated that the low level of P determined in these soils (Andosols) might be associated with the fixing effect of allophane, which has sometimes retained up to 2,000 mg kg^{-1}. Similar results were found by Tamayo-Vélez et al. (2022), who evaluated the leaf nutritional content in avocado trees in the high Andean region in Colombia.

According to Salazar-García and Lazcano-Ferrat (2003), Maldonado-Torres et al. (2007) and Campos and Calderón (2015) the recommended reference levels of nutrients in leaves for Hass avocado vary for N (1.9 – 2.31%), P (0.11 – 0.18%), K (0.5 – 1.4%), Ca (1.0 – 3.0%), Mg (0.3 - 0.8%), Fe (85 – 114 mg kg^{-1}), B (30 - 240 mg kg^{-1}), Zn (20 – 150 mg kg^{-1}) and Mn (30 – 500 mg kg^{-1}). From these reference values and according to the mineral composition of the leaf, only values below the reference were observed for P (278 and 625 trees ha^{-1}), which indicates that despite having observed significant differences in the concentration of nutrients in the leaf in response to the planting densities, in general the observed values were within the reference ranges for an optimal condition for the Hass cultivar water (Maldonado-Torres et al. 2007).

Nutritional balance index

There were significant differences in plant density in the nutritional balance index (NBi) for N, Ca, Mg, S, Fe, Mn, Zn, and B. While NBi for P and K did not present significant differences (*P*>0.05) for any of the plant densities evaluated. Similarly, the 2019M harvest season showed the highest NBi for all mineral nutrients except boron (Table 4).

Balanced and timely nutrition is essential to ensure the yield and quality of avocados. The avocado cultivar, the management practices, and the environmental conditions where it is cultivated influence the concentration of nutrients in the leaves and fruits (Salazar-García et al. 2019). Hence, in assessing the nutritional status of plants, Manzoor et al. (2022) recommend using the nutritional balance index (NBI) to seek the plants' nutrient balance. Leaf nutritional content allows the avocado leaves, according to the nutrient balance index proposed by Kenworthy (1973), as scarce (15-49%), below normal (>49-83%), normal (>83-117%), above normal (>117-151%), and excess (>151-185%). According to our results, although there were differences between plant densities, the N, P, K, Ca, and Fe generally had normal balances, while Mg, S, and Mn were below normal, indicating possible nutritional deficiency without symptoms being observed. Similar results were reported by Tamayo-Vélez et al. (2022), who evaluated the effect of rootstock/scion compatibility in the same regions. These authors found that the N, P, Ca, Fe, and Zn showed normal NBi, while K, Mg, S, Mn, and B were below normal, indicating possible nutritional deficiency without symptoms being observed.

Table 4. Nutritional balance index (NBi) for the avocado *cv*. Hass leaf for different plant densities and harvest seasons.

Treatment	NB	PB	KB	CaB	MgB	SB	FeB	MnB	ZnB	BB
Plant density*/ *P values*	<0.001	0.07	0.403	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
204	94^{bc}	114	114	81^{b}	66^{b}	54^{c}	80^{d}	82^{a}	154^{b}	80^{b}
278	91^{cd}	68	74	78^{bc}	63^{b}	47^{e}	128^{b}	66^{b}	90^{c}	66^{e}
333	97^{b}	85	69	111^{a}	73^{a}	66^{b}	90^{cd}	73^{b}	119^{c}	70^{d}
400	101^{a}	99	81	113^{a}	73^{a}	73^{a}	87^{d}	69^{b}	213^{a}	84^{a}
625	93^{bc}	68	86	75^{c}	64^{b}	52^{cd}	146^{a}	65^{b}	100^{c}	74^{c}
816	88^{d}	81	78	82^{b}	72^{a}	50de	102^{c}	49^{c}	92^{c}	67^{e}
Year/*P* values**	<0.001	0.644	0.228	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
2019M	96^{ab}	73	78	112^{a}	78^{a}	74^{a}	154^{a}	83^{a}	167^{a}	70^{c}
2020S	90^{c}	97	77	80^{cd}	66^{b}	53^{b}	96^{c}	62^{c}	104^{c}	81^{a}
2020M	98^{a}	88	71	84^{c}	69^{b}	52^{b}	104^{c}	63^{c}	107^{c}	74^{b}
2021S	93^{bc}	83	114	78^{d}	66^{b}	54^{b}	96^{b}	57^{c}	127^{bc}	75^{b}
2021M	93^{bc}	88	79	95^{b}	69^{b}	53^{b}	78^{c}	71^{b}	135^{b}	68^{d}
Mean	94	86	84	90	70	57	106	67	128	74

* Trees ha^{-1} **Main harvesting (M). Secondary harvesting (S). Values with similar letters for each nutrient within location or rootstock/scion relation are significantly different (HSD, *P*<0.05).

Plant density is known to affect plant traits and growth above ground significantly. Nevertheless, understanding the effects of sowing density on fruit and leaf nutrient accumulation is essential in determining an adequate fertilization program to guarantee acceptable yield and fruit quality (Hecht et al. 2019). More plants at higher plant densities require and take up more nutrients. This does not necessarily occur proportionally due to competition, which can be asymmetric when plants of different sizes compete. Instead, the competition might trigger similar root growth and canopy plasticity responses as low resource availability significantly changes the root and canopy system architecture and functioning (Hecht et al. 2019; Tun et al. 2018; Cano-Gallego et al. 2023) and soil elements availability (Reddy et al. 2014).

Fruit mineral composition: The plant density significantly does not affect the nutrients in the avocado fruit for the minerals K, Ca, S, and Fe. Conversely, the contents of N, P, Mg, Na Cu, Mn, Zn, and B were significantly affected by plant density. However, a homogeneous behavior was not observed in the variation of this variable; in general, the plant densities of 333 and 400 trees ha^{-1} presented high contents of all nutrients in fruit, except for Cu, whose contents were medium (Table 5). A similar result was reported: nitrite content in the fruit is also the lowest in the low-density treatment in cucumber (Ding et al. 2022), and a positive relation was found between leaf N to pulp N concentrations (Arpaia et al. 1996).

Regarding the nutritional composition in each fruit tissue, the highest contents of all the nutrients were observed in the seed

Table 5. Effect of plant density on *cv.* Hass avocado fruit mineral composition assessment in Colombia. Values are averages of 3 years (2019-2021).

Treatment	N	P	K	Ca	Mg	Na
	(%)					
Plant density*/*P* values	<0.001	<0.001	<0.001	0.121	<0.01	<0.001
204	0.8895^{a}	0.0805^{bc}	1.3450^{a}	0.1196	0.1087^{b}	0.0049^{bc}
278	0.7693^{c}	0.0662^{c}	1.2425^{a}	0.1510	0.1049^{b}	0.0072^{ab}
333	0.8730^{ab}	0.1111^{a}	1.3300^{a}	0.1397	0.1150^{b}	0.0037^{bc}
400	0.8982^{a}	0.1079^{a}	1.3589^{a}	0.1168	0.2116^{a}	0.0015^{c}
625	0.8251^{abc}	0.0618^{c}	1.3579^{a}	0.1368	0.0987^{b}	0.0108^{a}
816	0.8081^{bc}	0.1009^{ab}	1.3241^{a}	0.1426	0.1156^{b}	0.0031^{bc}
Fruit tissue/*P* values	<0.001	<0.001	<0.001	<0.001	<0.001	<0.05
Peel	0.835^{c}	0.083^{b}	1.115^{c}	0.123^{b}	0.095^{b}	0.004^{ab}
Pulp	0.917^{bc}	0.105^{a}	1.726^{a}	0.106^{b}	0.108^{b}	0.008^{a}
Seed coat	0.973^{a}	0.086^{b}	1.487^{b}	0.255^{a}	0.255^{a}	0.005^{ab}
Seed	0.650^{d}	0.078^{b}	0.978^{d}	0.052^{c}	0.045^{b}	0.004^{ab}
Treatment	**S**	**Fe**	**Cu**	**Mn**	**Zn**	**B**
	(%)	(mg kg^{-1})				
Plant density*/*P* values	0.0997	<0.01	<0.001	<0.001	<0.001	<0.001
204	0.072	28.840^{ab}	12.194^{a}	19.344^{a}	20.055^{ab}	65.922^{a}
278	0.070	28.777^{ab}	4.228^{cd}	18.270^{a}	16.459^{c}	40.065^{c}
333	0.072	23.929^{b}	6.313^{bc}	17.313^{a}	20.205^{ab}	53.865^{b}
400	0.080	26.087^{ab}	6.867^{b}	16.893^{a}	21.881^{a}	63.952^{a}
625	0.067	31.063^{a}	2.640^{d}	15.496^{b}	17.784^{bc}	63.553^{a}
816	0.063	27.594^{b}	3.605^{d}	11.448^{b}	16.268^{c}	40.903^{c}
Fruit tissue/*P* values	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Peel	0.054^{b}	32.256^{b}	8.304^{a}	10.424^{b}	18.269^{b}	54.254^{b}
Pulp	0.090^{a}	24.442^{c}	4.469^{b}	7.072^{bc}	15.905^{c}	50.048^{b}
Seed coat	0.088^{a}	39.548^{a}	7.834^{a}	42.567^{a}	32.387^{a}	84.661^{a}
Seed	0.051^{b}	14.613^{d}	3.291^{b}	5.780^{c}	8.541^{d}	29.877^{c}

*Trees ha^{-1} ** Values with similar letters for each nutrient within location or rootstock/scion relation are significantly different (HSD, P<0.05).

coat and pulp. In contrast, the peel and seed only presented higher values than the rest of the tissue in Na levels. Similar results were reported by Tamayo-Vélez et al. (2022), who found, in general, the highest nutrient contents in the seed coat. The seed coat provides an interface between the embryo and the external environment, can explain altered processes in fruits during embryogenesis, dormancy, and germination, and has a role in determining seed size (Haughn and Chaudhury 2005).

The concentration of elements in avocado fruits was in decreasing order of K>N>Ca>Mg>P>S>B>Fe>Zn>Mn>Cu. In this regard, Reddy et al. (2014) found a similar element concentration in avocado *cv*. Hass and Fuerte (Mg>Ca>Zn>Fe>Mn>Cu). Since the mesocarp is the edible part of the avocado fruit, its quality at consumption maturity usually identifies whether adequate orchard management was carried out (Salazar-García et al. 2019). It suggested that avocado fruits are nutritional as a good dietary source of the micronutrients Cu and Mn, contributing 75 and 34% towards dietary reference intake for these elements by avocado fruit consumption (Reddy et al. 2014). Mesocarp concentrations of both N and Ca are relevant for postharvest fruit quality (Salazar-García et al. 2019). Fruit from trees high in N showed increased susceptibility to chilling injury and a shorter ripening time (Kruger et al. 2016). Otherwise, high Ca levels have decreased cold-induced disorders (Salazar-García et al. 2019).

Some antagonistic and synergistic relationship between the soil exchangeable minerals and mineral concentration in avocado *cv*. Hass fruit has been reported by Reddy et al. (2014). Soil Al excess reduces *cv*. Hass fruit Cu (-0.8) and Fe (-0.9), Fe excess reduces Cu (-0.9) and Zn (-0.7), Ca excess reduces Mn (-0.7), and Mg excess reduces Mg (-0.8). On the other hand, the synergistic effects include soil Al excess increase in the Mn (0.8) and Cu (0.9) fruit concentration, Soil Mg excess increase in Mn (1.0) and Cu (0.9) fruit concentration, and soil Ca excess increase in Mn (0.7), Ca (0.7) and Cu (0.7) fruit concentration. The local soil presented strongly acid soils with high organic matter content, high and very high Ca, Mg with medium to low ranges, and high K. Minor elements are reported to have very high contents of iron, medium to high for manganese and zinc, and medium to low for copper and boron (Tamayo-Vélez et al. 2022). Ding et al. (2022) demonstrated that changing plant density has significantly improved the quality of the cucumber fruit.

In the case of avocado *cv*. Hass, it has been observed that increasing the N concentration in the leaf leads to a higher N content in the pulp (Tamayo et al. 2018). This finding was further confirmed in this study, where the highest leaf and fruit N concentrations were observed at intermediate plant densities (333 and 400 trees ha^{-1}). This suggests that plant density can play a significant role in determining fruit quality, with intermediate plant densities leading to higher N concentrations in the fruit.

Correlation matrix

The correlation matrix shows that some elements are associated with others in avocado leaves and fruits (Figure 1). The conventional approach to interpreting a correlation coefficient correlation selected was a very strong correlation (greater than 0.900), strong correlation (between 0.700 and 0.899), moderate correlation (between 0.400 and 0.699), and weak correlation (between 0 and 0.399) (Schober et al. 2018). S is positive and strongly associated with Ca in leaves and moderately associated with Mg, Zn, and N. Mg is moderately associated with Ca. On the other hand, P and Fe show a negative moderate association. In addition, some elements, mainly Fe and K, show no or minimal correlation with others (Figure 1A). In fruit, Zn is positive and strongly associated with Mn and B and moderately associated with Fe, Ca, and N. B is moderately associated with N, Mn, and K. Conversely, no negative association was observed, and P shows no or minimal correlation with other (Figure 1B). Lazare et al. (2020) reported significant correlations in most nutrients with the others in *cv*. Hass leaves with the others. The strongest correlations (more than 0.7) were between Mg and Ca, P and Cu, Mg and Mn, N and P, and Mg and N.

Silber et al. (2018) demonstrated high Zn concentration in the productive organs of avocados probably because Zn is transported in the phloem, unlike the other elements that are transported in the xylem. Zn is involved in diverse plant functions such as photosynthesis, sucrose and starch formation, protein metabolism, membrane integrity, auxin metabolism, flowering, and seed production. Zn shows a significant correlation with N, P, and B in leaves, while in fruits, with Cu, Fe, and S (Figure 1).

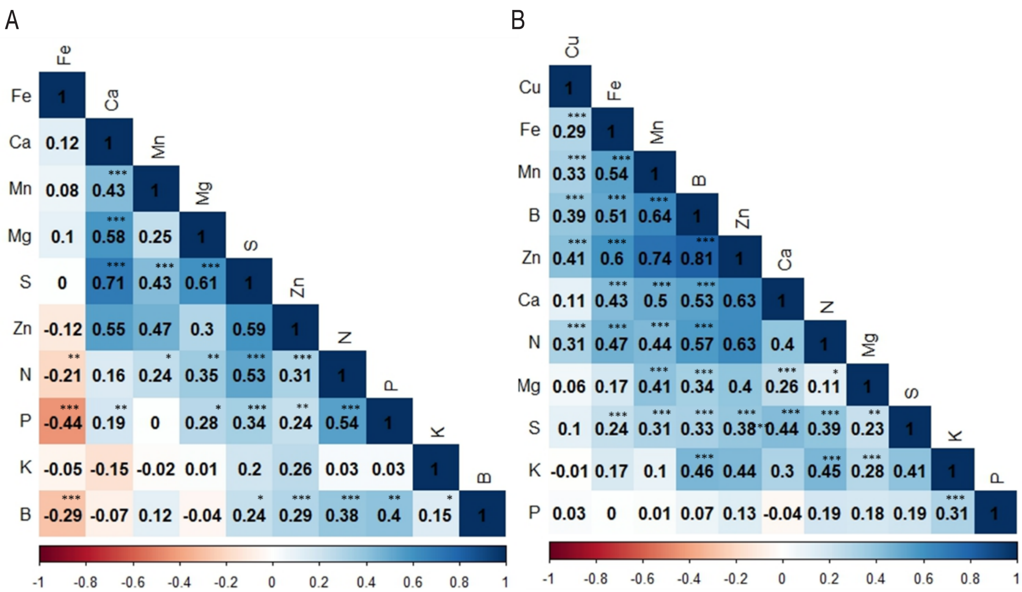

Figure 1. Pearson pairwise correlation matrix among all the nutrient contents in leaves (A) and fruit (B) of avocado *cv.* Hass. Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

Fatty acid composition of avocado fruit mesocarp

Figure 2 summarizes the contents of fatty acids present in the pulp of the fruits for the plant densities (Figure 2A) and harvest seasons (2019M and 2020S) (Figure 2B). The fruit harvested from trees in high densities presented the highest concentrations (g 100 g^{-1}) of Palmitoleic acid (3.01), Palmitic acid (7.15), Elaidic acid (1.61), and saturated fatty acid (7.38). On the contrary, the plant density of 333 and 400 trees ha^{-1} had the lowest contents for this same group of acids (Palmitoleic acid - 0.55 g; Palmitic acid - 1.63 g; Linoleic acid - 1.11 g; Oleic acid – 2.63 g; Elaidic acid - 0.44 g; and saturated fatty acid - 1.73 g). Regarding the harvest season, in the 2020S, higher content of Palmitoleic acid (2 g), Palmitic acid (5.38 g), Linolenic acid (1.86 g), Elaidic acid (1.17 g), Stearic acid (0.19 g), and saturated fatty acid (5.57 g) were recorded. The 2019M harvest only presented higher values for Oleic acid (2.63 g).

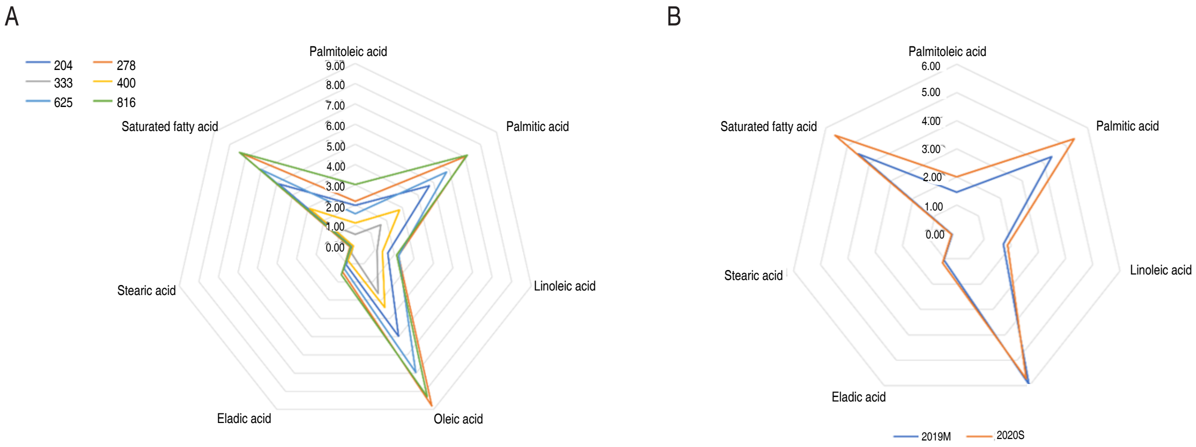

Figure 2. Fatty acid (g 100 g^{-1}) composition of avocado fruit mesocarp for A) plan density (tree ha^{-1}) and B) harvest season (2019M and 2020S). *Main Harvesting (M). Secondary Harvesting – Mitaca (S).

Vitamin E content in avocado fruit mesocarp

Vitamin E content is presented descriptively in Figure 3. The concentrations of vitamin E (mg α-tocopherol 100 g^{-1}) were similar in the plant densities of 204 (1.42), 278 (1.45), 400 (1.36), and 625 (1.415) trees ha^{-1}. The lowest values (0.905) were reached in the density of 333 trees ha^{-1}. Similar to what was observed for fatty acid concentration, a lower fatty acid level was observed at this plant density. Regarding harvest time, vitamin E contents were observed for 2019M (1.24) and 2020S (1.35). It has been reported that vitamin A contents rank for 1.78–3.5 mg α-tocopherol 100 g^{-1} (Jimenez et al. 2020). Nonetheless, this study's overall vitamin E content was low (0.90–1.45).

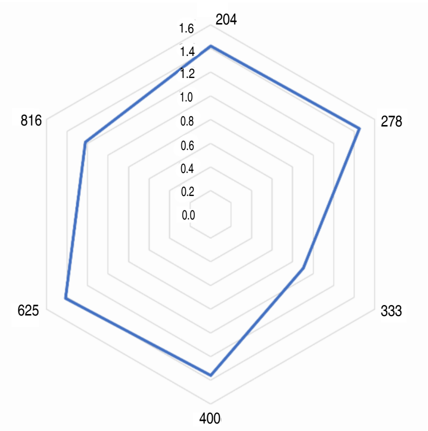

Figure 3. Vitamin E content (mg α-tocopherol 100 g^{-1}) in avocado fruit mesocarp for plant density (tree ha^{-1}).

Avocado fruit calibers

For many years, the profitability of avocado production was measured in terms of total fruit yield. However, this parameter has lost importance due to the avocado market. Harvest time, size, and fruit quality (external and internal) are considered the main factors for successful avocado marketing (Salazar-García and Lazcano-Ferrat 2001). However, the percentage of avocado fruits by size showed no significant differences due to plant distances. The harvest factor significantly affected most of the percentage proportions of each caliber in the avocado fruit. Despite the effect of the harvest season, a homogeneous behavior was not observed in the variation of this variable (Table 6).

Related to crop yield, it is reported that planting density affects avocado fruit yield. Cano-Gallego et al. (2023) found that yield per tree and number of avocado fruits per tree are negatively affected by the increase in planting densities. In addition, fruit quality parameters show better results at intermediate planting densities of 333 and 400 trees per hectare. The intermediate densities of 333 and 400 trees produced the highest fruits per tree (323) and the highest fruit yield (19 t ha^{-1}).

Table 6. Percentage proportions of the weight of each caliber in avocado trees *cv.* Hass for five plant densities during five harvests (2020M, 2020P, 2021M, and 2021P).

Treatment	C14	C16	C18	C20	C22	C24
Plant density*/*P values*	0.19	<0.05	<0.01	<0.01	<0.001	0.11
204	1.05	3.17^{ab}	7.84^{a}	7.59^{ab}	14.91^{a}	6.75
278	0.34	1.45^{b}	7.84^{b}	5.97^{b}	9.79^{b}	7.33
333	1.55	4.02^{a}	8.36^{a}	7.32^{ab}	10.91^{ab}	7.01
400	0.89	2.85^{ab}	8.65^{a}	10.58^{a}	14.43^{a}	8.66
625	1.01	2.66^{ab}	7.99^{a}	6.86^{b}	14.89^{a}	7.40
816	1.17	2.03^{ab}	6.12^{ab}	5.64^{b}	12.63^{ab}	8.57

(Table 6)

Treatment	C14	C16	C18	C20	C22	C24
Year/*P values***	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
2019M	2.34^{a}	6.11^{a}	12.21^{a}	9.93^{a}	14.80^{a}	7.27^{ab}
2020S	0.89	1.75^{b}	5.53^{b}	7.63^{ab}	15.61^{a}	8.11^{ab}
2020M	0.48^{b}	2.04^{b}	5.86^{b}	6.33^{b}	10.41^{b}	6.52^{b}
2021S	0.89^{b}	1.88^{b}	6.86^{b}	6.84^{b}	12.65^{ab}	9.09^{a}
2021M	0.41^{b}	1.70^{b}	5.24^{b}	5.91^{b}	11.18^{b}	7.12^{ab}
Treatment	**C26**	**C28**	**C30**	**C32**	**DC**	**IC**
Plant density*/*P values*	0.089	0.881	0.123	<0.001	<0.05	<0.05
204	7.01	9.59	10.60	21.94^{b}	5.36^{ab}	4.18^{ab}
278	6.85	8.44	12.15	32.15^{a}	5.75^{a}	5.92^{a}
333	6.56	9.87	10.30	24.26^{ab}	5.23^{ab}	4.60^{ab}
400	7.85	9.72	10.16	18.80^{b}	3.15^{b}	4.25^{ab}
625	8.27	9.42	11.92	22.23^{b}	3.74^{ab}	3.60^{ba}
816	8.27	9.48	10.88	26.79^{ab}	3.73^{ab}	4.69^{b}
Year/*P values***	0.557	<0.001	<0.001	<0.001	<0.001	<0.001
2019M	7.07	7.89^{ba}	7.85^{b}	18.56^{b}	2.89^{c}	3.07^{b}
2020S	7.00	9.50^{ab}	11.94^{a}	23.94^{ab}	3.89^{bc}	4.21^{ab}
2020M	7.55	8.63^{ab}	11.42^{a}	28.73^{a}	6.21^{a}	5.82^{a}
2021S	7.74	11.65^{a}	11.42^{a}	23.48^{ab}	3.44^{c}	4.09^{ab}
2021M	7.98	11.65^{ab}	12.39^{a}	27.11^{a}	6.03^{ab}	5.51^{a}

*Trees ha^{-1} **Main Harvesting (M). Secondary Harvesting "Mitaca" (S). *** Values with similar letters for each nutrient within location or rootstock/scion relation are significantly different (HSD, P<0.05).

CONCLUSION

The planting density significantly affected the leaf and fruit nutritional composition except for Ca; thus, the nutritional balance index (NBi) for N, Ca, Mg, S, Fe, Mn, Zn, and B. The highest nutrient concentrations in the leaves and fruits of Hass avocado and the lowest saturated fatty acid contents in fruits were obtained at the intermediate plant densities of 333 and 400 trees per hectare. On the other hand, the percentage of avocado fruits by size showed no significant differences due to plant distances. There were intermediate planting densities (333 and 400 trees ha^{-1}). The dynamics of the accumulation of nutrients in avocado leaves and fruit through the different leaf ontogeny and fruit development are recommended to determine if planting density through the Hass avocado phenology is related to the nutritional contents of the leaf and fruit.

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CONFLICT OF INTERESTS

The manuscript was prepared and reviewed with the participation of the authors, who declared that there exists no conflict of interest that puts at risk the validity of the presented results.

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