Original Article
Food Science and Technology

ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)

DOI: https://doi.org/10.1590/fst.93621

Volatile compounds of unripe fruits from different cultivars (*Persea americana* Mill.)

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Abstract

Avocado is one of the most demanded fruits worldwide. Comparative studies on the morphology and genetics of avocado have pointed out similarities and differences between different cultivars. Plant volatile compounds may facilitate cultivar differentiation. The volatile composition of leaves, flowers and fruit mesocarp of different cultivars has been reported, but the volatile chemical composition of immature fruits is unknown. This information may be relevant for studies on the chemical ecology of seed boring insects. Therefore, in this work the volatile chemical composition of three avocado cultivars was compared. A total of 31 compounds in developing fruits of Mexican, Fuerte and Hass avocado cultivars were identified by gas chromatography (GC) coupled to mass spectrometry (MS). Of these compounds, α-copaene, β-copaene and β-caryophyllene are among the most abundant in the extracts of the three avocado cultivars. Estragole, which has a characteristic aniseed aroma, was the most abundant compound in the Mexican cultivar (62.61%), while in Fuerte it was found in traces and in Hass it was not detected. The volatile profile of the Mexican cultivar was different from that of the Fuerte and Hass cultivars.

Keywords: *Persea americana*; cultivars; gas chromatography; chemical compound.

Practical Application: The composition of volatile compounds facilitates differentiating the most representative avocado cultivars in the industry of this crop: Mexican, Fuerte and Hass. This information may also be relevant for use in chemical ecology studies in the management of seed borers that attack this crop.

1 Introduction

Avocado is one of the most important fruit trees in the world (Bost et al., 2013). *Persea americana* Mill. is distributed from the mountainous areas of Mexico to the lowlands of Central America (Guatemala and Costa Rica) (Popenoe & Williams, 1947; Knight, 2002). The species is polymorphic and comprises several taxa considered botanical varieties or subspecies, which were called "horticultural" races (Scora & Bergh, 1991). Considering its center of origin, three races are currently recognized: Mexican (*P. americana* var. *drymifolia*), Guatemalan (*P. americana* var. *guatemalensis* Williams) and West Indian (*P. americana* var. *americana* Mill.) (Popenoe, 1934; Storey et al., 1986; Chen et al., 2009).

In general, the three races have characteristics that differentiate them, such as size and shape of the fruit, texture and color of the epicarp, size of the seed, among others (Lahav & Lavi, 2002). Currently, new cultivars obtained by hybridization of various materials collected in Mexico and Central America are used in modern avocado plantations (Knight, 2002; Lahav & Lavi, 2002; Galindo-Tovar et al., 2008).

The avocado fruits of the Mexican, Fuerte and Hass cultivars are susceptible to attack by seed boring insects (Equihua et al., 2007). In the field, it has been observed that the small avocado seed borer *Conotrachelus perseae* (Barber) (Coleoptera: Curculionidae) is more attracted to Mexican avocado trees than to Fuerte or Hass (personal communication of technicians working in the Mexican campaign against regulated pests of the avocado, 2019). In this sense, the behavior of this borer led us to suppose that there may be significant differences in the volatile chemical composition that the fruits of the mentioned cultivars emit.

Comparative studies on the morphology and genetics of avocado have pointed out similarities and differences between cultivars (Alcaraz & Hormaza, 2007; Abraham et al., 2018; Boza et al., 2018), although they have precursors in common, as in the case of cv. Fuerte and Hass, which originated from the Mexican and Guatemalan races (Bergh & Ellstrand, 1986).

The volatile chemical composition of the plants could also provide useful information on the differences between avocado cultivars. Several studies have been carried out on the volatiles present in leaves, flowers and fruits (King & Knight, 1987; King & Knight, 1992; Sagrero-Nieves & Bartley, 1995; Pino et al., 2000; Pino et al., 2004; Pino et al., 2006; El-Mageed, 2007; Liu et al., 2021), but there is no specific information on the chemical composition of immature fruits. These data may be useful for studies of the chemical ecology of herbivorous insects that attack this crop, in the context of managing insect pests with semiochemicals. For this reason, we identified and compared the volatile compounds present in immature fruits of

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Received 27 Nov., 2021
Accepted 07 Apr., 2022

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three of the most representative commercial avocado cultivars: Mexican, Fuerte and Hass.

2 Materials and methods

2.1 Plant material

The Mexican, Fuerte, and Hass avocado fruits collected for this study were in the development phase (light green). They had an equatorial diameter of 4 to 6 cm and were visibly healthy. 'Mexican' avocado was obtained from backyard trees in the town of Tequexquinahuac, Texcoco, State of Mexico (19°28'36" N, 98°49'37" W), and the other two cultivars were obtained from commercial avocado orchards located in Meyuca de Morelos, Coatepec de Harinas, State of Mexico (18°84'74" N, 99°77'69" W). All fruits were collected in August 2020. The harvested fruits were kept in polyethylene bags and transported in a cooler to the laboratory, where the chemical analyses were performed.

2.2 Collection of volatiles by dynamic headspace aeration

Volatiles were collected with the dynamic headspace aeration technique, as described by Cruz-López et al. (2006), with some modifications. For the analysis, 400 g of avocado fruits from each cultivar was used and placed separately in glass containers (39 cm long x 9 cm internal diameter) for sampling volatiles. An air stream filtered with activated carbon was passed (0.5 L/min) through the glass containers. Fruit volatiles were captured with Super Q adsorbent (25 mg) (Sigma Scientific LLC, Micanopy, Fl) over 24 h and subsequently eluted from the adsorbent with 400 µL of dichloromethane (Baker, HPLC grade, Sigma-Aldrich, Toluca, Mexico). In total, for each cultivar, five extracts, each composed of thirty 24-hour collections, were obtained. The extracts were concentrated to 100 µL with N_2 before chemical analysis.

2.3 Chemical analysis

The extracts were analyzed in a gas chromatograph (CG) (Varian Star 3400 CX) coupled to a mass spectrometer (MS) (Varian Saturn 4D, AC, USA). We used a non-polar DB5 column 30 m long x 0.25 mm in diameter, and 0.5 µm thick (J. & W Science Folsom, CA, USA). The carrier gas was He at a constant flow rate of 2 mL/min. The temperature program was 50 °C for 2 min, increasing 15 °C/min up to 280 °C and maintained for 10 min. Injector and ion source temperatures were 200 and 250 ºC, respectively. Helium was used as a carrier gas at a rate of 1 mL/min. Ionization voltage was 70 eV. The compounds were tentatively identified by comparison with the mass spectra of the NIST/EPA/NIH mass spectrometer library (version 2.0, 2002) and with the calculated retention indices. The identity of the compounds was confirmed by comparing them with the retention times and mass spectra of synthetic standards. Relative abundance (%) of the compounds was calculated with the peak areas of the identified compounds. The synthetic standards used were β-caryophyllene (98.5%), limonene (90%), ocimene (97%), β-pinene (98%), estragole (98%), nonanal (98%), β-myrcene (99%), α-pinene (98%), linalool (97%), α-copaene (98%), *n*-undecane, *n*-dodecane, *n*-tridecane, *n*-tetradecane, obtained from Fluka Chemicals (Columbus, Ohio USA) and Sigma Aldrich Chemical Company (Milwaukee, Wisconsin, USA).

2.4 Statistical analysis

The Random Forest method was applied to the areas of the identified compounds as a classification method to determine whether there were differences among avocado cultivars and to distinguish the variables that contribute to these differences. In addition, a multivariate analysis of variance with permutations (PERMANOVA) was performed in R v3.6.3 software (R Core Team, 2019) to identify significant differences ($p \leq 0.05$) among the cultivars.

3 Results and discussion

A total of 31 volatile compounds were identified in the avocado fruit extracts, including terpenes, hydrocarbons, phenylpropanoid (estragole), and alcohols (Table 1). The volatile chemical composition of avocado fruits is represented mostly by terpenes (Pino et al., 2000; Pino et al., 2004; El-Mageed, 2007; Niogret et al., 2013; Galvao et al., 2016); our findings agree with those results.

The major compounds in the volatile chemical composition of the three cultivars were α-copaene, β-copaene and β-caryophyllene (Table 1). These compounds have also been found as major compounds in leaves and fruits of different avocado cultivars (Sagrero-Nieves & Bartley, 1995; Pino et al., 2000; Pino et al., 2004; Ogunbinu et al., 2007; Torres-Gurrola et al., 2009).

In Hass and Fuerte avocado fruit extracts, the compounds identified were similar, but generally differed in abundance, except for linalool, ylangene, and (E)-α bergamotene, which were detected only in Hass fruit. In contrast, in the Mexican avocado extracts, estragole was the most abundant compound (62.61%), while in Hass fruits it was not detected and in the Fuerte extract it was found only in traces.

Previous studies have reported a volatile chemical diversity in leaves, flowers and mesocarp of avocado fruits (Scora & Scora, 1998; Sinyinda & Gramshaw, 1998; Pino et al., 2000; Moreno et al., 2003; Ogunbinu et al., 2007; Mahendran, 2016; Rincón-Hernández et al., 2011; Obenland et al., 2012; Campuzano-Granados & Cruz-López, 2021), but this is the first study on volatiles emitted by immature whole fruits of different cultivars. The information generated in this work, in addition to providing data on the differentiation of the volatile chemical profiles of three avocado cultivars, can be useful for control measures using attractants and management of avocado seed borers, considering that management of curculionids with semiochemicals has had positive results (Prokopy & Leskey, 1997; Leskey & Prokopy, 2001; Pinero & Prokopy, 2003; Leskey et al., 2005; Leskey & Prokopy, 2001; Hock et al., 2017). In addition, it has been shown that host volatile compounds act as attractants in synergy with pheromones in different species of phytophagous insects (Landolt & Phillips, 1997; Collatz & Dorn, 2013; Wibe et al., 2014; Ruiz-Montiel et al., 2017).

In the field, the cv. Mexican avocado is more attractive to the small seed borer weevil (*Conotrachelus perseae* Barber) than other cultivars such as Hass or Fuerte (Personal communication: technicians of the Mexican campaign against regulated avocado pests, 2020). This feeding preference towards Mexican cultivars

Table 1. Average relative abundance (± standard error) of volatile compounds identified in fruits of three avocado cultivars.

Number	Compound	TR	IR	Abundance[1] (%)		
				Mexican	Fuerte	Hass
1	α-Pinene*	4.83	939	3.44 ± 0.37	3.69 ± 1.61	2.01 ± 0.63
2	Bicyclo[2.2.1]heptane, 2,2-dimethyl-3-methylene-,	5.07	956	00	0.59 ± 0.16	0.27 ± 0.05
3	β-Pinene*	5.45	984	2.24 ± 0.51	1.50 ± 0.36	0.75 ± 0.15
4	1-Hepten-6-one, 2-methyl-	5.5	988	00	0.38 ± 0.1	0.63 ± 0.21
5	β-Myrcene*	5.54	991	0.18 ± 0.04	2.11 ± 0.13	1.70 ± 0.4
6	α-Thujene	5.8	1011	00	1.27 ± 0.28	2.31 ± 0.58
7	Limonene*	6.1	1035	0.55 ± 0.2	2.25 ± 0.53	2.19 ± 0.53
8	(Z)-β-Ocimene*	6.14	1039	0.79 ± 0.31	1.19 ± 0.31	1.17 ± 0.24
9	(E)-β-Ocimene*	6.28	1050	0.58 ± 0.15	2.36 ± 0.62	2.49 ± 0.67
10	*n*-Undecane*	6.9	1100	1.14 ± 0.28	0.81 ± 0.32	0.99 ± 0.68
11	Linalool*	6.95	1104	00	00	0.61 ± 0.13
12	Nonanal*	7	1109	3.05 ± 0.49	2.73 ± 0.48	10.50 ± 2.83
13	Hexanoic acid, butyl ester	7.95	1192	1.20 ± 0.2	1.47 ± 0.43	0.68 ± 0.22
14	*n*-Dodecane*	8.04	1200	0.48 ± 0.2	0.54 ± 0.12	0.86 ± 0.25
15	Estragole*	8.13	1209	62.61 ± 4.05	0.19 ± 0.1	00
16	*n*-Tridecane*	9.09	1300	2.40 ± 1.37	1.02 ± 0.3	1.44 ± 4.58
17	α-Cubebene	9.67	1359	00	5.60 ± 0.76	4.12 ± 0.67
18	Eugenol	9.89	1382	2.52 ± 1.01	00	00
19	Ylangene	9.93	1386	00	00	1.65 ± 0.64
20	α-Copaene*	9.99	1392	7.88 ± 0.42	10.58 ± 3.37	8.56 ± 2.8
21	*n*-Tetradecane*	10.07	1400	1.76 ± 0.16	3.33 ± 0.17	00
22	*Z*-α-Bergamotene	10.3	1423	0.51 ± 0.02	1.10 ± 0.1	0.74 ± 0.19
23	β-Caryophyllene*	10.45	1441	1.31 ± 0.31	12.64 ± 1.89	9.29 ± 1.91
24	*E*-α-Bergamotene	10.48	1445	00	00	0.67 ± 3.84
25	α-Caryophyllene	10.78	1477	00	2.37 ± 0.21	1.74 ± 0.34
26	Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-	10.92	1492	1.43 ± 0.16	2.0 ± 0.35	2.01 ± 0.51
27	β-Copaene	11.01	1502	2.55 ± 1.2	16.05 ± 2.55	13.82 ± 2.82
28	γ-Elemene	11.14	1517	00	4.0 ± 1.28	3.21 ± 0.85
29	Tricyclo[4.4.0.02,7]dec-3-ene, 1,3-dimethyl-8-(1-methylethyl)-, stereoisomer	11.29	1535	0.87 ± 0.16	3.70 ± 0.38	4.41 ± 0.54
30	cis-Calamenene	11.35	1542	2.20 ± 0.3	1.25 ± 1.09	0.87 ± 1.31
31	Farnesene	11.67	1579	0.21 ± 0.06	15.14 ± 3.79	20.16 ± 4.99

[1] The relative abundance (%) was calculated with the average of the peak areas of the identified compounds of five extracts corresponding to each cultivar. TR = rRetention time; IR = calculated retention index. *Compounds confirmed by comparison with synthetic standards.

may be related to the abundance of estragole, an anise-scented compound that has been detected only in the Mexican avocado cultivars (Pino et al., 2006; Pereira et al., 2013). However, it should not be ruled out that this feeding preference of the borers towards Mexican avocado fruits may also be related to characteristics of the fruits of the Mexican race, such as the very thin soft exocarp, which can facilitate oviposition of *C. perseae* females on avocado fruits. Future studies are needed on the effect of estragole on avocado seed borers, which are the main quarantine pests of this crop.

According to the statistical analysis, there were no significant differences in the volatile chemical composition between Fuerte and Hass fruits (F2.12 = 0.62, P = 0.57), while the Mexican cultivar was statistically different from both Hass (F2.12 = 5.55, P ≤ 0.01) and Fuerte (F2.12 = 4.32, P ≤ 0.01). These differences are reflected in Figure 1, where each point represents the compounds of the five analyzed replicates (extracts) from each cultivar. In general, the points corresponding to Fuerte and Hass are close or overlapping, which indicates the similarity between the compounds of these two cultivars. On the other hand, the points that represent cv. Mexican are more dispersed and far from the other cultivars.

To estimate which compounds influenced these differences, the mean decreases in precision (A) and the mean decrease in Gini (B) were used in the random forest model (Figure 2). The results are similar for both criteria and only the order of the variables changes. According to Figures 1-2, the compounds are placed in order of relevance from the top; the first 10 had the most influence on the differences between cultivars: n-tetradecane, (E)-α-bergamotene, ylangene, α-caryophyllene, estragole, eugenol, linalool, α-thujene, α-cubebene, γ-elemene and bicyclo[2.2.1]heptane, 2,2-dimethyl-3-methylene. Most of the

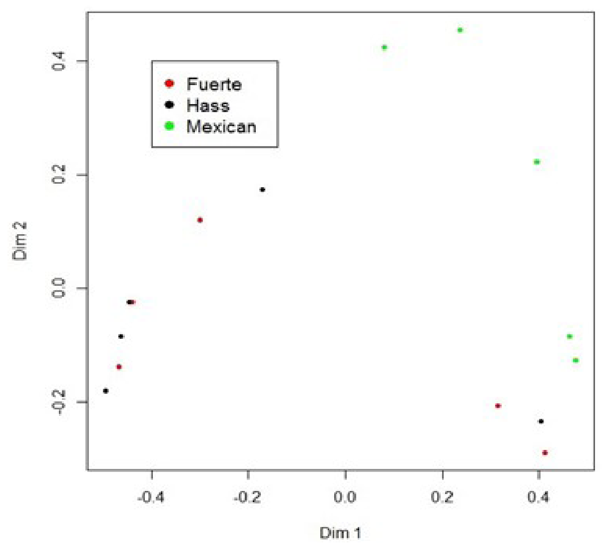

Figure 1. Distribution of volatile compounds of avocado fruits cv. Mexican, Fuerte and Hass, in a random forest analysis. Each dot represents the compounds of an extract or repetition of each avocado cultivar.

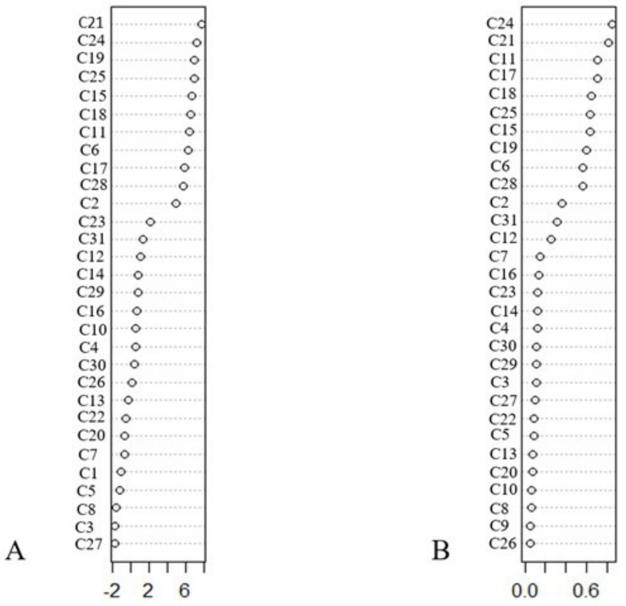

Figure 2. Mean decrease in accuracy (A) and mean decrease in Gini (B) in a random forest model. The numbers correspond to the compounds (C) identified in fruits of three avocado cultivars (see Table 1).

compounds that marked differences were found in Fuerte and Hass fruits but not in Mexican avocado, while estragole and eugenol were detected only in the extracts of Mexican avocado fruits (Table 1). The remaining compounds had little influence on the differences found.

The chemical variation of volatile compounds in Fuerte and Hass fruits relative to those of the Mexican variety may be due to the origin of their parents. In the case of Hass, it is a hybrid whose genetic material is 58% Guatemalan race and 42% Mexican race, while Fuerte avocado is made up of 99% Mexican race (Chen et al., 2009; Tremocoldi et al., 2018). That is, less than 50% of the Hass genetic material corresponds to the Mexican race. This may be the reason that the estragole compound, which characterizes the Mexican avocado, has not been detected. In this regard, Niogret et al. (2013) and Bravo-Monzón & Espinosa-García (2008) found that estragole in leaves of *Persea americana* var. drymifolia is the most abundant compound, while in Hass leaves it was detected only in traces. In contrast, Guzmán-Rodríguez et al. (2020) reported that this compound was one of the most abundant in Hass leaf volatiles.

As already mentioned, estragole was the main compound in Mexican avocado fruits. In other studies, it has been pointed out that Mexican avocado leaves are rich in estragole, a compound with an anise-like aroma, and in leaves of the Guatemalan or Antillean race, its concentration is minimal or not present (King & Knight, 1992; Pino et al., 2006; Pereira et al., 2013). Sagrero-Nieves & Bartley (1995) identified 30 volatile compounds in Mexican avocado leaves by gas chromatography coupled to mass spectrometry. They highlighted estragole as the major compound (78.12%). In addition, they noted that, of the compounds identified, α-cubebene (3.58%), methyl eugenol (3.37%) and β-caryophyllene (2.10%) were also among the most abundant. Likewise, Torres-Gurrola et al. (2009) performed an analysis of *P. americana* var. drymifolia leaves and found estragole as the major compound (22 to 72%), followed by caryophyllene with 9 to 16%. Thus, it has been suggested that estragole is genetically based in the Mexican race and is useful as a taxonomic marker to distinguish this cultivar from others (King & Knight, 1987; Scora & Bergh, 1991).

4 Conclusions

We found that estragole is a compound that characterizes the cv. Mexican avocado, while in Fuerte and Hass avocado fruits it was almost or completely absent. However, although Hass is a Mexican-Guatemalan hybrid and the Mexican race predominates in Fuerte, the compounds linalool, ylangene and (E)-α-bergamotene, that were not found in cv. Mexican avocado fruits were detected in the other two cultivars. This information facilitates differentiation of these avocado cultivars and can also be used to implement a management strategy with attractants in the control of the avocado seed borer weevil.

Conflict of interest

No potential conflict of interest was reported by the author(s).

Acknowledgements

The authors thank the Consejo Nacional de Ciencia y Tecnología (CONACYT), Colegio de Postgraduados and El colegio de la Frontera Sur (ECOSUR). We also thank M. C. Javier Valle Mora for his collaboration with the statistical analysis.

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Erratum
Food Science and Technology

ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)

DOI: https://doi.org/10.1590/fst.93621er

ERRATUM: Volatile compounds of unripe fruits from different cultivars (*Persea americana* Mill.)

Due to author's honest mistake the article "Volatile compounds of unripe fruits from different cultivars (*Persea americana* Mill.)" (DOI https://doi.org/10.1590/fst.93621), published in Food Science and Technology, 42, e93621, 2022, was published with an error.

On page 1, where the text reads:

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It should read:

Reyna VARGAS-ABASOLO[1], Leopoldo CRUZ-LÓPEZ[2*], Julio Cesar ROJAS[2], Héctor GONZÁLEZ-HERNÁNDEZ[1], Armando EQUIHUA-MARTÍNEZ[1], Jesús ROMERO-NÁPOLES[1]

The authors apologize for the errors.