Main components of the essential oil from leaves, peel, and pulp of the Mexican autochthonous avocado (P. americana var. drymifolia, Schltdl. & Cham.)

Principales Componentes del aceite esencial de las hojas, cáscara y pulpa del aguacate autóctono mexicano (P. americana var. drymifolia, Schltdl. & Cham.)

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Abstract

In this paper, the extraction and analysis of the volatile compounds of the leaves, peel, and pulp of Persea Americana var. drymifolia (avocado[3]) are presented. The essential oils, EO, from these parts of the plant and fruit were extracted by hydrodistillation and their composition was determined through a gas chromatography coupled with a mass spectroscopy. The results showed EO yields of 1.18±0.24, 0.74±0.05, and 0.28±0.17% (db) for the leaf, peel, and pulp, respectively. The leaves, peel, and pulp volatile compounds constituting their flavor and aroma; were identified: 20, 31, and 31 compounds, respectively. The main compounds were benzene derivatives: 86.75, 73.08, and 60.24%; terpene derivatives: 6.51, 21.38, and 36.51% in the leaf, peel, and pulp Eos, respectively. Individually, the highest contents were estragole, ledene oxide (II), aromadendrene, β-pinene, methyleugenol, and benzaldehyde, 4-methoxy-, among others. The toxicological characteristics of the identified majority volatile components, the bioactivation of food-borne genotoxic carcinogens, and the toxicity risks involved in the consumption of the majority volatile component, estragole, found in the EOs of autochthonous avocado peel and pulp were discussed.

Keywords: Persea Americana var. drymifolia essential oils in avocado fruit, peel, and pulp, autochthonous avocado, alkenylbenzenes, estragole

Resumen

En este trabajo, se presenta la extracción y análisis de los compuestos volátiles de las hojas, cáscara y pulpa de Persea americana var drymifolia (aguacate[4] autóctono). Los aceites esenciales, AE, de estas partes de la planta y fruto se extrajeron por hidrodestilación y su composición se determinó mediante cromatografía de gases acoplada a espectroscopía de masas. Los resultados mostraron rendimientos de AE de 1.18±0.24, 0.74±0.05 y 0.28±0.17% (base seca, bs) para la hoja, cáscara y

[3] *Avocado, a word of Nahuatl origin, ahuacatl, fruit of the avocado. A tree of America, of the laurel family, eight to ten meters tall, with alternate, leathery, evergreen leaves, dioecious flowers and edible fruit (https://dle.rae.es/aguacate?m=form)*

[4] Aguacate, palabra de origen náhuatl, *ahuacatl*, fruto del aguacate. Árbol de América, de la familia de las lauráceas, de ocho a diez metros de altura, con hojas alternas, coriáceas, siempre verdes, flores dioicas y fruto comestible (https://dle.rae.es/aguacate?m=form)

pulpa, respectivamente. Los compuestos volátiles de hojas, cáscara y pulpa que constituyen su sabor y aroma se identificaron, siendo 20, 31 y 31 compuestos, respectivamente. Los principales compuestos fueron: Derivados del benceno: 86.75, 73.08 y 60.24%; derivados terpénicos: 6.51, 21.38 y 36.51% en el extracto de hoja, cáscara y pulpa, respectivamente. Individualmente, los mayores contenidos fueron estragol, óxido de ledeno (II), aromadendreno, β-pineno, metileugenol y benzaldehído, 4-metoxi-, entre otros. Se discutió la información sobre las características toxicológicas de los componentes volátiles mayoritarios identificados, la bio-activación de carcinógenos genotóxicos transmitidos por alimentos y los riesgos de toxicidad asociados al consumo del componente volátil mayoritario, el estragol, presente en el extracto de cáscara y pulpa de aguacate autóctono.

Palabras clave. Aceites esenciales de Persea americana var. Drymifolia en fruto, cáscara y pulpa, aguacate autóctono, alquenilbencenos, estragol

Introduction

The origin of the avocado is possibly Mesoamerica, since its name comes from the Nahuatl word ahuacatl, a tree fruit that have the shape of testicles (Cabrera, 2002). Mexico is the main producer and exporter of avocado (more than 2.3 million tons in 2020, around 32% of the world production of this fruit), produced in almost all the states of the country, but mainly in the state of Michoacán, where its success has caused the botanical transformation of a large area of natural ecosystems (Pérez, 2019).

In recent years, the avocado has occupied a prominent place worldwide in the cultivation and trade of fruits. In Mexico, its cultivation and production have grown significantly due to its progressive acceptance in consumption in the internal and external markets, due to its organoleptic characteristics, as well as the progressive knowledge of its nutritional and functional qualities (Bhuyan et al., 2019; Jiménez-Patiño et al., 2020; Lara-García et al., 2021). From an economic perspective, avocado is currently a relevant export product in Mexico, generating income and foreign exchange in significant amounts that have led it to be considered as a true "green gold". This has also led to its cultivation experiencing high annual growth rates, mainly Hass variety which is the most commercially accepted. In traditional producing regions and in many others, its promotion has led to the undermining of the regional natural biodiversity by displacing not only other more traditional crops, but also endemic avocado varieties with various socioeconomic and environmental consequences (Barrientos-Priego, 2010; Barrientos-Priego and López-López, 2010; Salgado-Garciglia, 2017).

The pre-Hispanic cultures settled in Mesoamerica had extensive knowledge about the avocado. According to the Florentine Codex, three types of avocado were distinguished, according to their characteristics, the aoacatl which could correspond to the so-called Mexican race, the tlacacolaocatl, the Antillean race and the quilaoacatl for the Guatemalan race.

Currently in Mexico, there is also a large number of hybrids with mixed traits which causes problems for their location in one race or another. The avocado called 'Hass' is, for example, one of these hybrids -the most advanced one-, from the Guatemalan and Mexican races, which has been estimated to have, between 10 and 15%, of genes from the Mexican race (Barrientos-Priego and López-López, 2010).

The autochthonous or Creole avocado is a small avocado from the highlands of Mexico, and is grown in Chile, in California in the U.S., in the south of France, in Italy, and in Algeria too. It has an elongated shape and a thin, smooth, dark-colored peel. Its peel and pulp have a strong and outstanding aroma and flavor defined as aniseed, the pulp has an appreciated almost creamy texture (Gupta et al., 2018).

Research on the composition and properties of creole avocado var. drymifolia are relatively scarce with respect to those directed to Hass avocado. However, various studies and some practical evidence have shown that various parts of the avocado plant var. drymifolia, have, in addition to its nutritional and functional qualities, properties against various herbivorous predators, insects, and pathogens.

Such properties make this plant, potentially important for applications in agriculture and medicine, among other fields of use (Bravo-Monzon and Espinosa-Garcia 2008; Corrales-García et al., 2019; García-Moreno et al., 2017; Granados-Echegoyen et al., 2015; Niogret et al., 2013; Ochoa-Zarzosa et al., 2021; Ramos-Aguilar et al., 2021a,b; Rincón-Hernández et al., 2011; Sagrero-Nieves and Bartley, 1995; Torres-Gurrola et al., 2011).

Among the compounds from plants with biological activity of interest and application, essential oils have been recognized and used since the Middle Ages. They are relative ease to obtain. As well as, they have multiple applications. And they have bactericidal, virucidal, fungicidal, antiparasitic, insecticidal, repellent and aromatic properties and applications in medicine, and agrochemical, pharmaceutical, health, cosmetic, and food industries, etc. (Báez-Magaña et al., 2019; Bakkali et al., 2008; Djilani and Dicko 2012; Friedman et al., 2002; Lara-Marquez et al., 2020; Nerio et al., 2010; Raut and Karuppayil 2014; Sarkic and Stappen 2018; Sharma et al., 2021).

However, the components of essential oils also involve risk. Various authors and investigations have shown that some of these components could have toxic effects at the cellular, organic or systemic levels. These effects ranging from mild to severe, such as irritation, corrosion, sensitization, and even carcinogenicity or teratogenicity, which has limited their use in various applications and caution is recommended in the consumption of foods that naturally or artificially contain them (Raut and Karuppayil, 2014; Vigan, 2010).

The investigation of the volatile compounds of P. americana var. drymifolia is scarce. The existing reports have focused preferentially on the essential oil extracted from the leaves of the plant (Bravo-Monzon and Espinosa-Garcia, 2008; Granados-Echegoyen et al., 2015; King and Knight 1992; Niogret et al., 2013; Sagrero-Nieves and Bartley, 1995; Torres-Gurrola et al. 2011; among others). However, the Mexican avocado landrace fruit, known in some Mexican rural areas as “odorous” avocado, has a characteristic aroma that is shared by the leaves, with the traditionally edible portions of the plant i.e. peel and pulp; although to date there are no further reports on the compounds that constitute the essential oil extracted from the latter to date.

At present, the expectations faced by autochthonous avocados in Mexico are not favorable because of their current conservation status, which is very precarious. Their original cultivated areas have decreased in recent years for various reasons attributable to the dynamics of changes in agricultural and livestock activities, and urban growth, but mainly due to the effect of the substitution of traditional creole cultivars for improved hybrid cultivars. The commercial success of the latter in the international market has resulted in their predominance in the regions of high avocado production in the world, as is the case in Mexico, where the Hass variety covers more than 90% of the currently existing orchards (Torres-Gurrola et al., 2009). One of the public politics adopted in the country to counteract this trend, has been the establishment of germplasm banks.

Another possible impact in the rescue of this phytogenetic richness is the generation of greater knowledge and its dissemination, about the characteristics, nutritional and medicinal, properties and potential of the plant and their various portions (Solís-Fuentes et al., 2024). An extended use, a higher and better valuation, and a growing demand of P. americana var. drymifolia in the markets can propitiate its greater conservation, cultivation, and production (Corrales-García and Méndez-Zúñiga, 2020).

The objective of this work was to analyze and evaluate the composition of the volatile components of the essential oil, EO, extracted from the leaf and the portions of the peel and pulp of the fruit of Mexican autochthonous avocado known as “criollo” in Spanish.

Materials and methods

Autochthonous criollo avocado samples

The avocado samples Persea americana var. drymifolia were obtained at the municipality of Jalacingo, in the central region of the state of Veracruz, Mexico.The fruits were selected considering their state of maturation, according to their physical appearance, with mesocarp firm to the finger touch, and with a black-purple peel. The fruits were separated into their fractions of pulp, peel, and seed. Leaf samples were collected in the same producing region too.

Mass percentage of each portion and chemical analysis

Each one of the anatomical parts of the fruits, were weighed, and their percentage participation in the mass of complete fruit, was estimated. Moisture content for the samples of leaves, peel, pulp, and seed, as well as crude fat content of peel, pulp and seed were realized following the official analysis techniques (Horwitz, 2010). The measured values are reported as means and standard deviation of at least 3 replicates.

Obtaining essential oils from the leaf and from the peel, pulp, and seed portions

The extraction of the essential oils from the leaf and peel samples were made through steam distillation. Pulp and seed portions of essential oils were extracted with the help of a hydro-distillation apparatus. Briefly, in each case, the plant materials were washed and cut with scissors into small pieces (for the pulp portion this was not necessary). Wet sample was weighed and placed inside the extraction chamber and sufficient water was added in a stirring mechanism. The sample was heated to a boiling point and the distillate was collected in a glass container, being careful to keep the organic phase formed taking care that when the trap cap was opened. Once the extraction was finished (approximately after 90 min), the obtained distillate presented an amount of emulsified oil, so it was extracted with methylene chloride, dried with anhydrous Na_2SO_4, and subsequently, the excess of solvent was removed with the help of a rotary steam apparatus. The processed samples were stored in containers hermetically sealed under refrigeration (minus 5±2°C) and protected from light until analysis.

Estimation of essential oil yield

The yield of essential oil, EO, extracted from each of the samples of leaf, peel, pulp, and seed studied were estimated using the relationship: Yield (%) = [mass EO/mass of sample (dry basis, db)] x100.

Analysis of volatile components by gas chromatography coupled to mass spectroscopy

For the analysis of the volatile organic compounds constituents of the respective essential oils, a gas chromatograph Pegasus® III GC-TOFMS (LECO) was used. It was equipped with a column Agilent DB5, 121-5022, 20 m x 0.18 mm x 0.18 µm film thickness (J&W Scientific, Folsom, CA, US). The mass spectrometer provided included a full spectrum scan detector (SCAN) of 50 to 500 -m/z. The apparatus had a ChromaTOF® software. The operating conditions in the chromatograph were: An initial temperature of 40 °C for 3 min, with a heating rate of 20 °C/min up to 300 °C for 5 min; the carrier gas was helium at 1.0 mL / min, the injector temperature was 300 °C and, an ionization source of electronic impact was used.

Results and discussion

Characteristics and EO yields of leaves, peel, and pulp of autochthonous criollo avocado

The studied samples of odorous Mexican autochthonous avocado were physiologically mature fruits, with dark skin, bright green flesh, and with a strong and characteristic aroma. The avocados had an average length of 7.27±1.16 cm. This is an intermediate size for this variety because data reported this race ranging from 4 to 12 cm in length (Campos-Rojas et al., 2011). The measurements taken for the widest or equatorial diameter of the fruit had, on average, 6.9±0.58 cm. The seed in these avocados has a size that covers an important part of the fruit, this portion had a length of 5.16±0.71 cm and a diameter of 4.47±0.53 cm (González-Rodríguez, 2017). The leaf samples were fresh, green, and mature, and it was observed that they were smaller and thinner than those of the Hass variety.

Table 1 shows the percentage, moisture, and crude fat content of each one of the portions of the fruit, as well as the yields of the essential oil extracted from the peel, pulp, seed, and leaf of the Criollo avocado. The portions of pulp and peel, traditionally edible, together represented little more than 67% of the mass of a complete fruit. The essential oil (EO) content was higher in the leaf (1.18±0.24%), followed by the peel (0.74±0.05%), the pulp (0.28±0.17%), and the seed with the lowest percentage (0.04±0.1). Compared to other plant constituents, the EO content of plant material is often low and varies in each species, but typically ranges from 1 to 3% of the plant mass. The percentages obtained here are in the levels commonly reported for EO contents in various portions, generally the leaves of various plants. According to some used criteria, values greater than 0.5% could be considered a high yield. Small values are generally found in oils distilled from fruits, seeds, buds, and flowers (Kumar and Tripathi, 2011).

Table 1. Percentage of the total mass of the fruit, moisture, crude fat, and essential oil yield, extracted from the peel, pulp, seed, and leaf of P. americana var. drymifolia

	Peel	Pulp	Seed	Leaves
Mass, g*	4.98±1.72	32.45±10.53	17.39±5.76	--
% of the fruit	8.93	58.10	31.15	--
Moisture	69.06±1.42	79.83±0.43	66.23±0.68	61.17±0.27
Crude fat**	6.07±0.85	34.91±3.26	1.00±0.19	--
Essential oil***	0.74±0.05	0.28±0.17	0.04±0.1	1.18±0.24

* % Wet basis (wb); ** % Dry basis (db); ***% Essential oil (m/m), dry basis

Essential oil composition

As it is known, EOs are mixtures of a large number (sometimes hundreds) of chemical compounds that can be grouped into a few groups: Aliphatic compounds, terpenes, and terpene and benzene derivatives, although sometimes some other compounds present in the mixture have little resemblance to any of these groups (Kumar and Tripathi, 2011). The chromatograms resulting from the analysis of the studied EOs, showed a large number of signals corresponding to a large number of compounds (Figure 1). However, only the largest peaks were considered, which corresponded to at least 0.05% of the total area in the case of the leaf EO, larger to 0.10 in the peel, and greater than 0.15% of the pulp. Table 2 shows 59 volatile compounds included and identified in the essential oils extracted from these parts of the Mexican autochthonous avocado, plant and fruit.

Leaf EO composition

One total of twenty-three volatile compounds were identified for the EO of the avocado leaves. Of these 4.45% of the total area of the identified compounds, in the chromatogram, corresponded to aliphatic compounds, 6.51% terpenes and terpene derivatives, and 86.75% benzene derivatives compounds. For the leaf, 81.14% of its EO was p-allyl anisole (1-methoxy-4(2 propenyl) benzene), more widely known as estragole; followed by methyleugenol (2.96%), calamenene (1.85%), 3-hexen-1-ol, acetate, (Z)- (1.73%) and 2-nonanone (1.46%), followed by lower percentages of 4-carene, pentafluoropropionic acid, hexyl ester, β-pinene, eucalyptol, and others as shown in Table 2.

These results are consistent with the predominance of estragole, which is a component of several trees and plants, including turpentine (pine oil), anise, fennel, bay, tarragon, and basil (Arsenijević et al., 2025). Very often, they are used in the preparation of fragrances, and other products. King and Knight (1992) considered that estragole could be up to 1% by mass of the fresh leaf of the Mexican criollo avocado. Rincón-Hernández et al. (2011) reported between 75-86% of estragole in leaf EOs. Torres-Gurrola et al (2009) found a great variability in the estragole contents of samples from different cultivation sites in Mexico with values between 22 and 72% of this compound in leaf oil samples of criollo avocado populations stored at INIFAP germplasm bank, a governmental institute in Guanajuato, Mexico. It should be highlighted that the presence and concentration of estragole, the main compound in leafs of criollo avocado, has a genetic basis and a great variability in the foliar chemical profile in this and other volatile compounds, such as: Sabinene and β-cubebene, in addition to caryophyllene, p-cymene, and β-phellandrene (and chavicol-methyl-ether).

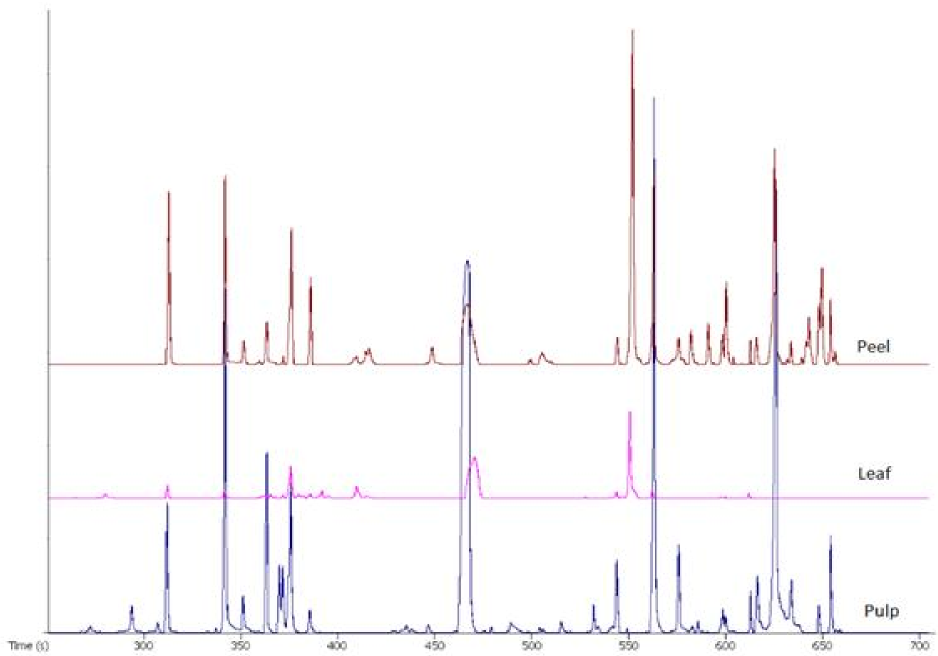

Figure 1. Processed chromatograms for the signals to identify the Kovats retention indices of the essential oils volatile compounds from leaves, peel, and pulp of Mexican autochthonous avocado

Table 2. Compounds identified in the essential oil from the leaf, peel, and pulp of Mexican autochthonous avocado

Compounds		RT, s*	Formula	Area, %**		
				Leaf	Peel	Pulp
1	Pentafluoropropionic acid, hexylester	264.6	$C_9H_{13}F_5O_2$	1.17		
2	Ethanone,1-(2-methyl-2-cyclopenten-1-yl)-	272.5	$C_8H_{12}O$			0.78
3	Ethane, 1,1,2,2-tetrachloro-	293.8	$C_2H_2Cl_4$			1.54
4	α-Phellandrene	307.2	$C_{10}H_{16}$			0.26
5	α-Pinene	312.2	$C_{10}H_{16}$	0.33	1.28	1.07
6	1,3,5-Cycloheptatriene, 3,7,7,-trimethyl-	337.3	$C_{10}H_{14}$			0.18
7	β-Pinene	342.05	$C_{10}H_{16}$	1.00	1.24	4.23
8	β-Myrcene	351.6	$C_{10}H_{16}$		0.45	0.80
9	3-Thujene	359.5	$C_{10}H_{16}$	0.60		
10	3-Hexen-1-ol, acetate, (Z)-	361.5	$C_8H_{14}O_2$	1.73		
11	γ-Terpinene	363.3	$C_{10}H_{16}$		1.53	1.53
12	Acetic acid, hexylester	365.3	$C_8H_{16}O_2$	0.05		
13	o-Cimene	369.9	$C_{10}H_{14}$			0.50
14	m-Cimene	371.5	$C_{10}H_{14}$	0.13		0.69
15	Cyclohexene, 4-ethenyl-1,4-dimethyl-	374.5	$C_{10}H_{16}$			2.06
16	Eucalyptol	376.1	$C_{10}H_{18}O$	0.82	1.46	2.06
17	trans-β-Ocimene	379.8	$C_{10}H_{16}$	0.53		
18	3-Carene	385.8	$C_{10}H_{16}$	0.74	1.55	0.63
19	4-careno (1S,3R,6R)-(-)-	391.9	$C_{10}H_{16}$	1.26		
20	2-Nonanone	409.8	$C_9H_{18}O$	1.46	0.45	
21	1,6-Octadien-3-ol, 3,7-dimethyl-, acetate	414.4	$C_{12}H_{20}O_2$		0.27	
22	2-octeno, 1-(metoximetoxi)-(E)	414.7	$C_{10}H_{20}O_2$	0.02		
23	Nonanal	416.2	$C_9H_{18}O$		0.89	
24	Cosmene	435.3	$C_{10}H_{16}O$			1.68
25	Pinecarbone	446.8	$C_{10}H_{14}O$			0.32
26	Bornylchloride	448.7	$C_{10}H_{17}Cl$		0.26	

Table 2. Compounds identified in the essential oil from the leaf, peel, and pulp of Mexican autochthonous avocado

Compounds		RT, s*	Formula	Area, %**		
				Leaf	Peel	Pulp
27	Estragole	467.7	$C_{10}H_{12}O$	81.14	67.97	52.43
28	2-Nonene	479.3	C_9H_{18}			0.27
29	Benzaldehyde, 4-methoxy-	489.2	$C_8H_8O_2$			4.86
30	1,3-Cyclooctadiene (Z,Z)	499.7	C_8H_{12}		0.11	
31	Bicyclo[2.2.1]hept-2-ene, 1,7,7-trimethyl-	503.8	$C_{10}H_{16}$			1.02
32	Anetol	504.0	$C_{10}H_{12}O$		0.37	
33	2-Dodecanone	505.4	$C_{12}H_{24}O$		1.75	
34	2,4-Decadienal, (E,E)-	506.1	$C_{12}H_{20}O$		1.90	
35	D-Verbenone	515.2	$C_{10}H_{14}O$			0.32
36	Fenol, 4-(2-propenil)-acetate	527.7	$C_{11}H_{12}O_2$	0.66		
37	Cubenene	531.9	$C_{15}H_{24}$			0.50
38	α-Cubebene	543.0	$C_{15}H_{24}$	0.35		1.67
39	Copaene	544.2	$C_{15}H_{24}$		0.32	
40	Methyleugenol	552.0	$C_{11}H_{14}O_2$	2.96	3.44	
41	Aromadendrene	562.8	$C_{15}H_{24}$	0.53	2.39	6.31
42	1,4,7-Cycloundecatriene,1,5,9,9-tetramethyl-, Z,Z,Z-	575.7	$C_{15}H_{24}$			1.63
43	α-Caryophyllene	575.7	$C_{15}H_{24}$		1.71	
44	Cyclohexene, 6-ethenyl-6-methyl-1-(1-methylethyl)-3-(1-methylethylidene)-, (S)-	582.2	$C_{15}H_{24}$		0.56	
45	Germacrene D	585.8	$C_{15}H_{24}$			0.43
46	Isoledene	591.2	$C_{15}H_{24}$		0.43	
47	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S, cis)-	600.0	$C_{15}H_{24}$			0.47
48	Calamenene	600.566	$C_{15}H_{22}$	1.85	0.81	
49	α-Calacorene	608.2	$C_{15}H_{20}$		0.28	
50	(E,E)-geranyllinalool	612.6	$C_{20}H_{34}O$	0.34	0.22	
51	3-Methoxycinnamaldehyde	615.4	$C_{10}H_{10}O_2$		0.21	0.79
52	Ledene oxide (II)	625.2	$C_{15}H_{24}O$		4.61	7.25
53	Seychellene	633.7	$C_{15}H_{24}$		0.096	
54	p-Methoxybenzoic acid, 2-methylphenyl ester	635.1	$C_{15}H_{14}O_3$			0.97
55	Tetracyclo[6.3.2.0(2,5).0(1,8)]tridecan-9-ol, 4,4-dimethyl-	642.8	$C_{15}H_{24}O$		1.15	
56	α-Eudesmol	647.9	$C_{15}H_{26}O$		0.49	0.46
57	4,4-dimethyl-3-(3-methylbut-3-enylidene)-2-methylenebicyclo[4.1.0]heptanes	649.7	$C_{15}H_{24}O$		0.81	
58	(-)-Spathulenol	654.1	$C_{15}H_{24}O$		0.54	
59	7-Tetracyclo[6.2.1.0(3.8)0(3.9)]undecanol, 4,4,11,11-tetramethyl-	654.3	$C_{15}H_{24}O$			1.62
Total of identified compounds				20	31	31
Area of identified compounds, %				97.71	99.55	99.33
Aliphatic compounds, %				4.451	4.99	2.58
Terpene and derivatives, %				6.51	21.38	36.51
Benzene and derivatives, %				86.75	73.08	60.24
Miscellaneous, %					0.11	

*RT: Retention time;** Percentage of the total area of the compounds identified in the respective chromatogram

Regarding the other compounds identified in this work, there are coincidences in their presence in the EO from the leaves, even when they differ in the percentage of participation reported. Rincón-Hernández et al. (2011) highlighted greater relevance for β-caryophyllene (1.4-3.1%), β-pinene (1.3-1.8%) and α-pinene (0.8-1.5%). Guzmán-Rodríguez et al. (2020) found greater percentages of: Estragole 61.2, 68.0, and 81.5%, caryophylene 19.4, 5.2, and 3.2%, (+) - 4-carene 7.5, 6.8, and 2.7% and γ- terpinen 6.9, 8.0, and 2.7 % in samples of drymifolia, “Hass”, and “Mendez” avocado varieties, respectively.

Peel EO composition from the fruit of Mexican landrace avocado

Table 2 also contains compounds identified in the essential oil from the peel of the studied fruit of Mexican criollo avocado. It shows that 4.99% of the total area corresponded to aliphatic compounds, 21.38% were terpenes and terpene derivatives, 73.06% of benzene derivatives and 0.11% were miscellaneous compounds. This oil contains, although less than in the leaf, a high percentage of p-allyl anisole, around 68%, followed in abundance by ledene oxide (4.60%), methyleugenol, and aromadendrene (3.43 and 2.38%, respectively), 2,4-decadienal, (E,E)-(1.90), 2-dodecanone (1.74%), followed by α-caryophyllene , 3-carene, γ-terpinene, eucalyptol, α-pinene, and others with percentages <1% , for a total of 31 volatile compounds.

No references of studies of the essential oil of the criollo avocado peel were found. From the reports reviewed, it is evident that in recent years the research interest in this part of the fruit has been towards the phytochemical composition, mainly of compounds with antioxidant properties (polyphenols, sterols, carotenoids, chlorophylls, cyanidins, enzymes, etc.), mainly of varieties of greater commercial interest (Prabha et al., 1980; Ramos-Aguilar et al., 2021b; Trujillo-Mayol et al., 2020). This scarcity of research for the Mexican criollo is relevant due to the characteristic aroma and flavor of the peel; traditionally also an edible part of this avocado, with an aroma that is shared with those registered for the plant leaf and the fruit pulp.

Pulp essential oil composition from criollo avocado

The few reports in the literature on volatile compounds in avocado pulp refer to varieties other than criollo avocado. Generally, they are hybrids, such as the Australian cultivar Sherwil (Whitfield et al., 1980), identified pentanal, hexanal, (E)-2-hexenal, β -caryophyllene, and α-copaene). Cultivars California and Hass studied by Pino et al. (2000), and the Cuban cultivar Moro by Pino et al. (2004). They found higher contents of terpene types such as (E)-nerodilol, β-caryophyllene, β-pinene, trans-α-bergamotene and β -bisabolene, for the former and (Z)-nerodilol, (E,E)-2,4-decadienal, (E,E)-α-farnesene, β-caryophyllene, caryophyllene oxide and α-copaene as major volatile compounds in the Moro variety.

Authors de-Sousa-GALVAO et al. (2016) studied, by simultaneous distillation and extraction with a solvent technique, the volatile compounds from the pulp of avocado fruit of four cultivars, Barker, Collinson, Fortuna, and Geada. Some of the main volatile compounds that were common in all the studied cultivars were hexanal (3.5-30.3%), ethyl acetate (7.4-19.8%), methyl dodecanoate (5.8-6.3), 2,5-dimethyl furan (3.0-8.7%), 1,3-butanediol (5.2-2.4%), β-caryophyllene (3.0-14.5%), among others.

In a recent report, Kilic-Buyukkurt (2021) investigated the aroma profile of cold-pressed avocado oil from Turkey using the ‘Headspace solid-phase microextraction method’ (HS-SPME), without specifying the part of the fruit and variety of origin of the oil. This author identified through a gas chromatography-mass spectrometry, 19 different compounds, with tridecane (23.2%), hexanal (12.8%), and (Z)-5-tridecene (11.6) as the major constituents, in addition to dodecane, undecane, and 2-hepatanone, among others.

Table 2 shows the 31 identified constituents of the essential oil of criollo avocado pulp, in the present study. By groups of compounds, the pulp EO contains 2.58% of aliphatic compounds, 36.51% of terpenes and terpene derivatives, and 60.24% benzene derivatives. Here estragole was also the main compound (52.43%), followed by ledene oxide II (7.25%), aromadendrene (6.31%), benzaldehyde 4-methoxy (4.86%), α-pinene (4.23%), cyclohexene, 4-ethenyl-1,4-dimethyl- and eucalyptol (2.06% each one), cosmene (1.68%), α-cubebene (1.67%), with lower percentages of 1,4,7-cycloundecatriene,1,5,9,9-tetramethyl-, Z,Z,Z-Ethane, 1,1,2,2-tetrachloro, and γ-terpinene, among others.

Main volatile components of peel and pulp of Creole avocado

According to the results obtained, the EO from leaves and the fruit portions of peel and pulp of the Mexican criollo avocado var. drymifolia have high contents of compounds derived from benzene, specifically alkenylbenzenes and mainly estragole. This result is in accordance with various investigations and reports. They showed a content between 3 and 85% of estragole in the EO of criollo avocado leaf (EMEA, 2004; King and Knight, 1992; Rincón-Hernández et al., 2011; Torres Gurrola et al., 2009). On the other hand, no reports on the volatile compounds that make up the aroma and flavor of the peel and pulp of Mexican criollo avocado, were found.

Simple compounds derived from benzene, as safrole, methyleugenol, elemicin, and apiol, are secondary metabolites commonly found in various herbs and species but also, although in smaller quantities, in agricultural products such as tomatoes, apples, oranges, bananas, and grapes, among many other fruits, since they are important components in the aroma and flavor of many food products. The type and quantity of these compounds in each one of these natural sources, depends on a number of factors: The geographic region of origin (soil, humidity, solar irradiance, etc.), their genetic variability, the physiological state, and part (fruits, seeds, flowers, leaves, stems, roots) of the plant, harvest conditions, processing, and storage, among others (Martins et al., 2018; Torres-Gurrola et al., 2009).

Alkenylbenzene compounds in avocado var. drymifolia

The interest in alkylbenzenes dates back several decades when safrole was found to induce liver tumors in rats and was evaluated by the International Agency for Research on Cancer (IARC) in the 1980s (Martins et al., 2018). In addition to the controlling alkenylbenzene, estragole, in the studied avocado peel and pulp EOs, o-cymene, m-cymene, anethole, methyleugenol, calamenene, and α-calacorene, among other aromatic derivatives, were also identified in smaller amounts.

Estragole is a phenylpropanoid chemically known also as tarragon, methyl-chavicol, p-allyl anisole, among other names (R&DChemicals, 2022). It consists of a benzene ring replaced by a methoxy and a propenyl group (Figure 2).

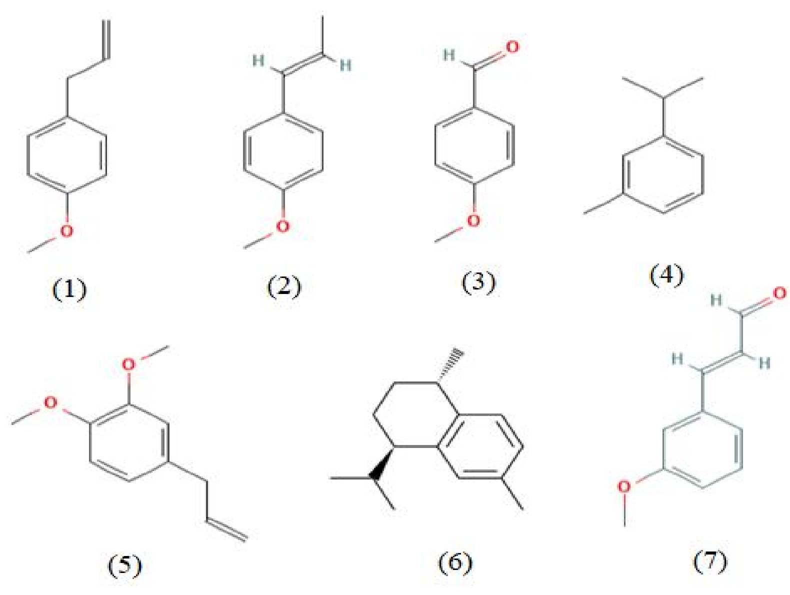

Figure 2. Chemical structure of the major benzene derivatives identified in the essential oils of peel and pulp of Mexican autochthonous avocado: 1) Estragole, 2) Anethole, 3) Benzaldehyde 4-Methoxy, 4) m-Cimene, 5) Mehyleugenol, 6) Calamenene, 7) p-Methoxybenzoic acid 2-methylphenilester

It is a natural constituent of a number of aromatic plants species and many trees and their essential oils. Some plants containing estragole are widely used as culinary herbs or additives in food preparation, and as medicinal plants in folk medicine. The group of plants with estragole contents comprises turpentine (pine oil), anise, fennel, laurel, tarragon, and basil (Ocimum basilicum), Rav; ensara anisata (madeira), Croton zehntneri, nutmeg, coriander, cinnamon, Melissa officinalis, Tamarindus indica, Zingiber officinalis, black pepper, and many other spices and species (Alves-Júnior et al., 2020; Arsenijević et al., 2025Dief et al., 2025; EMEA, 2004; Siano et al., 2003). EOs of some of these plants are used in aromatherapy and as flavoring additives in pharmaceutical, cosmetic, and food products, and as an important constituent of insecticides (Ebadollahi, 2020).

Estragole is described, in scientific reports, as a compound with a broad biological activity, such as antioxidant and antimicrobial, anxiolytic, skeletal muscle contracturant, and visceral muscle relaxant, as well as an anti-inflammatory and gastric protector (Alves-Júnior et al., 2020; Batista et al., 2024). However, like safrole and other alkenylbenzenes (such as methyleugenol and isosafrole), estragole is also considered a genotoxic liver carcinogen in rats. When this alkenylbenzene was administered to laboratory rats in pure form and at high doses together with its proximate carcinogenic metabolite 1′-hydroxyestragole for 12 to 15 months it caused a significant increase in the incidence of hepatocellular carcinomas (Akermann et al., 2025; Martins et al., 2018; Miller et al., 1983). Notwithstanding the foregoing, for several well-founded reasons, its potential toxicity in humans is, at present, still under debate (Yadav et al., 2021).

The hepatocarcinogenicity of some alkenylbenzenes such as estragole and methyleugenol, among others, has been attributed to their bioactivation by cytochrome P450 enzymes that lead to the formation of the proximate carcinogen. With estragole for example, 1'-hydroxyestragole is formed and is subsequently activated with the participation of sulfotransferase enzymes (SULT) to the carcinogenic metabolite 1'-sulfooxyestragole with the ability to bind covalently to other endogenous nucleophiles including DNA leading, in the latter case, to DNA adduct formation. The sulfonation pathway is essential for the genotoxicity (Alhusainy et al., 2010, 2013; Bergau et al., 2021; Miller, 1994; Miller et al., 1983; Phillips et al., 1981, 1984; Smith et al., 2002).

Alkenylbenzenes and the matrix effect

Eisenreich et al. (2021) conducted a discussion on the various aspects that currently prevent the conclusive evaluation of the adverse effects on human health of alkenylbenzenes present in food. Their arguments allow to say that although the toxicity of single safrole or single estragole has been verified in animals in the laboratory, the toxicological action of these compounds when they are constituent part of more complex matrices has not been fully studied and confirmed in humans.

Some studies have shown that the genotoxic potential of alkenylbenzenes can be reduced by other plant or food components, which act as sulfotransferase inhibitors (Alhusainy et al., 2013; Eaton et al., 1996; Gori et al., 2012; Huang et al., 2009; Marabini et al., 2019; van den Berg et al., 2013). In this regard, several studies have shown the inhibitory effect of naturally occurring flavonoides in foods and plants on the enzymatic sulfonation reaction (Alhusainy et al., 2013; Bertelli et al., 2021; Ghazali and Waring, 1999; Morimitsu et al., 2004; Rietjens et al., 2011; Wan and Jiang, 2018).

Alhusainy et al. (2013) and Al-Subeihi et al. (2013) reported the inhibition of SULT mediated estragole and methyleugenol on DNA adduct formation by the flavone nevandesin (a trimethoxyflavone) showing that this flavonoid, like quercetin, kaempferol, myricetin and apigenin have an inhibitory effect on the the bioactivation of food-borne genotoxic carcinogens like estragole or methyleugenol.

Other aspects that have impeded the final evaluation of the risk of consuming foods that naturally contain these compounds, are: The limited knowledge of their content of alkenylbenzenes, the difficulties in establishing the possible exposure doses that derive from consumption habits, and the scarcity of studies related to the carcinogenicity of the different alkenylbenzenes (Eisenreich et al., 2021).

In the specific case of the result of this work, it corroborated the high content of estragole and other alkenylbenzenes, in the essential oil of the leaves (already known for decades), and they also shown the relatively high content of these compounds in the EOs extracted from the edible portions of the pulp and peel of the Mexican criollo avocado.

It is important to note that P. americana in its different varieties, including the drymifolia variety, have shown, through several studies, that the plant and its fruit have antioxidant phenolic compounds, especially, flavonoids (Bhuyan et al., 2019; Dabas et al., 2013; Prabha et al., 1980; Ramos-Aguilar et al., 2021a,b; Rodríguez-Carpena et al., 2012; Trujillo-Mayol et al., 2020).

As tarragon, basil, and fennel, and now criollo avocado fruit, and their essential oils, with known high alkenylbenzenes content, have one ancestral consumption in their complex biological matrices, showing up to now, in the absence of scientific evidence to the contrary, minimal risks of its toxicity. Different as presented by single alkenylbenzenes in laboratory animals (Marabini et al., 2019; Schrader, 2003).

Conclusions

- The Mexican criollo avocado is an endemic plant of Mexico whose economic, cultural, and functional biochemical characteristics are of great importance since pre-Hispanic times and it represents a phytogenetic richness
- The main attribute of this avocado variety, that distinguishes it from others widely traded varieties, resides in its aroma and flavor of its fruit -peel and pulp- and leaves
- Even though the constituent volatile components of the leaves have been analyzed for a long time, for the special repellent properties of the plant, against insects and microorganisms, the corresponding components of the aroma of the peel and pulp of the fruit had not been widely established
- In this research, the extraction yield and analysis of the composition of the essential oil from leaves, peel, and pulp, were done. The yields in essential oil were 1.18±0.24, 0.74±0.05, and 0.28±0.17% (db) for the leaf, peel, and pulp, respectively
- There were identified 59 different volatile compounds in the analyzed parts of the plant. The majority of the compounds were benzene derivatives: 86.75, 73.08, and 60.24%; terpene derivatives: 6.51, 21.38, and 36.51% in the leaf, peel and pulp EOs, respectively. Individually, the highest contents were estragole, ledene oxide (II), aromadendrene, β-pinene, methyleugenol, and benzaldehyde, 4-methoxy-, among others
- The toxicological characteristics of the identified volatile components, the bio-activation of food-borne genotoxic carcinogens, and the toxicity risks involved in the consumption of some major volatile components such as estragole, found in the EOs of criollo avocado peel and pulp were discussed. In the light of some recent scientific contributions about the character chemoprotector and inhibitory of other natural components, like flavonoids, and the matrix effect of plants and food, in the case of the Mexican autochthonous avocado it seems that the risk might be minimized.

Glossary

Acronyms or terms	Meaning
Db	Dry basis
EO	Essential oil
HS-SPME	Headspace solid-phase microextraction method
INIFAP	Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias (Mexico)
IARC	International Agency for Research on Cancer
SULT	Sulfotransferase enzymes
USAII	Spanish acronym for Unidad de Servicios de Apoyo a la Investigación y a la Industria (Research and Industry Support Services Unit) of the Faculty of Chemistry -Facultad de Química in Spanish- of the National Autonomous University of Mexico, UNAM in Spanish
Wb	Wet basis

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