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ARTICULO DE INVESTIGACION

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Análisis de los compuestos volátiles de la guayaba ácida (*Psidium acidum* [DC.] Landrum) mediante microextracción en fase sólida del espacio de cabeza

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ABSTRACT

Application of headspace-solid phase microextraction combined with gas chromatography coupled to flame ionization, mass spectrometry and olfactometric detectors was used to analyze, for the first time, the volatile compounds of sour guava (*Psidium acidum* [DC.] Landrum) and to estimate the most odor-active compounds. According to the global odor trapped in the fiber, a comparison between 100 µm PDMS, 65 µm PDMS/DVB, 50/30 µm DVB/CAR/PDMS and 85 µm CAR/PDMS fibers showed that the 50/30 µm DVB/CAR/PDMS fiber was the most adequate to analyze the volatile compounds of this fruit. The analyses led to the identification of 128 compounds, including 49 esters (88.7 % of the total volatile composition), 20 terpenes (0.9 %), 14 alcohols (3.8 %), 14 aldehydes (0.7 %), 13 acids (2.4 %), 7 ketones (0.5 %), 5 hydrocarbons (0.1 %), 3 oxides (2.4 %), 2 furans (0.1 %) and one S-compound (traces). Major components (> 8 %) were (*Z*)-3-hexenyl acetate, butyl butanoate, butyl acetate, methyl octanoate, methyl hexanoate and hexyl acetate. Twenty-six of them were considered as odor-active compounds and contribute to the typical sour guava aroma, from which the most important were (*Z*)-3-hexenyl acetate, hexyl acetate, 3-methylbutyl butanoate and (*Z*)-3-hexenyl hexanoate. The relevance of aliphatic esters, particularly those related to C6 compounds, as odor-active compounds of sour guava fruit was tentatively demonstrated

Keywords: sour guava; *Psidium acidum*; volatile compounds; headspace-solid phase microextraction.

RESUMEN

La aplicación de la microextracción en fase sólida del espacio de cabeza combinada con la cromatografía de gases acoplada con detectores de llama de hidrógeno, masas y olfatométrico fue empleada para analizar, por primera vez, los compuestos volátiles de la guayaba ácida (*Psidium acidum* [DC.] Landrum) y para estimar los compuestos más activos en el aroma de la fruta. De acuerdo con el olor global atrapado en la fibra, una comparación entre las fibras de 100 µm PDMS, 65 µm PDMS/DVB, 50/30 µm DVB/CAR/PDMS y 85 µm CAR/PDMS mostró que la fibra de 50/30 µm DVB/CAR/PDMS fue la más adecuada para analizar los compuestos volátiles de esta fruta. El análisis permitió la identificación de 128 compuestos, incluyendo 49 ésteres (88.7 % de la composición volátil total), 20 terpenos (0.9 %), 14 alcoholes (3.8 %), 14 aldehídos (0.7 %), 13 ácidos (2.4 %), 7 cetonas (0.5 %), 5 hidrocarburos (0.1 %), 3 óxidos (2.4 %), 2 furanos (0.1 %) y un compuesto azufrado (trazas). Los componentes mayoritarios (> 8 %) fueron el acetato de (*Z*)-3-hexenilo, butanoato de butilo, acetato de butilo, octanoato de metilo, hexanoato de metilo y acetato de hexilo. De ellos, 26 fueron considerados como compuestos activos del aroma y contribuyen al típico aroma de la guayaba ácida, de los cuales los más importantes fueron el acetato de (*Z*)-3-hexenilo, acetato de hexilo, butanoato de 3-metilbutilo y hexanoato de (*Z*)-3-hexenilo. La relevancia de los ésteres alifáticos, particularmente aquellos relacionados a los compuestos C6, como compuestos activos del aroma de la guayaba ácida fue tentativamente demostrada.

Palabras claves: guayaba ácida; *Psidium acidum*; compuestos volátiles; microextracción en fase sólida del espacio de cabeza.

INTRODUCTION

Psidium is a genus of at least 60 species and perhaps as many as 100, ranging from Mexico and the Caribbean to Argentina and Uruguay (Landrum, 2017). A few species have been introduced as cultivated plants in the Old World and Pacific Island tropics and subtropics, and some are weedy invasives (Global Invasive Species Database, 2020). Taxonomic studies of *Psidium* have been numerous in the last few years with several new species being described (Landrum, 2017).

Recently, *Psidium acutangulum* DC. as it has traditionally been recognized east of the Andes, was divided into two species: *P. acutangulum* and *P. acidum* (DC.) Landrum; and some of the populations of *Psidium* from western Ecuador on the Pacific slope that have previously been identified as *P. acutangulum*, should be recognized as a new species (Landrum, 2016).

P. acidum is native to tropical South America and it is a large shrub or small tree, commonly known in some American countries as "guayaba de monte", "sacha guayaba", "guayaba agria" and "guayaba ácida". The fruit is a smooth spherical berry (6-7 cm across), glabrous, green turning to yellow when ripe, with persistent calyx remnants at the apical end and yellowish-white, very acidic but strongly flavored pulp containing a few hard and triangular seeds (Trujillo et al., 2018). The fruit is consumed fresh, but commonly it is used to prepare juices and sorbets.

No work has yet been published in the literature to characterize the volatile compounds of sour guava fruit. Therefore, the aim of the present study was to analyze its volatile compounds and to investigate the aroma-active compounds by headspace-solid phase microextraction (HS-SPME).

MATERIALS AND METHODS

Samples and chemicals

Sour guava fruits were grown using standard agricultural practices in the University of Ciego de Avila, Cuba. Fruits were harvested at fully yellow maturity stage and immediately transported to the laboratory. Fruits were cut, and the peel and seeds were removed. The pulp was homogenized using a commercial blender. Plant materials were deposited in the Julián Acuña Galé herbarium (Ignacio Agramonte University, HIPC-Thiers, 2018; voucher HPC-12030).

Reference compounds were purchased from Sigma-Aldrich (Steinheim, Germany) and Merck (Darmstad, Germany), and some others were generously given by Robertet (Grasse, France). A normal paraffin solution (C_5-C_{24}) was supplied by Sigma-Aldrich. Sodium chloride was provided by Merck (Darmstadt, Germany).

Standard chemical analysis

Total soluble solids, total acidity (as anhydrous citric acid), pH and ascorbic acid content were assayed in fruit samples according to official methods (AOAC, 2019).

Headspace solid-phase microextraction analysis

Volatile compounds from the fresh fruit homogenate headspace were extracted using four SPME fiber coatings: 100 µm PDMS, 65 µm PDMS/DVB, 50/30 µm DVB/CAR/PDMS, and 85 µm CAR/PDMS (Supelco, Park, Bellefonte, Pa.). All the fibers were conditioned before use and cleaned between analyses by inserting them into the GC injector. HS-SPME extraction was performed at 40 °C on 3 g of pulp, 3 mL distilled water and 1 g NaCl contained in a 15-mL vial sealed with a PTFE-lined screw cap. A pre-extraction time of 10 min, and an extraction time of 30 min under magnetic stirring at 600 min^{-1} were applied. The sampling conditions were chosen after preliminary GC-FID analyses and were like those reported in other studies (Pino & Febles, 2013; Pino & Bent 2013; Pino, 2014).

GC-FID and GC-MS analysis

A Hewlett-Packard 6890N series II (Agilent, Santa Clara, CA, USA) gas chromatograph equipped with a 30 m x 0.25 mm x 0.25 µm DB-5ms (J & W Scientific, Folsom, CA) and a flame ionization detector (FID) was used. Oven temperature was held at 50 °C for 2 min and then raised to 280 °C at 4 °C/min and held for 10 min. Carrier gas (hydrogen) flow rate was 1 mL/min. Injector and detector were set at 250 °C. Injection was in splitless mode (2 min) and with a recommended liner of 0.75 mm. Linear retention indexes were calculated using a mixture of normal paraffins. quantitative determinations were based on the normalization method assuming similar calibration factors for all the compounds.

GC-MS analysis was performed on a QP-2010 Ultra (Shimadzu, Japan) with the same capillary column and chromatographic parameters as for the GC-FID. Helium carrier gas flow rate was 1

mL/min. MS data were recorded in a mass range 35-350 u, with electron energy of 70 eV and ion source and connecting parts temperature, 250 °C. The identification of compounds was achieved by comparison of their linear retention indexes and mass spectra with those shown by reference standards when they were available. In other cases, comparison was made with those in commercial databases (NIST 05, NBS 75 k, Wiley 6 and Adams 2001).

HS-SPME direct gas chromatography-olfactometry (GC-O)

A Hewlett-Packard 6890N series II gas chromatograph equipped with a FID and a sniffing port joined to the injector by a short stainless-steel capillary (25 cm x 0.4 mm i.d.). The flow rate of the carrier gas (H_2) was 25 mL/min, and the oven temperature was kept at 250 °C. The three SPME extracts were introduced in successive sequences into the GC port at 250 °C. Because no chromatographic separation was carried out by the short capillary, volatile compounds arrived simultaneously at the sniffing port. Here, for each SPME extract, a trained panel of three sniffers perceived and evaluated the resulting global odor. Fibers were kept in the GC inlet until the end of the sensorial stimulus.

Sensory analysis sessions were performed only after a suitable training: sniffers were first familiarized with fresh fruit and asked to agree on a common list of descriptors. A similarity test was performed in triplicate on the same homogenate. Sniffers were asked to smell the reference pulp (2 mL) contained in a plastic cup sealed with a pierced cap at 25 °C. They had to memorize the odor and then describe it using the descriptors list. Then they evaluated with the direct GC-O
device the different extracts, rating their similarity to the reference using a 10-cm scale ranging from 0 (close to the reference) to 10 (far from the reference). Sniffers had to smell the reference before each sample evaluation. All analyses were replicated three times.

Gas chromatography-olfactometry of HS-SPME extract

The odor-active compounds of DVB/CAR/PDMS SPME extracts were analyzed by GC-O on the above mentioned GC-FID equipped with the same capillary column mentioned above, connected via two fused silica capillaries (25 cm x 0.25 mm i.d.) to a FID and a sniffing port described earlier (Pino & Febles, 2013). After sampling, the SPME fiber was desorbed for 5 min into the injection port at 250 °C. Operating conditions were the same as described before for GC-FID. The GC effluent was split 1:1 between the FID and the sniffing port (both at 250 °C). Injection volume was 1 µL. For each odor stimulus, the three sniffers recorded the detection time and odor description. GC-O frequency analysis was performed following the methodology described earlier (Chaintreau, 2001). Detected odors (quality and retention times) were marked in the chromatogram. Each sample was sniffed in triplicate by each sniffer. Zones of the chromatogram which were detected with the same descriptor, at least three times, were considered as odor zones.

RESULTS AND DISCUSSION

Four fibers, coated with 100 µm PDMS, 65 µm PDMS/DVB, 50/30 µm DVB/CAR/PDMS and 85 µm CAR/PDMS, were assessed for the isolation of the volatile compounds. The similarity scaling obtained for the four SPME global odors with respect to the reference sample were DVB/CAR/PDMS (1.0 ± 0.2), PDMS/DVB (3.5 ± 0.3), CAR/PDMS (7.2 ± 0.3) and PDMS (8.2 ± 0.5) and. The reason might be that the distribution coefficient of three phase between coating and sample exist in the process of extraction. Thus, the 50/30 µm DVB/CAR/PDMS fiber was selected in this study.

Table 1 shows the 128 volatile compounds isolated from sour guava fruit, representing 99 % of the isolated compounds, as well as the odor detected by sniffers. According to their chemical group, the volatile compounds are classified as 49 esters, 20 terpenes, 14 alcohols, 14 aldehydes, 13 acids, 7 ketones, 5 hydrocarbons, 3 oxides, 2 furans and one S-compound.

Table 1. Chemical composition and GC-O evaluation of the volatile compounds in sour guava fruit

Compound	LRI	Area %	Detection frequency	Odor description
Acetic acid	645	0,1		
Butan-1-ol	669	0,6		
1-Penten-3-ol	683	0,1		
Propyl acetate	707	0,1		
Methyl butanoate	729	0,3	3	Fruity
Dimethyl disulfide	742	tr		
2-Methylpropyl acetate	768	tr		
Hexane-2,3-dione	786	tr		
(*Z*)-3-Hexenal	796	tr		
Hexanal	802	0,2	6	Green, fruity
Ethyl butanoate	805	0,1	7	Fruity
Butyl acetate	811	9,3	7	Strong fruity
1-Methoxyhexane	819	1,2		
1-Methoxy-3-hexene	826	1,2		
(*Z*)-1-Methoxy-2-hexene	833	tr		
2-Pentyl acetate	843	tr		
(*E*)-3-Hexen-1-ol	855	tr		
(*E*)-2-Hexenal	856	tr		
(*Z*)-3-Hexen-1-ol	859	1,4	5	Grassy-green
(*E*)-2-Hexen-1-ol	862	0,1		
Hexan-1-ol	871	1,0		
3-Methylbutyl acetate	881	tr		
3-Methyl-3-buten-1-yl acetate	887	tr		
Propyl butanoate	899	tr		
Heptanal	902	tr		
Butyl propanoate	907	0,4	3	Faintly sweet
(*Z*)-2-Penten-1-yl acetate	910	0,1		
Pentyl acetate	915	0,5	3	Fruity
Methyl hexanoate	924	8,6	7	Pineapple
Methyl (*Z*)-3-hexenoate	927	0,2		
α-Thujene	930	tr		
Benzaldehyde	960	tr		
Methyl (*E*)-2-hexenoate	966	tr		
6-Methyl-5-hepten-2-one	986	0,3		
6-Methyl-5-hepten-2-ol	992	tr		
Butyl butanoate	995	11,0	7	Fruity, pear-like
Hexanoic acid	1000	0,1		
(*Z*)-3-Hexenyl acetate	1005	24,6	9	Green, fruity
Hexyl acetate	1009	8,4	9	Fruity, floral
(*E*)-2-Hexenyl acetate	1019	0,1		
(*Z*)-4-Hexenyl acetate	1022	tr		
p-Cymene	1025	0,1		
Methyl heptanoate	1026	0,1	4	Fruity
3-Cyclohexenyl acetate	1028	0,2		

Table 1. (continued)

Compound	LRI	Area %	Detection frequency	Odor description
1,8-Cineole	1030	tr		
2-Ethyl-1-hexanol	1033	0,4		
Bergamal	1044	tr		
Phenylacetaldehyde	1052	tr		
Butyl crotonate	1054	tr		
3-Methylbutyl butanoate	1058	0,4	9	Ripe fruity
4-Methoxy-2,5-dimethyl-3(2*H*)-furanone	1061	0,1		
Acetophenone	1065	tr		
Octan-1-ol	1068	tr		
Linalool	1097	0,3	6	Floral
Nonanal	1101	0,3	8	Citrus-like
2,6,6-Trimethyl-2-hydroxy-cyclohexanone	1108	tr		
(*Z*)-3-Hexenyl propanoate	1111	0,2	5	Ripe pear-like
Heptyl acetate	1115	tr	5	Sweet, apricot-like
2-Ethylhexanoic acid	1120	tr		
exo-Fenchol	1123	tr		
Methyl octanoate	1127	8,7	5	Fruity, orange-like
4-Ketoisophorone	1145	tr		
2-Ethylhexyl acetate	1155	tr		
Benzyl acetate	1162	tr		
Borneol	1169	0,1		
Nonan-1-ol	1172	tr		
Methyl phenylacetate	1179	tr		
iso-Menthol	1183	tr		
(*Z*)-3-Hexenyl butanoate	1186	5,0	4	Green, fruity
Butyl hexanoate	1188	2,4		
α-Terpineol	1189	tr		
Hexyl butanoate	1191	3,7	5	Fruity, apricot-like
(*E*)-2-Hexenyl butanoate	1194	0,4		
Ethyl octanoate	1197	tr		
Decanal	1202	0,1	7	Citrus-like
Octyl acetate	1214	0,4	5	Fruity
β-Cyclocitral	1220	tr		
Geraniol	1252	tr		
2-Phenylethyl acetate	1257	0,1		
(*E*)-2-Decenal	1266	tr	4	Fatty
(*Z*)-3-Hexenyl 2-methylbutanoate	1272	tr		
Pentyl hexanoate	1282	0,1		
Methyl decanoate	1326	0,1		
Citronellyl acetate	1354	tr		
Neryl acetate	1362	tr		
Decanoic acid	1375	0,2		
α-Copaene	1377	tr		
(*Z*)-3-Hexenyl hexanoate	1380	0,9	9	Fully-green
Geranyl acetate	1382	tr		

Table 1. (continued)

Compound	LRI	Area %	Detection frequency	Odor description
(*Z*)-3-Hexenyl (*Z*)-3-hexenoate	1384	tr		
Hexyl hexanoate	1387	0,3	6	Herbaceous
Butyl octanoate	1389	1,3		
Octyl butanoate	1394	tr		
n-Tetradecane	1400	tr		
Dodecanal	1409	tr		
(*E*)-Caryophyllene	1420	tr		
2-Phenylethyl butanoate	1441	tr		
Geranyl acetone	1453	0,5		
2,6-Dimethyl-2,6-undecadien-10-ol	1459	0,2		
Undecanoic acid	1464	0,1		
Dodecan-1-ol	1471	tr		
(*E*)-β-Ionone	1487	0,1	8	Woody, fruity
Pentyl octanoate	1490	tr		
β-Selinene	1493	tr		
α-Selinene	1498	tr		
n-Pentadecane	1500	tr		
β-Bisabolene	1506	tr		
Tridecanal	1510	tr		
δ-Cadinene	1525	tr		
Dihydroactinidiolide	1537	tr		
Dodecanoic acid	1566	0,4		
(*Z*)-3-Hexenyl octanoate	1570	0,1		
Hexyl octanoate	1579	tr		
n-Hexadecane	1600	tr		
Tetradecanal	1613	0,1		
Benzophenone	1628	0,1		
Tridecanoic acid	1662	0,1		
n-Heptadecane	1700	tr		
Tetradecanoic acid	1779	0,1		
(*E,E*)-Farnesyl acetate	1843	tr		
Pentadecanoic acid	1868	0,1		
Hexadecan-1-ol	1876	tr		
(*E,E*)-Farnesyl acetone	1922	tr		
(*Z*)-9-Hexadecenoic acid	1953	0,1		
Hexadecanoic acid	1960	0,4		
Heptadecan-1-ol	1975	tr		
(*Z*)-9-Octadecenoic acid	2140	0,5		
Octadecanoic acid	2200	0,2		

LRI: Linear retention indices in DB-5ms capillary column. tr: <0.1%.

The semi-quantitative distribution of the fruit volatiles chemical families included esters (88.7 %), alcohols (3.8 %), acids (2.4 %), oxides (2.4 %), terpenes (0.9 %), aldehydes (0.7 %), ketones (0.5 %), hydrocarbons (0.1 %), furans (0.1 %) and S-compound (traces). Major components (> 8 %) were (*Z*)-3-hexenyl acetate, butyl butanoate, butyl acetate, methyl octanoate, methyl hexanoate and hexyl acetate. The GC-O was performed to categorize the volatile compounds according to their odor potency. Twenty-six compounds exhibited frequency factors ≥ 3 from a maximum of nine and therefore, they should contribute to the overall sour guava aroma. Of them, (*Z*)-3-hexenyl acetate (green, fruity), hexyl acetate (fruity, floral), 3-methylbutyl butanoate (ripe fruity) and (*Z*)-3-hexenyl hexanoate (fully green) were the most-odor active compounds. (*Z*)-3-Hexenyl acetate and hexyl acetate were among the most abundant in the composition and had high detection frequencies. It is interesting to note that 3-methylbutyl butanoate and (*Z*)-3-hexenyl hexanoate, which very low proportions also had high detection frequencies. Besides this, the relevance of aliphatic esters, particularly those related to C6 compounds, as odor-active compounds of sour guava fruit was tentatively demonstrated.

Esters seemed to be the important aroma compounds in fruits by their fruity notes. Some of them have been reported as key compounds in *Psidium* spp. fruits (Steinhaus *et al.*, 2008, 2009; Pino & Bent, 2013; Buranelo-Egea *et al.*, 2014; Cuadrado-Silva *et al.*, 2017).

A quantitative approach based on absolute concentrations and the calculation of odor activity values combined with sensory studies needs to be done to determine the actual contribution of these volatile compounds to sour guava fruit, including model and omission sensory experiments.

CONCLUSIONS

A total of 128 volatile compounds were identified, for the first time, in sour guava fruit, including 49 esters, 20 terpenes, 14 alcohols, 14 aldehydes, 13 acids, 7 ketones, 5 hydrocarbons, 3 oxides, 2 furans and one S-compound. Twenty-six of them were considered as aroma-active compounds, from which the most important were (Z)-3-hexenyl acetate, hexyl acetate, 3-methylbutyl butanoate and (*Z*)-3-hexenyl hexanoate. The relevance of aliphatic esters, particularly those related to C6 compounds, as odor-active compounds of sour guava fruit was tentatively demonstrated.

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