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Characterization of volatile compounds in guava (*Psidium guajava* L.) varieties from Colombia

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Key words: guava, *Psidium guajava*, volatile compounds, HS-SPME, GC-MS.

RESUMEN. La guayaba (*Psidium guajava* L.), con su sabor único y que recuerda al quince-banano, es una fruta subtropical económicamente importante en muchos países tropicales en todas las estaciones. En este trabajo se aislaron los constituyentes volátiles de tres variedades de guayaba colombianas: Coronilla (comúnmente guayaba común), Palmira ICA-1 (comúnmente llamada guayaba pera) y Glum Sali (comúnmente llamada guayaba manzana), mediante microextracción en fase sólida del espacio de cabeza usando fibras de 100 mm de polidimetilsiloxano y se analizaron por cromatografía de gases-espectrometría de masas. Para cada extracción, 30 g de frutas y 90 mL de disolución de NaCl 20 % se mezclaron por 10 min y se centrifugaron a 3 000 r/min durante 10 min . El sobrenadante (7 mL) se colocó en un vial de 15 mL, se mantuvo cerrado por 15 min para alcanzar el equilibrio y posteriormente, se expuso la fibra en el espacio de cabeza durante 30 min . El vial fue continuamente agitado a 100 r/min y mantenido a 40 °C . La desorción se hizo durante 2 min en el inyector a 250 °C . Se identificaron 97 compuestos, 19 de ellos se reportan por primera vez en la guayaba. Cada variedad posee una composición típica, caracterizada por una relación específica de los componentes mayoritarios y las clases presentes de sustancias. Las variedades Palmira ICA-1 y Coronilla poseen mayor cantidad de compuestos volátiles que la variedad Glum Sali, particularmente ésteres, mientras que en la variedad Glum Sali predominan el hexanal, 2*E*-hexenal y los ácidos. Los compuestos volátiles mayoritarios fueron el acetato de 3*Z*-hexenilo, acetato de 3-fenilpropilo, acetato de (*E*)-cinamilo y hexanal.

ABSTRACT. The guava (*Psidium guajava* L.), which has unique and quince-banana like flavor, is an economically important subtropical fruit in many tropical countries on all seasons. Volatile constituents from three Colombian varieties of guava fruits: Coronilla (commonly named *guayaba común*), Palmira ICA-1 (commonly named *guayaba pera*) and Glum Sali (commonly named *guayaba manzana*) were isolated by headspace-solid phase microextraction using 100 mm polydimethylsiloxane fibers and analyzed by gas chromatography-mass spectrometry. For each extraction, 30 g of fruits and 90 mL of 20 % NaCl solution were blended for 10 min and then centrifuged at 3 000 r/min for 10 min . A 7 g supernatant was immediately placed in a 15 mL vial. The sample was kept for 15 min to achieve the equilibrium and after them, the SPME fiber was exposed to the headspace of the sample to adsorb the volatiles for 30 min . Vials were continuously swirled with an agitation speed of 100 r/min and at 40 °C . Desorptions for 2 min into the GC injector were made at 250 °C . Ninety-seven compounds were identified in the present study, 19 of them for the first time as volatile constituents of guava. Each variety has a typical composition, characterized by a specific ratio between the main components and classes of substances. Palmira ICA-1 and Coronilla varieties had higher amount of volatile compounds than Glum Sali variety, particularly esters; while in Glum Sali variety were predominant hexanal, 2*E*-hexenal and acids. Major volatiles in all varieties were either 3*Z*-hexenyl acetate, 3-phenylpropyl acetate, (*E*)-cinnamyl acetate and hexanal.

INTRODUCTION

Among the many attractive and desirable attributes that create demand for fruits from the tropics and subtropics, their characteristic flavor is the most noticeable to consumers. In addition, these fruits are often inexpensive, extremely rich in vitamins, and can be used in a wide range of food products.

Guava is the fruit of *Psidium guajava* L., a tree native of Central America. It is grown in many tropical and subtropical areas of the world. The round-oval, green-yellow fruit is valued for its light yellow or pink pulp, having a flavor impression often described as “quince-banana” like. On a worldwide scale, guava is produced in much smaller quantity than other major tropical fruits, but it is economically important in certain countries. In addition to its consumption as fresh fruit, guava is processed into many different foods such as jams, jellies, nectars and juices.

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Among publications about guava volatiles,[1-13] most concern identification and distribution of volatile components of fresh fruits. The most typically utilized methods for isolation and concentration had been liquid-liquid extraction, simultaneous distillation-extraction, vacuum distillation and purge-and-trap. The majority of these methods are time-consuming, require exhaustive concentration steps and require dedicated headspace sampling devices. An alternate sampling procedure, headspace-solid phase microextraction (HS-SPME), has the potential to reduce the time investment in sampling and should work well in combination with rapid separation and detection systems.[14,15]

The aim of this study was to analyze the volatile constituents of three Colombian varieties of guava fruits: Coronilla, Pera and Manzana, by HS-SPME combined with GC-MS.

MATERIALS AND METHODS

Fruits

Fresh ripe fruits from the varieties: Coronilla, Palmira ICA-1 (commonly named *guayaba pera*) and Glum Sali (commonly named *guayaba manzana*) were picked from the same bushes grown in Coello (Tolima province) Colombia. The fruits were collected, provided and identified by Colombian Institute of Agriculture (ICA). These fruits were transported and immediately after arrival they were checked and tested, so that the isolation of volatile compounds was concluded within 24 h after harvest.

Headspace-solid phase microextraction

The SPME holder and fibers were purchased from Supelco Inc. (Bellefonte, USA). Fibers of 100 µm polydimethylsiloxane (PDMS) were used. The fibers were activated according to the manufacturer's instructions. Preliminary assays were carried out in order to establish the experimental conditions for HS-SPME of guava volatiles, particularly the temperature, and the equilibration and sampling times. For each extraction, 30 g of fruits (without seeds) and 90 mL of 20 % NaCl solution were blended with a Braun MR 400 juicer for 10 min and then centrifuged at 3 000 r/min for 10 min . A 7 g supernatant was immediately placed in a 15 mL vial. In each extraction the sample was kept for 15 min to the headspace of the sample to adsorb the volatiles for 30 min . Vials were continuously swirled with an agitation speed of 100 r/min at 40 °C . Desorptions for 2 min into the GC injector were made at 250 °C . Analyses were made three times for different batches of fruits.

Gas chromatography-mass spectrometry

An HP 6890 Series II with a HP-5973N mass detector and a fused silica HP-5MS capillary column (60 m X 0.25 mm i.d. X 0.25 mm film thickness) were used. The temperature program was 2 min isothermal at 50 °C and then 40-220 °C at a rate of 4 °C /min . The carrier flow rate (helium) was 1 mL/min . Injector and detector temperatures were kept at 220 °C . The injection was in splitless mode (splitless time = 2 min). Chromatographic retention indices were calculated of separated compounds relative to a C_8-C_{25} n-alkanes mixture. Mass spectra were recorded in the electron-impact (EI) mode at 70 eV by 1.8 scans/s, and the mass range used was m/z 35-300.

Qualitative and quantitative analysis of volatile compounds

Constituents were identified by comparison of their mass spectra with those in NIST/EPA/NIH or our FLAVORLIB data base (from reference standards), and confirmed in many compounds by their relative retention indices with authentic standards. Mass spectra from the literature[16] were also compared.

Semi-quantitative determinations were carried out in terms of relative (percent) areas in the chromatograms. The data from triplicate analyses was transformed ($y = \arcsin p^{1/2}$), statistically processed by two-way ANOVA and Duncan's test for significant differences.

RESULTS AND DISCUSSION

Table 1 shows the semi-quantitative composition of guava fruit varieties. In general, more than 95 % of the total composition was identified. In terms of total area (which represents the total amount of volatiles extracted from the same quantity of fruits for each variety), Palmira ICA-1 and Coronilla varieties have higher amount of volatile compounds than Glum Sali variety. Ninety-seven compounds were identified, 19 of them for the first time as volatile constituents of guava. Each variety main components. It would seem from these data that there are significant variations among the varieties. In total, 41 esters, 18 terpenes, 17 carbonyls, 12 acids, four alcohols, two phenols, two furans and one component with miscellaneous structure were identified.

Qualitatively and quantitatively, esters occupy a special place among the guava fruit volatiles (Table 1). Among these components, 3*Z*-hexenyl acetate, 3-phenylpropyl acetate and (*E*)-cinnamyl acetate presented the largest amount. Other major esters were ethyl butanoate (in Coronilla variety), ethyl hexanoate and hexyl acetate. Ethyl hexanoate was considered the important flavor component in guava juice,[3] while 3-phenylpropyl acetate was considered a characteristic sweet flavor of ripening guava from Amami Island.[6] All these esters had been reported in higher amounts in other studies.[3,4,12,13] In general, Coronilla and Palmira ICA-1 varieties had higher concentrations than Glum Sali variety.

Carbonyls were the second important class of compounds in the quantitative distribution of the studied varieties. Among these components, hexanal and 2*E*-hexenal presented the largest amount, particularly in Glum Sali variety. These C_6 aldehydes were found as major compounds in Brazilian guava[4] and it had been suggested that the presence of high amounts of C_6 aldehydes and alcohols involved enzymic oxidation and reduction of C_6 compounds. The presence of hexanoic acid and many esters with C_6 moieties agrees with this hypothesis.

In total, 12 acids were identified, one of them, 2-ethylhexanoic acid, reported for the first time in guava. Glum Sali variety had major proportion of acids than the other two varieties.

Another important class of compounds was the terpenes. Limonene and β-caryophyllene were the major terpene hydrocarbons. They were also identified as major constituents in previous studies.[1,2,10] On the other hand, 1,8-cineol, which has been reported in previously as a major volatile in guava from Taiwan,[10] was not found.

A further important group of guava volatiles was represented by the cinnamyl derivatives, including cinnamaldehyde, cinnamyl alcohol, ethyl (*E*)-cinnamate, and esters of

Table 1. Volatile compounds in guava (GC-MS peak area %).

Compound	IK	Coronilla	Glum Sali	Palmira ICA-1
Acetaldehyde	435	0.3 a	1.1 b	0.2 a
Ethanol	537	0.3 a	1.7 b	0.2 a
Methyl acetate	545	0.2	t	0.2 a
Acetic acid	600	0.4 a	1.6 b	0.3
Ethyl acetate	605	0.9	1.7	0.2
Methyl propanoate	646	t	nd	nd
2-Ethylfuran	702	0.2	t	0.2
Ethyl propanoate	717	0.3 a	nd b	nd b
Methyl butanoate	729	0.4 a	nd b	nd b
2-Methylpropyl acetate	776	0.2	nd	t
Hexanal	802	14.7 a	40.4 b	14.7 a
Ethyl butanoate	804	4.3	t	t
Butyl acetate	812	t	nd	nd
2-Furfural	830	t	t	nd
3-Methyl-2-butyl acetate[1]	846	t	nd	nd
2E-Hexenal	855	0.4 a	5.2 b	0.6 a
3Z-Hexenol	859	0.3 a	0.2 a	0.2 a
1-Hexanol	871	0.2 a	0.1 a	0.2 a
3-Methylbutyl acetate	876	0.1 a	nd a	0.1 a
2-Methylbutyl acetate[1]	881	t	nd	nd
Propyl butanoate[1]	899	t	nd	nd
Ethyl pentanoate	901	0.1	nd	t
γ-Butyrolactone[1]	915	0.1 a	2.6 a	0.1 a
Methyl hexanoate	926	0.6 a	0.2 b	0.6 a
α-Pinene	939	0.5 a	0.6 a	0.3 a
2E-Heptenal	957	t	t	nd
Benzaldehyde	960	0.2 a	0.1 a	0.2 a
Hexanoic acid	975	t	0.1	t
6-Methyl-5-hepten-2-one	985	t	nd	t
Myrcene	991	nd a	nd a	0.1 b
2-Pentylfuran	992	0.1 a	nd b	nd b
Butyl butanoate	995	0.1	0.1	t
Ethyl hexanoate	998	3.1 a	0.2 b	1.2 c
α-Phellandrene[1]	1003	0.2 a	0.5 a	0.2 a
3Z-Hexenyl acetate	1005	15.9 a	6.3 b	23.4 c
Hexyl acetate	1009	2.0 a	nd b	1.2 a
ρ-Cymene	1025	0.2 a	nd b	0.2 a
Limonene	1028	3.5 a	2.0 b	4.7 c
5-Ethyl-2(5H)-furanone	1030	t	t	t
(Z)-β-Ocimene	1036	t	nd	nd
Butyl 2-methylbutanoate[1]	1044	0.1	nd	t
(E)-β-Ocimene	1050	t	nd	0.1
γ-Terpinene	1060	0.1 a	0.1 a	0.1 a
Acetophenone	1066	nd a	0.1 b	nd a
Terpinolene	1089	t	nd	t
Methyl benzoate	1091	0.2 a	nd b	0.1 a
Linalool	1097	0.2 a	0.3 a	0.1 a
Nonanal	1101	0.4 a	0.5 a	0.3 a

of cinnamic acids,[4] and the production of cinnamyl esters or cinnamates was assumed to have relationship with cinnamyl alcohols or cinnamic acids. These aromatic compounds may play an important role in the characteristic sweet flavor of ripe guavas.

Comparing these results with a previous work of volatiles from Palmira ICA-1 and Glum Sali guava fruits isolated by liquid-liquid extraction[13] it was found some similar results. Again, it was found that Palmira ICA-1 has higher content of volatiles than Glum Sali. Esters e.g. 3*Z*-hexenyl acetate and (*E*)-cinnamyl acetate were found as major compounds in both studies. On the other hand, hexanal and 3-phenylpropyl acetate were reported in lower concentration in previous work.[13] The present study applied the HS-SPME analysis, which did not cause a loss of volatiles during extraction and separation, and no artifacts appeared. Hence, this procedure is very suitable for detection of changes of volatiles during processing.

CONCLUSIONS

Ninety-seven compounds were identified in the present study, 19 of them for the first time as volatile constituents of guava. Each variety has a typical composition, characterized by a specific ratio between the main components and classes of substances. Palmira ICA-1 and Coronilla varieties had higher amount of volatile compounds than Glum Sali variety, particularly esters; while in Glum Sali variety were predominant hexanal, 2*E*-hexenal and acids. Major volatiles in all varieties were either 3*Z*-hexenyl acetate, 3-phenylpropyl acetate, (*E*)-cinnamyl acetate and hexanal.

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Table 1. *(Continued)*

Compound	IK	Coronilla	Glum Sali	Palmira ICA-1
Cis-rose oxide[1]	1108	t	nd	t
2-Ethylhexanoic acid[1]	1122	0.1	0.6	t
Methyl octanoate	1127	0.2 a	nd b	0.4 c
Benzoic acid	1165	0.1	t	t
Ethyl benzoate	1173	0.5 a	0.2 b	0.2 b
Octanoic acid	1175	0.3 a	0.8 b	0.2 a
3Z-Hexenyl butanoate	1186	0.3 a	nd b	0.5 a
α-Terpineol	1188	0.1	0.4	t
Butyl hexanoate[1]	1189	0.1 a	nd b	nd b
Ethyl octanoate	1197	0.5 a	0.5 a	1.1 b
Decanal	1202	0.6 a	0.9 a	0.5 a
Octyl acetate	1214	0.3	nd	nd
Ethyl 2-phenylacetate	1247	t	nd	nd
2-Phenylethyl acetate	1258	0.1	t	0.2
2E-Decenal	1264	0.1	0.4	t
(E)-Cinnamaldehyde	1270	t	nd	t
Nonanoic acid	1271	0.2 a	0.7 b	0.1 a
(Z)-Theaspirane	1294	1.2 a	nd b	3.3 c
(E)-Cinnamyl alcohol	1304	0.9 a	1.2 b	0.9 a
Eugenol	1359	0.1 a	nd b	nd b
Decanoic acid	1371	t	0.9	t
3-Phenylpropyl acetate	1378	11.9	2.4	5.2
3Z-Hexenyl hexanoate	1383	0.4 a	nd b	0.4 a
Hexyl hexanoate[1]	1385	0.1	nd	t
Ethyl decanoate	1396	0.1 a	nd b	0.2 a
Methyl eugenol	1404	nd a	0.3 b	nd a
Dodecanal[1]	1410	0.1 a	0.4 b	nd c
(E)-β-Caryophyllene	1419	0.8 a	0.5 a	0.7 a
Dihydro-β-ionone[1]	1436	0.2 a	nd b	0.4 a
(E)-Cinnamyl acetate	1446	12.4 a	4.2 b	26.6 c
Geranyl acetone	1455	1.5 a	1.3 a	1.9 b
Ethyl (E)-cinnamate	1465	t	0.2	nd
γ-Decalactone	1467	0.4 a	nd b	nd b
(E)-β-Ionone	1489	1.5 a	0.2 b	2.0 a
α-Selinene	1498	0.1	nd	nd
β-Calacorene[1]	1566	t	nd	nd
3-Phenylpropyl isobutanoate[1]	1569	t	nd	nd
Dodecanoic acid	1571	0.5 a	0.9 b	0.4 a
3Z-Hexenyl octanoate[1]	1580	t	nd	0.1
Caryophyllene oxide	1583	0.2 a	nd b	nd b
Benzophenone[1]	1628	t	0.1	t
(E)-Cinnamyl butanoate[1]	1643	nd a	nd a	0.1 b
Cubenol	1647	0.1	nd	nd
Methyl *cis*-dihydrojasmonate[1]	1656	0.2 a	0.8 b	0.1 a
Tridecanoic acid	1672	t	t	t
Tetradecanoic acid	1780	2.2 a	2.3 a	0.9 b
Pentadecanoic acid	1878	1.4 a	2.3 b	0.4 c
Ethyl 9Z-hexadecenoate[1]	1990	1.9 a	2.7 b	0.5 c
Hexadecanoic acid	1992	7.2 a	8.7 b	2.2 c
Total area	—	$157 \cdot 10^6$	$43 \cdot 10^6$	$230 \cdot 10^6$

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