

## Characterization of Volatiles in Costa Rican Guava [*Psidium friedrichsthalianum* (Berg) Niedenzu] Fruit

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Volatile compounds were isolated from Costa Rican guava fruit by simultaneous steam distillation–solvent extraction according to the Likens–Nickerson method. Compounds were identified by capillary GC-MS and sensorially characterized by sniffing-GC. One hundred and seventy-three components were identified in the aroma concentrate, from which (*E*)- $\beta$ -caryophyllene,  $\alpha$ -terpineol,  $\alpha$ -pinene,  $\alpha$ -selinene,  $\beta$ -selinene,  $\delta$ -cadinene, 4,11-selinadiene, and  $\alpha$ -copaene were found to be the major constituents. The amounts of aliphatic esters and terpenic compounds were thought to contribute to the unique flavor of this fruit.

**KEYWORDS:** Volatiles; Costa Rican guava; aliphatic esters; terpenic compounds

### INTRODUCTION

Among the many attractive 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 and extremely rich in vitamins and can be used in a wide range of food products.

*Psidium friedrichsthalianum* (Berg) Niedenzu, commonly named the Costa Rican guava, is a shrub or tree ~8 m high, found in forests from Chiapas, Mexico, to Panama, from 90 to 1000 m. It is frequently cultivated for its fruits, which are made into juices, especially in Costa Rica (1, 2). This plant is a member of the same botanical family (Myrtaceae) as guava, and its fruit is often described as being more aromatic than common guava.

Despite the characteristic desirable flavor of this fruit, the chemical composition of the volatile compounds of Costa Rican guava has not previously been the object of study. Different authors (3–6) have analyzed the volatiles of other *Psidium* species, in which terpenoid constituents were found to be dominant. Therefore, in the present study we investigated the volatile flavor constituents of this fruit cultivated in Cuba.

### EXPERIMENTAL PROCEDURES

**Materials.** Fruits were collected mature from the National Botanical Garden near Havana and immediately processed. Diethyl ether was purchased from Merck (Darmstadt, Germany).

**Sample Preparation.** After addition of an internal standard (methyl undecanoate, 2 mg), pulp (200 g) was blended with distilled water (800 mL), adjusted to pH 7, and simultaneously distilled and extracted for 90 min in a Likens–Nickerson microapparatus with 25 mL of diethyl ether (previously redistilled and checked as to purity). The volatile

concentrate was dried over anhydrous sulfate and concentrated to 0.6 mL on a Kuderna–Danish evaporator with a 12-cm Vigreux column and then to 0.2 mL with a gentle nitrogen stream.

**GC and GC-MS Analyses.** A Konik 4000A HRGC equipped with a 30 m  $\times$  0.25 mm (0.25- $\mu$ m film thickness) DB-1 Chrompack fused-silica capillary column and a flame ionization detector (FID) was used. Injector and detector temperatures were 250 °C. Oven temperature was held at 60 °C for 10 min and then raised to 280 °C at 2 °C/min and held for 40 min. Carrier gas (hydrogen) flow rate was 1 mL/min. These conditions were used for quantitative analysis, by the internal standard method. The recovery of the method was determined by the standard addition technique applied to a sample. The analytes [ $\alpha$ -pinene, limonene, ethyl hexanoate, 1-hexanol, (*Z*)-3-hexenol, ethyl octanoate, (*E*)-1-caryophyllene, and  $\alpha$ -terpineol] were added at two different concentrations. The average recoveries were ~89–102%, and their relative standard deviations were <1.0%.

GC-MS analyses were done on a Hewlett-Packard model 5890 series II or model 6890 series II GC coupled to an HP 5972 or HP 5973 mass spectrometer. They were fitted with a CP-SIL-5CB Chrompack fused-silica column (50 m  $\times$  0.32 mm, 0.4- $\mu$ m film thickness) or an AT-Wax Alltech fused-silica column (60 m  $\times$  0.32 mm, 0.25- $\mu$ m film thickness). Temperature programming was performed from 60 °C (10 min) to 280 °C at 3 °C/min and held for 60 min in the apolar column and from 65 °C (10 min) to 250 °C at 2 °C/min and held for 60 min in the polar column. Injector temperature was 250 °C; transfer line temperature, 250 °C; and carrier gas (helium) flow rate, 1 mL/min. Mass spectra were obtained at 70 eV. Linear retention indices were calculated against those of *n*-paraffins (7). Compounds were identified by comparing their spectra to those of the Wiley library or our IDENT library and also, in many cases, by comparison of their GC Kovats index to those of standard compounds and data from the literature (8, 9).

**GC-Sniffing Analysis.** Odors of the separated components were assessed at an odor port following GC using a Hewlett-Packard model 5890 series II GC equipped with a thermal conductivity detector (TCD). Separations were done with a methyl siloxane HP-1 fused-silica column (30 cm  $\times$  0.53 mm, 2.65- $\mu$ m film thickness). An injector temperature of 250 °C, a detector temperature of 280 °C, and an oven temperature

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Table 1. Volatile Compounds of Costa Rican Guava

compound	retention index <sup>a</sup>		ID <sup>b</sup>	concn (ppm)	odor description	compound	retention index <sup>a</sup>		ID <sup>b</sup>	concn (ppm)	odor description
	RI <sub>1</sub>	RI <sub>2</sub>					RI <sub>1</sub>	RI <sub>2</sub>			
ethanol	377	854	A	6.92		$\alpha$ , $p$ -dimethylstyrene	1067	1425	A	0.03	
acetaldehyde	381	669	A	0.11		<i>cis</i> -linalooloxide (furanoid)	1068	1449	A	0.06	
1-propanol	517	1026	A	0.07		terpinolene	1076	1274	A	0.34	
ethyl formate	530	724	A	0.14		propyl hexanoate	1079	1307	A	0.10	
diacetyl	538	951	A	0.07		2-phenylethanol	1081	1888	A	0.13	fresh, flowery
acetic acid	547	1443	A	0.10	vinegar	ethyl heptanoate	1082	1324	A	0.03	
ethyl acetate	581	825	A	0.32	estery, fruity	nonanal	1085	1381	A	t	
isobutanol	592	1064	A	0.27	caramel	linalool	1086	1530	A	0.18	floral
isopentana	617	864	A	t <sup>c</sup>		$\alpha$ -fenchol	1094	1565	A	0.72	camphoraceous
1-butanol	619	1126	A	8.65	winey	myrcenol	1100		B	t	
2-pentanone	653	944	A	t		methyl octanoate	1109	1381	A	1.09	winey-fruity
acetoin	667	1273	A	0.08		<i>cis</i> - $\beta$ -terpineol	1126		C	0.19	
ethyl propanoate	681	925	A	0.03		isoborneol	1138	1633	A	0.06	
methyl butanoate	696	955	A	0.04		isobutyl hexanoate	1140	1343	A	0.05	
3-penten-2-one	698		C	0.04		borneol	1151	1680	A	0.61	camphoraceous
3-methylbutanol	700	1187	A	1.33	winey	terpinen-4-ol	1157	1664	A	0.68	spicy
					alcoholic	<i>p</i> -cymen-8-ol	1158	1829	A	t	
2-methylbutanol	705		B	0.14		octanoic acid	1166	2042	A	0.10	acid, rancid
pentyl methyl ether	708	774	C	t		$\alpha$ -terpineol	1171	1679	A	18.28	floral, citrus
1-entanol	735	1238	A	0.04		hexyl butanoate	1775	1405	A	0.13	
ethyl carbonate	747	1083	C	t		butyl hexanoate	1176	1403	A	0.52	fruity
( <i>E</i> )-3-hexenal	769		C	0.05		$\gamma$ -terpineol	1177		C	0.20	
hexanal	772	1062	A	0.01		ethyl octanoate	1181	1425	A	1.01	fruity-winey
ethyl butanoate	781	1025	A	4.58	fruity	octyl acetate	1189	1464	A	0.03	
ethyl lactate	787	1333	A	0.04		3-phenylpropanol	1205	2016	A	0.03	
butyl acetate	791	1052	A	0.07		2-phenylethyl acetate	1221	1795	A	t	
furfural	795	1448	A	1.03	burnt	isopentyl hexanoate	1238	1447	A	t	
hexyl methyl ether	808	924	B	0.41		1-decanol	1258	1744	A	t	
( <i>E</i> )-3-hexenyl methyl ether	810	974	C	0.06		vitispirane	1268		C	0.93	
( <i>Z</i> )-3-hexenyl methyl ether	815	980	C	0.79		methyl geraniate	1305		B	0.02	
( <i>E</i> )-2-hexenal	817	1206	A	0.18		myrtenyl acetate	1306	1680	C	0.30	
( <i>E</i> )-3-hexenol	823	1364	A	0.15		$\alpha$ -campholenyl acetate	1317		C	0.03	
( <i>Z</i> )-3-hexenol	828	1368	A	1.51	green, grassy	dehydroionene	1335	1682	C	0.09	
1-hexanol	849	1343	A	2.33	fruity, grassy	3-phenylpropyl acetate	1345	1917	A	t	
<i>m</i> -xylene	849	1132	B	0.03		$\alpha$ -cubebene	1349	1447	A	0.21	
<i>o</i> -xylene	863	1174	B	0.03		decanoic acid	1360	2242	A	0.06	
propyl butanoate	865	1117	A	0.03		( <i>Z</i> )-3-hexenyl hexanoate	1365	1642	A	0.82	fruity-green
butyl propanoate	888	1129	A	t		$\alpha$ -ylangene	1369	1467	A	0.24	
tricyclene	899	949	B	t		hexyl hexanoate	1371	1597	A	0.17	
methyl hexanoate	904	1175	A	0.10		butyl octanoate	1373	1601	A	0.40	
$\alpha$ -thujene	918	1019	B	t		$\alpha$ -copaene	1374	1476	A	10.69	woody
5-methylfurfural	924	1556	A	0.03		$\beta$ -bourbonene	1381	1503	C	1.00	
benzaldehyde	924	1508	A	0.03		ethyl decanoate	1383	1625	A	0.94	
$\alpha$ -pinene	928	1012	A	15.61	terpene, pine-like	$\beta$ -elemene	1388	1573	B	1.99	woody
						( <i>Z</i> )- $\beta$ -caryophyllene	1399		C	0.28	
$\alpha$ -fenchene	937	1045	B	0.16		( <i>E</i> )- $\beta$ -caryophyllene	1410	1576	A	35.81	woody-spicy
camphene	940	1052	A	0.30		$\beta$ -cubebene	1426	1524	B	0.47	
3-hydroxy-2-pyrone	959	1997	C	0.17		aromadendrene	1437	1589	B	0.58	
2-methyl-6-heptenone	962	1326	A	t		$\alpha$ -hmulene	1443	1651	A	4.39	woody
$\beta$ -pinene	965	1091	A	3.24	woody	<i>allo</i> -aromadendrene	1457	1627	B	0.74	
butyl butanoate	978	1207	A	0.54		4,11-selinadiene	1473	1656	B	10.78	woody
ethyl hexanoate	90	1225	A	7.49	fruity-winey	germacrene D	1476	1684	A	0.87	
ethyl ( <i>E</i> )-3-hexenoate	981	1291	A	t		$\beta$ -selinene	1483	1695	B	11.21	woody
ethyl ( <i>Z</i> )-3-hexenoate	982	1295	A	t		$\alpha$ -selinene	1491	1699	B	13.84	woody-spicy
( <i>E</i> )-3-hexenyl acetate	983	1298	A	0.05		$\alpha$ -muurolene	1491	1704	A	0.80	
( <i>Z</i> )-3-hexenyl acetate	984	1307	A	0.21	green	$\beta$ -bisabolene	1505	1716	A	0.84	
octanal	985	1275	A	t		$\gamma$ -cadinene	1508	1727	A	2.44	
hexanoic acid	988	1825	A	0.10	rancid	<i>trans</i> -calamenene	1511	1802	C	0.67	
$\alpha$ -phellandrene	993	1157	A	0.14		$\delta$ -cadinene	1518	1741	A	11.00	dry-woody
$\alpha$ -terpinene	1006	1167	A	0.23		zonarene	1518		C	0.45	
<i>p</i> -cymene	1007	1258	A	0.20		cubenene	1526	1761	C	1.08	
octyl methyl ether	1008	1132	C	t		$\alpha$ -cadinene	1527	1766	A	0.48	
1,4-cineole	1010	1164	A	0.16		$\alpha$ -calacorene	1530	1888	B	1.18	woody
$\beta$ -phellandrene	1016	1196	A	0.17		( <i>E</i> )-nerolidol	1549	2015	A	0.48	
1,8-cineole	1022	1193	A	0.20	camphoraceous	dodecanoic acid	1558	2448	A	0.11	
limonene	1023	1187	A	2.13	citrus	caryophyllene formate	1559		C	4.94	camphoraceous
( <i>Z</i> )- $\beta$ -ocimene	1026	1228	A	0.03		spathulenol	1563	2074	A	0.88	
2,5-dimethyl-4-methoxy-3(2 <i>H</i> )-furanone	1027		B	0.02	caramel	( <i>Z</i> )-3-hexenyl octanoate	1564	1838	A	0.34	
acetophenone	1030	1643	A	0.02		hexyl octanoate	1571	1796	A	0.91	green
( <i>E</i> )- $\beta$ -ocimene	1037	1244	A	0.26		globulol	1573	2064	A	1.25	
isopentyl butanoate	1041	1255	A	0.01		ethyl dodecanoate	1577	1824	A	0.10	
$\gamma$ -terpinene	1046	1236	A	0.35		$\beta$ -caryophyllene epoxide	1578	1954	A	2.05	woody-spicy
1-octanol	1054	1544	A	0.30		viridiflorol	1583	2041	A	2.43	sweet-woody
<i>trans</i> -linalooloxide (furanoid)	1055	1425	A	0.05		neointermedeol	1600	2093	C	2.34	sweet-woody

Table 1. (Continued)

compound	retention index <sup>a</sup>		ID <sup>b</sup>	concn (ppm)	odor description	compound	retention index <sup>a</sup>		ID <sup>b</sup>	concn (ppm)	odor description
	RI <sub>1</sub>	RI <sub>2</sub>					RI <sub>1</sub>	RI <sub>2</sub>			
5-guaien-11-ol	1600	2101	C	1.28		α-bisabolol	1667		C	0.11	
1,4,9-cadalatriene	1607	1962	C	0.78		epi-α-bisabolol	1671		C	0.24	
caryophyllenol	1614	2299	C	0.67		junipercamphor	1678		B	0.04	
cubanol	1619	2015	A	1.16		aromadendrene oxide	1702		C	0.17	
γ-eudesmol	1620	2136	A	3.51	sweet-woody	tetradecanoic acid	1743	2624	A	1.35	
T-muurolo	1622	2154	A	4.81	woody	octyl octanoate	1767	1998	A	0.06	
epi-α-cadinol	1628	2198	C	2.86	spicy	pentadecanoic acid	1845	2756	A	0.14	
δ-cadinol	1631	2167	C	2.33	spicy	(Z)-3-hexenyl undecanoate	1860	2125	A	0.10	
T-cadinol	1633	2139	A	1.44		hexadecanoic acid	1952	2862	A	3.60	waxy
β-eudesmol	1636	2195	A	0.80		ethyl hexadecanoate	1983	2224	A	0.09	
5-epi-neointerdeol	1639	2219	C	5.64	woody	fanesyl butanoate	2015		C	0.05	
α-eudesmol	1640	2186	A	1.00		linoleic acid	2113		A	0.51	
14-hydroxy-9-epi-(E)-caryophyllene	1654		C	0.46		ethyl linoleate	2145	2491	A	0.85	

<sup>a</sup> RI<sub>1</sub> and RI<sub>2</sub> = retention indices on CP-SIL-5CB and AT-Wax capillary columns, respectively. <sup>b</sup> The reliability of the identification proposal is indicated by the following: A, mass spectrum and Kovats index agreed with standards; B, mass spectrum and Kovats index agreed with literature data; C, mass spectrum agreed with mass spectral database. <sup>c</sup> t = trace (<0.01 ppm).

program of 60–280 °C at 3 °C/min were used. Sample size for each injection was ~0.5–1 μL. Odor assessments were made by one flavorist, who was familiar with tropical fruit flavors.

The coincidence of an identified peak with some odor impression does not imply that this specific compound is solely responsible for the odor. The possibility of an underlying minor component strongly influencing the sensory results can never be ruled out.

## RESULTS AND DISCUSSION

The volatile constituents of fresh Costa Rican guava fruit were obtained by simultaneous steam distillation–solvent extraction and analyzed by GC and GC-MS using fused-silica capillary columns. Valid aroma concentrates were prepared by using well-established procedures that have been previously reported (3, 4, 10). In addition, the pH of the sample was adjusted to 7.0 to avoid transformation of terpenoids at the natural pH of the pulp (11). The concentrate was found, on appropriate redilution with water, to possess characteristic fruit aroma.

Table 1 shows the identified compounds and the sensory descriptions of the corresponding peaks at the sniffing port of the GC, according to their order of elution on a CP-SIL-5CB column. Identification of these compounds was based on GC-MS and retention index information on both columns. One hundred and seventy-three volatile compounds were identified for the first time in Costa Rican guava.

A quantitative analysis of the volatile compounds is also shown in Table 1. The yield of total volatiles, estimated by the addition of a measured amount of internal standard to the pulp, was 238 mg/kg of fruit pulp (excluding skin and seeds). In comparison with other closely related fruits, this is a high level. Thus, for example, values reported were 35–61 mg/kg for the common guava (3) and 110 mg/kg for strawberry guava (4), using the same isolation procedure.

Among the compounds found in this study, a large number of terpenes and terpenic derivatives were identified. Major constituents were (E)-β-caryophyllene, α-terpineol, α-pinene, α-selinene, β-selinene, δ-cadinene, 4,11-selinadiene, and α-co-paene. Many of the terpene and terpenic derivatives found in this fruit that are strong contributors to tropical fruit aromas have also been found in a variety of other guava species (3–6).

The flavor of the Costa Rican guava has been described as sweet with strong fruity, woody-spicy, and floral notes with some green and camphoraceous character. It can be concluded

from the results shown in Table 1 that the aliphatic esters contribute much to its typical flavor. Many of the esters found in this fruit that are strong contributors to tropical fruit aromas have also been found in a variety of other tropical and subtropical fruits. Methyl and ethyl esters similar to those found in this study have been identified as important contributors of tropical fruit flavors in guava (3), strawberry guava (4), papaya (12), acerola (13), passion fruit (15), lulo (15), and soursop (16).

The following compounds are thought to contribute to the complexity of the Costa Rican guava flavor. Fruity notes are due to the presence of many aliphatic esters, whereas floral notes can be attributed to α-terpineol and linalool. Woody and spicy notes can be ascribed to the presence of many sesquiterpenes, particularly (E)-β-caryophyllene and its epoxide, whereas green notes are due to (Z)-3-hexenol and its esters. The camphoraceous note is due to caryophyllene formate, α-fenchol, 1,8-cineole, and borneol, whereas the pine-like note can be ascribed to the presence of α-pinene.

Although it is well-known that specific odors of pure substances can change when they are mixed, our results suggest that the aliphatic esters and terpenic compounds contribute much to the typical Costa Rican guava flavor. However, it was not possible to find one or more impact compounds.

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