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Volatile Compounds of *Psidium salutare* (H.B.K.) Berg. Fruit

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Volatile compounds were isolated from *Psidium salutare* fruits by simultaneous steam distillation−solvent extraction according to the Likens−Nickerson procedure. Compounds were identified by capillary GC and GC-MS. One hundred and fifty compounds were identified in the aroma concentrate, from which limonene, myrcene, and α-pinene were found to be the major constituents in the fruit.

KEYWORDS: ***Psidium salutare* (H.B.K.) Berg.; Myrtaceae; fruit volatiles; limonene; myrcene; α-pinene**

INTRODUCTION

The species *Psidium salutare* H.B.K., commonly called "guayabita del pinar", very much resembles the guava in size of tree and general growth. The small tree, which is endemic of the western part of Cuba, produces a thin-skinned, green fruit about the size of a little olive, with a short sharp point at the flower end. The fruit has little flesh and a pleasant sweet flavor. It is very aromatic and commonly used to prepare an appreciated liquor (*1*). These aromatic properties led us to investigate its composition to determine the volatile constituents of the fruit. To date, the aroma of *P. salutare* has not been the subject of a previous study.

EXPERIMENTAL PROCEDURES

Materials. Fruits were collected at mature stage from a commercial plantation in Pinar del Río in Cuba's western region and immediately processed. Pure reference standards of acetaldehyde, acetone, acetic acid, methyl acetate, 3-buten-2-one, butanal, propanol, ethyl acetate, 1-butanol, 2-pentanone, pentanal, acetal, 2-pentanol, 2,5-dimethylfuran, 1-pentanol, (*Z*)-2-penten-1-ol, propanoic acid, hexanal, ethyl butanoate, furfural, 3-methyl-2-butenol, 3-methyl-2-butenal, butanoic acid, 1-hexanol, α-pinene, *β*-pinene, ethylbenzene, camphene, 6-methyl-5-hepten-2-one, pentanoic acid, myrcene, α-terpinene, 1,8-cineole, (*E*)-2-hexenal, 2-heptanol, 2-heptanone, 2,4-hexadienal, α-terpinene, *δ*-3-carene, *γ*-terpinene, myrcene, *p*-cymene, phenol, hexanal, (*Z*)-3-hexenol, 1-octanol, benzaldehyde, fenchone, linalool, camphor, borneol, nerol, neral, geraniol, α-terpineol, carvone, ethyl salicylate, *trans*-carveol, thymol, carvacrol, 1,2,4-triethylbenzene, methyl decanoate, geranyl acetate, vanillin acetate, (*E*)-nerolidol, (*E*,*E*)-farnesol, isopropyl palmitate, (*E*)-phytol, benzyl benzoate, tetradecanoic acid, palmitic acid, and linoleic acid were purchased from Aldrich (Steinheim, Germany). Limonene, 3-methylbutanal, (*E*)-*β*-caryophyllene, and α-humulene were obtained from Sigma (Steinhem, Germany), and *cis*-linalool oxide, *trans*-linalool oxide, *β*-fenchol, and terpinen-4-ol were purchased from Fluka (Buchs, Switzerland). Diethyl ether was purchased from Merck (Darmstadt, Germany).

Isolation of Volatile Compounds. After the addition of an internal standard (methyl undecanoate, 2 mg), ground fruits (200 g) were blended with distilled water (800 mL), adjusted to pH 7.0, 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.

Gas Chromatography. A Konik 4000A HRGC equipped with a 30 m × 0.25 mm (0.25 *μ*m film thickness) CP-SIL-5CB (5% phenyl-polymethylsiloxane) 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. Quantitative data were obtained by electronic integration of FID areas without the use of correction factors. The recovery of the method was determined by the standard addition technique applied to a sample. The analytes [α-pinene, limonene, ethyl butanoate, 1-hexanol, (*Z*)-3-hexenol, (*E*)-*β*-caryophyllene, (*E*)-nerolidol, and α-terpineol] were added at two different concentrations. The average recoveries were ∼88−102%, and their relative standard deviations were <10%.

Gas Chromatography—Mass Spectrometry. GC-MS analyses were done on a Hewlett-Packard model 5890 series II or model 6890 GC coupled to an HP 5972 or HP 5973 mass spectrometer. They were fitted with a CP-SIL-5CB (5% phenyl-polymethylsiloxane) Chrompack fused-silica column (50 m × 0.32 mm, 0.4 *μ*m film thickness) or an AT-Wax (100% polyethylene glycol) Alltech fused-silica column (60 m × 0.32 mm, 0.25 *μ*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. The injector temperature was 250 °C and the transfer line temperature, 250 °C. Carrier gas (helium) flow rate was 1 mL/min. Mass spectra were obtained at 70 eV. Linear retention indices (Kovats indices) were calculated against those of *n*-paraffins. Component identification was carried out by comparing the relative retention indices and mass spectra of reference compounds in both columns. Mass spectra of published data were also compared (*2*, *3*).

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Table 1. Concentrations of Fruit Volatiles of *P. salutare*

compound	retention index[a] RI_1	retention index[a] RI_2	concn (mg/kg)	compound	ention index[a] RI_1	ention index[a] RI_2	concn (mg/kg)
ethanol	377	854	0.25	*p*-mentha-1,5-dien-8-ol	1136		0.09
acetaldehyde	381	669	0.02	δ-terpineol	1141	1638	0.01
acetone	481	810	t[b]	borneol	1151	1680	0.09
acetic acid	547	1423	0.05	terpinen-4-ol	1157	1664	5.08
methyl acetate	506	813	t	(*Z*)-3-hexenyl butanoate	1165	1442	0.09
3-buten-2-one	511	908	t	α-terpineol	1171	1679	2.29
butanal	518	813	0.03	hexyl butanoate	1174	1398	0.15
1-propanol	524	1026	t	isobornyl formate	1178	1596	0.09
ethyl acetate	581	825	0.29	*trans*-piperitol	1180	1469	0.09
3-methylbutanal	617	864	0.02	*trans*-carveol	1192	1818	0.09
1-butanol	619	1126	t	2-heptyl butanoate	1194		0.05
2-pentanone	653	944	0.02	2-methylbutyl hexanoate	1196		0.09
pentanal	658	1002	0.19	nerol	1204	1757	0.15
2-pentanol	662	1084	t	carvone	1207	1700	0.34
2,5-dimethylfuran	667	1398	t	carvotanacetone	1213		0.05
1-pentanol	735	1238	t	piperitone	1218	1703	0.19
(*Z*)-2-penten-1-ol	736	1302	0.02	carvacrol methyl ether	1220		0.01
3-methyl-2-butenal	737		0.05	geraniol	1232	1830	0.34
3-methyl-2-butenol	739		t	neral	1239	1680	0.01
propanoic acid	741	1510	t	ethyl salicylate	1241	1787	0.01
(*E*)-3-hexenal	769		t	thymol	1263	2100	0.02
hexanal	772	1062	0.24	hexyl pentanoate	1266	1498	0.05
ethyl butanoate	781	1025	0.01	carvacrol	1272	2191	0.05
acetal	785		2.29	methyl decanoate	1306	1581	t
furfural	795	1448	0.02	α-cubebene	1349	1447	0.09
(*Z*)-2-hexenal	804	1226	0.05	geranyl acetate	1359	1735	0.05
(*E*)-2-hexenal	817	1206	1.17	α-longipinene	1365	1541	0.04
2-pentyl acetate	818	1055	0.09	α-copaene	1370	1229	2.00
(*Z*)-3-hexenol	828	1368	0.15	β-bourbenene	1374	1476	0.01
1-hexanol	849	1343	0.09	eucarvone	1382		0.05
butanoic acid	856	1610	t	1-methylhexyl hexanoate	1390		0.01
2-heptanone	859	1172	0.01	α-gurjunene	1401	1531	0.34
(*E*,*E*)-2,4-hexadienal	877	1400	t	(*E*)-β-caryophyllene	1410	1576	22.43
2-heptanol	884	1301	t	β-gurjunene	1427	1337	0.19
α-thujene	918	1019	2.88	aromadendrene	1437	1589	0.78
benzaldehyde	924	1508	0.05	α-humulene	1443	1651	2.79
α-pinene	928	1012	42.43	*allo*-aromadendrene	1457	1627	0.44
toluene	930		t	γ-muurolene	1467	1692	1.08
α-fenchene	937	1045	0.05	β-selinene	1483	1695	1.47
camphene	940	1052	0.05	valencene	1485	1722	0.05
6-methyl-5-hepten-2-one	951		0.01	α-selinene	1486	1729	1.51
phenol	956	1932	0.03	α-muurolene	1487	1457	0.98
sabinene	957	1108	0.06	γ-cadinene	1508	1727	3.13
β-pinene	965	1091	3.08	*trans*-calamenene	1511	1802	0.90
pentanoic acid	973	1711	t	vanillin acetate	1512		0.03
myrcene	981	1155	74.10	δ-cadinene	1518	1741	5.91
p-mentha-1(7),8-diene	992		0.74	cadina-1,4-diene	1521	1786	0.44
α-phellandrene	993	1157	4.00	α-calacorene	1526	1636	0.39
δ-3-carene	1001	1138	0.59	α-cadinene	1528	1766	0.29
α-terpinene	1006	1167	1.81	elemol	1532	2078	0.29
p-cymene	1007	1258	3.10	epiglobulol	1540	2000	0.90
1,8-cineole	1022	1193	0.60	(*E*)-nerolidol	1549	2015	27.27
limonene	1023	1187	79.33	spathulenol	1563	2074	0.29
(*Z*)-β-ocimene	1026	1228	26.05	β-caryophyllene epoxide	1569	1936	4.49
(*E*)-β-ocimene	1037	1244	5.72	globulol	1570	2064	4.69
γ-terpinene	1046	1236	15.01	viridiflorol	1583	2041	2.25
trans-linalool oxide (furanoid)	1055	1425	0.44	guaiol	1585	2060	0.83
1-octanol	1054	1544	0.05	ledol	1586	1936	1.47
α-pinene oxide	1060	1365	t	*neo*-intermedeol	1600	2093	0.59
fenchone	1066	1391	0.01	1-epicubenol	1617	1773	2.15
cis-linalool oxide (furanoid)	1068	1449	0.49	cubenol	1619	2015	5.62
terpinolene	1076	1274	1.42	γ-eudesmol	1620	2136	2.69
3-methyl-3-cyclohexen-1-one	1079		0.29	epi-α-cadinol	1628	2198	25.86
linalool	1086	1530	13.74	epi-α-muurolol	1630		2.70
hexyl propanoate	1089	1326	0.05	β-eudesmol	1636	2195	5.62
β-fenchol	1092	1576	0.05	α-cadinol	1642	2180	26.80
myrcenol	1097	1156	0.05	α-eudesmol	1644	2186	6.08
α-campholenal	1099	1477	0.06	(*E*,*E*)-farnesol	1699	1745	0.15
cis-*p*-menth-2-en-1-ol	1103		0.24	benzyl benzoate	1720	2579	0.03
camphor	1108	1484	0.05	tetradecanoic acid	1752	2679	0.01
(*E*,*E*)-alloocimene	1114	1366	0.15	carissone	1879		t
trans-*p*-menth-2-en-1-ol	1117	1378	0.09	palmitic acid	1952	2862	0.19
cis-β-terpineol	1125	1616	0.05	isopropyl palmitate	2011	2217	0.05
ipsdienol	1127		0.04	(*E*)-phytol	2101	2557	0.09
neo-alloocimene	1129	1384	0.05	linoleic acid	2113	3148	0.01

[a] RI_1 and RI_2 = retention indices on CP-SIL-5CB and AT-Wax columns. [b] t = trace (<0.01 mg/kg).

RESULTS AND DISCUSSION

The volatile constituents of *P. salutare* fruit were obtained by simultaneous steam distillation−solvent extraction and analyzed by GC and GC-MS using fused-silica capillary columns. A valid aroma concentrate was prepared by using well-established procedures that have been previously reported (*4*−*6*). In addition, the pH of the sample was adjusted to 7.0 to avoid transformation of terpenoids at the natural pH of the pulp (*7*). The concentrate was found, on appropriate redilution with water, to possess the characteristic fruit aroma.

Table 1 shows the identified compounds with their concentrations. Quantitations are based upon GC-FID peak integration data, so accuracy is potentially limited by a number of factors, including coelution of two or more compounds, possible sample discrimination during injection, peak broadening, and differences in FID response factors among the components. The quantitative data in **Table 1** show that in total ∼458 mg of volatile contituents was obtained per kilogram of fresh fruit.

A total of 150 compounds were identified for the first time in *P. salutare* fruit. In general terms, the fruits of *P. salutare* contain, besides mono- and sesquiterpenes, a variety of alcohols, ketones, and aliphatic esters. Quantitatively, the most abundant class of compounds identified was the terpenoids. Among them, limonene (17.3% of the total volatiles), myrcene (16.2%), and α-pinene (9.3%) were the major constituents. 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 (*4*, *6*, *9*).

It is our opinion that *P. salutare* fruit does not have easily identifiable flavor impact compounds; instead, its flavor would be the result of the presence of several components, particularly terpenoids, C_6 aldehydes, and fatty esters.

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