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Characterization of volatiles in Loquat fruit (*Eriobotrya japonica* Lindl.)

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Key words: *Eriobotrya japonica*, loquat volatiles, hexadecanoic acid, octadecanoic acid, decane, benzaldehyde, nonanal.

Palabras claves: *Eriobotrya japonica*, níspero del Japón, ácido hexadecanoico, ácido octadecanoico, decano, benzaldehído, nonanal.

RESUMEN: Entre los muchos atributos deseables y atractivos que generan la demanda de frutas tropicales y subtropicales, su aroma y sabor característicos son lo más atractivos para el consumidor. Además, estas frutas por lo general son baratas, extremadamente ricas en vitaminas y pueden ser usadas en una amplia variedad de productos, tales como bebidas, postres y derivados lácteos. El níspero del Japón es la fruta de la especie *Eriobotrya japonica* Lindl., un arbolito perenne nativo del Japón y que en la actualidad también se cultiva en otros países tropicales y subtropicales. El objetivo de este trabajo fue aislar e identificar los componentes volátiles del níspero del Japón cultivado en Cuba. Se aislaron los componentes volátiles del níspero del Japón mediante destilación/extracción con disolvente simultáneas de acuerdo a Likens-Nickerson y se analizaron por CG y CG-EM. De los 140 constituyentes separados, 128 fueron identificados. Se encontró una gran variedad de funciones químicas. Los ácidos fueron el grupo mayoritario de volátiles (57.4 %); la composición de los otros grupos de compuestos fue la siguiente; aldehídos y cetonas, 13.7 %; parafinas, 10.6 %; alcoholes, 5.5 %; terpenoides, 5.2 %; ésteres, 1.7 % y otros, 4.9 %. Los compuestos mayoritarios identificados fueron el ácido hexadecanoico, ácido octadecanoico, decano, benzaldehído y nonanal. La alta cantidad de ácidos grasos parecen contribuir al aroma y sabor característicos de esta fruta.

ABSTRACT: 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, are extremely rich in vitamins, and can be used in a wide range of products including beverages, desserts, and dairy products. Loquat is the fruit of *Eriobotrya japonica* Lindl., a small evergreen tree native to Japan and actually grown there as well as in various other tropical and subtropical countries. The purpose of this study is to isolate and identify volatile components in loquat fruit grown in Cuba. Volatile components were isolated from loquat fruit by simultaneous steam distillation/solvent extraction according to Likens-Nickerson and analyzed by GC and GC-MS. Among 140 constituents separated, 128 were identified. A rough survey of the chemical classes represented in the loquat flavor was as follows: Acids comprise the largest class of volatiles (57.4 %); the composition of the other classes of compounds was as follows: aldehydes and ketones, 13.7 %; paraffines, 10.6 %; alcohols, 5.5 %; terpenoids, 5.2 %;

esters, 2.7 % and others, 4.9 %. Hexadecanoic acid, octadecanoic acid, decane, benzaldehyde and nonanal were found to be the major constituents. The highly amounts of fatty acids was thought to contribute to the unique flavor of this fruit.

INTRODUCTION

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

Loquat is the fruit of *Eriobotrya japonica* Lindl., a small evergreen tree native to Japan and actually grown there as well as in various other tropical and subtropical countries. The fruits are oval in shape, slightly pear-shaped, 3 to 7 cm long. They vary from pale yellow to light orange in color on ripening. The tough, slightly woolly, thick skin is easily peeled from the firm flesh of the fruit. The flavor of the fresh fruit has been described as mild, slightly acid and apple-like¹. The fruits may be used very much in baking or preserving, and the partly ripe fruits make a firm, delicately flavoured jelly.

Various studies on the volatile compounds of loquat grown in different regions have been reported²⁻⁶. The purpose of this study is to isolate and identify volatile components in loquat fruit grown in Cuba.

MATERIALS AND METHODS

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 6, 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 in 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

The extract was analysed by GC using a Hewlett-Packard 6890 gas chromatograph equipped with a flame ionization detector (FID). The separations were performed using a SPB-5 column (30 m x 0.25 mm I.D., 0.25 μ m) with an oven temperature program of 60 °C (2 min), 4 °C/min to 250 °C (20 min). The carrier gas was helium with a flow-rate of 1 mL/min. The temperature of the injector and detector was 250 °C. The injection was made in the split mode (1:10 ratio). Linear retention indices were calculated against those of n-paraffins. 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 (α -pinene, limonene, 1-hexanol, (Z)-3-hexenol, benzaldehyde, methyl benzoate and α -terpineol) were added at two

different concentrations. The average recoveries were about 88-102 % and their relative standard deviations were lower than 10 %.

GC-MS analyses were performed with a Hewlett-Packard series 6890 (series II) gas chromatograph equipped with a HP-5973 mass-selective detector was used. The chromatographic conditions were the same as those described for GC-FID. The detector operated in impact electron mode (70 eV) at 230 °C. Detection was performed in the scan mode between 30 and 400 amu.

Compounds were identified by comparing their spectra to those of standard compounds, the Wiley library or our IDENT library and also by comparison of their GC Kovats index to those of standard compounds and data from literature^{7,8}.

RESULTS AND DISCUSSION

Valid aroma concentrate were prepared by using well-established procedures that have been previously reported^{9,10}. Besides, the pH of the sample was adjusted to 6.0 to avoid transformation of terpenoids at the natural pH of the pulp¹¹. The concentrate was found, on appropriate redilution with water, to possess characteristic fruit aroma.

Table 1 summarizes the qualitative and quantitative analyses of the fruit volatiles according to order of elution on the SPB-5 column. Identification of these compounds was based on GC/MS and retention index information. The yield of total volatiles, estimated by the addition of a measured amount of internal standard to the pulp, was 14.6 mg/kg of fruit pulp.

Among 140 constituents separated, 128 were identified. A rough survey of the chemical classes represented in the loquat flavor was as follows: Acids comprise the largest class of volatiles (57.4 %); the composition of the other classes of compounds was as follows: aldehydes and ketones, 13.7 %; paraffines, 10.6 %; alcohols, 5.5 %; terpenoids, 5.2 %; esters, 2.7 % and others, 4.9 %.

Major constituents found in loquat flavor were hexadecanoic acid (5.25

ppm), octadecanoic acid (2.18 ppm), decane (1.26 ppm), benzaldehyde (0.72 ppm) and nonanal (0.65 ppm). The volatiles from Spanish loquat fruit were dominated by hexanal, (E)-2-hexenal and benzaldehyde³, while for the Japanese fruit, 2-methylbutanoic acid, (E)-2-hexenol, octanoic acid and nonanoic acid⁴; (E)-2-hexenol, (Z)-3-hexenol and hexanol⁵ or (E)-2-hexenal, hexanal, hexanol, 2-methylbutanoic acid and (Z)-3-hexenol⁶ were the most abundant volatile compounds.

Some compounds present, e.g. furfural and some furanes are probably degradation products of ascorbic acid and sugars^{12,13}. Nevertheless, the concentrate was found, on appropriate redilution with water, to possess the characteristic loquat aroma.

It is not usually in fruit volatiles composition studies that fatty acids were an abundant group of compounds, as it found in the present study. The major representatives were hexadecanoic acid and octadecanoic acid, all of them reported in lesser amounts in previous studies³⁻⁶. These major fatty acids are not of aromatic importance, but the presence of higher amounts of other minor fatty acids could be responsible for the acidic and pungent notes observed in loquat fruit.

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Table 1. Volatile compounds of loquat fruit grown in Cuba

compounds	RI ^a	Means of identification ^b	Concentration (mg/kg)
acetaldehyde	435	A	0.02
ethanol	500	A	0.01
1-propanol	568	A	<0.01
diacetyl	612	A	0.02
ethyl acetate	615	A	0.12
isobutanol	622	A	0.17
1-butanol	668	A	<0.01
hexane	600	A	<0.01
pentanal	700	A	0.01
acetoin	720	A	0.05
2,5-dimethylfuran	723	C	<0.01
3-methyl-1-butanol	736	A	0.13
2-methyl-1-butanol	739	A	0.02
pyridine	752	A	0.04
1-pentanol	768	A	0.02
toluene	776	A	0.02
3-methyl-2-buten-1-ol	779	B	0.02
diethyl carbonate	784	C	<0.01
octane	800	A	0.26
2-furfural	829	A	0.18
3-methyl-1H-pyrrole	841	C	<0.01
(E)-2-hexenal	854	A	0.02
(Z)-3-hexen-1-ol	856	A	0.02
ethylbenzene	860	C	0.08
(E)-2-hexen-1-ol	865	A	0.12
1-hexanol	867	A	0.09
p-xylene	884	A	0.31
o-xylene	896	A	0.16
nonane	900	A	<0.01
heptanal	901	A	0.03
methional	905	A	<0.01

Table 1 (continued)

compounds	RI ^a	Means of identification ^b	Concentration (mg/kg)
(E,E)-2,4-hexadienal	909	A	<0.01
2-acetylfuran	910	A	<0.01
diethyl disulfide	922	A	<0.01
cumene	926	B	<0.01
α -thujene	931	A	<0.01
α -pinene	939	A	0.01
(Z)-2-heptenal	949	B	<0.01
camphene	953	A	<0.01
tetrahydrothiophen-3-one	954	C	<0.01
ethyl 3-hydroxy-3-methylbutanoate	955	C	<0.01
(E)-2-heptenal	958	A	0.05
benzaldehyde	960	A	0.72
5-methyl-2-furfural	962	A	<0.01
1-heptanol	969	A	<0.01
β -pinene	979	A	0.01
1-octen-3-ol	980	A	<0.01
hexanoic acid	980	A	<0.01
6-methyl-5-hepten-2-one	985	A	<0.01
myrcene	991	A	0.02
(E,E)-2,4-heptadienal	998	A	<0.01
decane	1000	A	1.26
δ -3-carene	1011	A	0.01
α -terpinene	1018	A	0.01
p-cymene	1020	A	0.04
limonene	1030	A	0.30
1,8-cineole	1032	A	0.09
benzyl alcohol	1033	A	0.09
cyclohexyl acetate	1043	C	0.10
2-phenylacetaldehyde	1046	A	0.05
(Z)- β -ocimene	1040	A	<0.01
γ -terpinene	1062	A	0.06
acetophenone	1065	A	<0.01
1-octanol	1070	A	0.03
m-cresol	1075	A	<0.01
p-tolualdehyde	1079	A	<0.01
terpinolene	1088	A	0.01
methyl benzoate	1091	A	0.06
linalool	1098	A	0.04
nonanal	1102	A	0.65
2-phenylethanol	1110	A	0.05
cis- β -terpineol	1144	A	0.01
benzyl cyanide	1150	C	<0.01
p-vinylanisole	1156	C	0.01
(E)-2-nonenal	1158	B	<0.01
ethyl benzoate	1170	A	<0.01
terpinen-4-ol	1177	A	0.09
octanoic acid	1180	A	<0.01
p-cymen-8-ol	1183	A	<0.01
α -terpineol	1189	A	0.01
methyl salicylate	1190	A	<0.01
ethyl octanoate	1195	A	<0.01
decanal	1204	A	0.02
benzothiazole	1221	A	0.01
2,3-dihydrobenzofuran	1226	C	<0.01

Table 1 (continued)

compounds	RI ^a	Means of identification ^b	Concentration (mg/kg)
3-phenylpropanol	1234	C	0.01
p-anisaldehyde	1252	A	<0.01
geraniol	1255	A	<0.01
(E)-2-decenal	1257	C	0.11
(E)-cinnamaldehyde	1266	A	<0.01
nonanoic acid	1280	A	0.03
(E)-anethole	1283	A	<0.01
(E,Z)-decadienal	1291	A	0.01
undecanal	1304	A	0.01
4-vinyl-2-methoxyphenol	1306	C	0.01
(E,E)-2,4-decadienal	1314	A	0.05
methyl decanoate	1326	A	<0.01
2,3-dihydro-1,1,4,6-tetramethyl-1H-indene	1350	C	0.01
dihydro-5-pentyl-2(3H)-furanone	1354	C	<0.01
(E)-2-dodecenal	1357	A	<0.01
methyl (E)-cinnamate	1379	A	0.02
ethyl (E)-cinnamate	1462	A	0.04
γ -muurolene	1477	A	0.02
(E)- β -ionone	1485	A	<0.01
δ -decalactone	1493	A	<0.01
α -muurolene	1499	A	0.01
cis- α -bisabolene	1504	A	<0.01
methyl dodecanoate	1525	A	<0.01
(E)-nerolidol	1564	A	0.02
dodecanoic acid	1580	A	0.04
longiborneol	1592	B	<0.01
epi- α -muurolol	1641	B	<0.01
α -muurolol	1645	B	0.01
α -cadinol	1653	A	<0.01
heptadecane	1700	A	<0.01
anthracene	1775	C	<0.01
tetradecanoic acid	1780	A	0.50
ethyl tetradecanoate	1795	A	<0.01
benzyl salicylate	1863	A	<0.01
pentadecanoic acid	1880	A	0.09
methyl hexadecanoate	1927	A	0.04
hexadecanoic acid	1980	A	5.25
heptadecanoic acid	2080	B	0.09
methyl linoleate	2090	C	<0.01
methyl octadecanoate	2127	A	<0.01
1-nonadecanol	2175	B	<0.01
ethyl linolenate	2178	A	<0.01
octadecanoic acid	2180	A	2.18

^aRetention index on SPB-5 capillary column.^bThe 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.