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Characterization of odor-active compounds in pechiche (*Vitex cymosa* Berteo ex Speng) fruit

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Abstract

Pechiche (*Vitex cymosa* Berteo ex Speng) is a small tree which grows wild in the provinces of the western coastal region of Ecuador. Volatile constituents of pechiche fruit were isolated by headspace-solid phase microextraction (HS-SPME) and analyzed by gas chromatography with flame ionization detector (GC-FID) and gas chromatography-mass spectrometry (GC-MS). The global odor captured by the DVB/CAR/PDMS fiber had a high similarity with respect to the original pulp. GC-O frequency analysis and odor activity value (OAV) were applied to find the odor-active compounds. A total of 109 volatile compounds comprising >99% of the composition were identified. The application of the GC-O and OAV strategies afforded 21 odor-active compounds, from which *(E)*-4-decenal, *(E)*-2-octenal, octanal, decanal, *(E)*-2-decenal, *(E)*-2-nonenal, 1-octen-3-one and *(E,Z)*-2,6-nonadienal were the most odor-active compounds.

Keywords: *Pechiche, Vitex cymosa, HS-SPME, GC-FID, GC-MS, GC-O, odor activity value*

1. INTRODUCTION

Flavor is one of the key characteristics of fruits. Generally, flavor consists of aroma, taste and sense, among which aroma is most important (Small, 2012). Many isolation techniques for aroma compounds had been applied to identify the odor-active compounds in fruits, including liquid-liquid extraction, simultaneous solvent-assisted flavor evaporation and simultaneous distillation and extraction (Laohaprasit et al., 2012; Yilmaztekin, 2014; Bonneau et al., 2016; Lasekan and Yap, 2018). However, these techniques are highly laborious, time-consuming, need the use of a solvent and require preconcentration of extracts. Contrasting to well-established approaches, headspace solid-phase microextraction (HS-SPME) has been widely used in the isolation of volatile compounds from various fruits (Pino and Bent, 2013; Steingass et al., 2015; Pino and Roncal, 2016; da Rocha et al., 2017; Sung et al., 2019). This technique is a rapid, simple, sensitive, and solvent-free procedure. Combining this isolation technique with gas chromatography-mass spectrometry (GC-MS) and gas chromatography-olfactometry (GC-O) produces a highly suitable method of identifying odor-active compounds.

Besides this, the use of the divinylbenzene /carboxen/polydimethylsiloxane (DVB / CAR / PDMS) fiber had been found effective for obtaining the real aroma profile of various fruits (Pino, 2014; Pino and Roncal, 2016). Pechiche (*Vitex cymosa* Berteo ex Speng), belong to the Lamiaceae family, is a small tree or shrub occurring in tropical and subtropical regions. This plant is endemic to Panama, Venezuela, the Netherlands Antilles, Brazil, Bolivia, Colombia, Ecuador, Peru, Paraguay and Argentina. In Ecuador, it is found growing wild in the provinces of Guayas, El Oro, Manabí and Los Ríos, all located in the western coastal region (Aguirre, 2012). The fruits are black or purple fleshy drupes, ovoid between 1.5-2 cm in length with a

bitter taste, but cooked fruits are prepared as a delicious dessert with a very sweet flavor and very popular on the Ecuadorian coast.

Although some studies reported the general chemical composition of pechiche fruit (Barzola-Miranda et al., 2018; Guevara et al., 2020), no previous reports were found in the literature about the volatile compounds of this fruit. Therefore, the aim of this study was to determine the volatile constituents profile of the pechiche fruit as well as to determine the odor-active compounds.

2. MATERIALS AND METHODS

2.1. Materials

Mature pechiche fruits were collected directly from plants grown in the orchard La Represa from the Universidad Técnica Estatal de Quevedo in the province of Los Ríos, Ecuador, in January 2019 (harvest time December-February).

A batch of 0.5 kg of fruits without damage was pureed in a Waring stainless-steel blender, then the whole pulp was passed through a 60-mesh sieve to yield the final pulp. The specimens were identified at the Universidad Técnica Estatal de Quevedo, where a reference vouchers is available in the center's herbarium.

Standards of chemicals were purchased from Sigma–Aldrich (St. Louis, MO) and some were generously given by Robertet (Grasse, France).

2.2. Standard chemical analysis

Total soluble solids and titratable acidity (expressed as anhydrous citric acid) were determined by official methods (AOAC, 2019).

2.3. Headspace solid-phase microextraction analysis

A DVB/CAR/PDMS fiber, 1 cm long, provided by Supelco (Bellefonte, PA, USA) was chosen for analyses. For each trial, pulp (3 g), Milli-Q water (3 mL) and sodium chloride (1 g) were placed into a 15-mL vial sealed with a PTFE-lined screw cap. The extractions were carried out under magnetic stirring at 600 min^{-1} at 40 °C for 30 min, after equilibration of the samples for 10 min at the same temperature. Assays were carry out by triplicate. These conditions were like those reported earlier (Pino and Bent, 2013; Pino, 2014).

2.4. GC/FID and GC/MS Analysis

GC-FID was performed on a HP-6890 (Hewlett-Packard Co., Palo Alto, CA, USA), equipped with a flame ionization detector and a DB-5 MS (30 m x 0.25 mm x 0.25 μm; J & W Scientific, Folsom, CA) capillary column. The oven temperature program was set at 50 °C for the first 2 min, rising to 250 °C at 4 °C/min and held for 10 min. The column oven was operated at 50 °C for 2 min, temperature programmed at 4 °C/min to 250 °C and held for 10 min. Carrier gas hydrogen with a flow rate of 1 mL/min. Injector and FID temperatures were 250 °C. Thermal desorption was carried out at 250 °C in splitless mode for 2 min, using and inlet liner of 0.75 μm i.d.

Linear retention indexes (LRI) of the compounds were determined by a mixture of *n*-alkanes (C_6–C_{28}) as references.

Quantitative analysis of the active odorants was done by calculating their relative quantitative correction factors (RQCFs) with the "single-point correction method", which is similar to the standard addition method (Liu et al., 2020). The method of obtaining RQCFs consisted of the following: a sample of the pulp and a similar sample with defined amounts of various authentic and an internal standard (5 μL of 2 mg/mL of methyl nonanoate in methanol) were analyzed by the HS-SPME-GC-FID to generate an area for each detected compound and quantitative correction factor for the internal standard. All measurements were done three times. GC-MS analysis was carried out on a using a QP-2010 Ultra (Shimadzu, Japan) system with a similar capillary column and chromatographic parameters as for the GC-FID. The MS worked in electron impact mode at 70 eV ionization energy and in scanned mode from m/z 35 to 350, at 1.3 scan/s. Ion source and interface temperature were 250 °C. Identification of compounds was achieved by matching lineal retention indexes and mass spectra with those of

chemical standards and commercial libraries (NIST 05, Wiley 6, NBS 75 k and Adams 2001).

2.5. Direct SPME-GC-O

The procedure was performed following the methodology described earlier (Pino and Roncal, 2016). A HP-6890 (Hewlett-Packard Co., Palo Alto, CA, USA) was used. The injector was connected via a deactivated fused silica capillary (25 cm × 0.25 mm) to a home-made sniffing port consisted of a cylindrically shaped aluminum device with a beveled top and a central drill hole housing the capillary. The oven was maintained at 250 °C. Because no chromatographic separation was carried out by the short capillary, volatile compounds arrived simultaneously at the sniffing port. Three trained sniffers perceived and evaluated the resulting global odor of pechiche fruits. Sniffers evaluated with the direct GC-O device the SPME extract and give their criteria about the similarity to the reference, after they smelled first the fruit pulp (3 g) contained in a plastic cup and memorize the odor.

2.6. Gas chromatography-olfactometry (GC-O)

The SPME extract was submitted to GC-O analysis on a Hewlett–Packard 6890N equipped with a with the same DB-5 ms column used in GC-FID. The effluent was split by the sniffing port previously described. Operating parameters were the same that those above-mentioned for GC-FID analyses. Each sniffing run was divided into two 30-min sessions to avoid fatigue among the two experienced sniffers. Assessors registered the detection time and described the perception for each odor stimulus. GC-O frequency analysis was made by using the procedure reported earlier (Chaintreau, 2001). All measurements were done three times. Odor active compounds were defined as those detected at the same retention times with the same descriptor, at least four times, by the two sniffers.

2.7. Determination of odor activity value (OAV)

The OAVs for some odorants were calculated by the ratio of the odorant content in the pulp and its odor detection threshold (Selli and Kelebek, 2011). The odor detection thresholds were taken from literature.

3. RESULTS AND DISCUSSION

3.1. Results of aroma analysis

The pulp of mature pechiche fruits was characterized by total soluble solids, 13.00% ± 0.02 and titratable acidity, 0.34% ± 0.03. No data were found in the literature for comparison. Considering that this study was to determine the odor-active compounds based on the use of HS-SPME-GC-O to analyze the volatiles from pechiche pulp, the global odor of the trapped volatiles was evaluated at the sniffing port (direct SPME-GC-O). The three experts agreed in a high similarity with respect to the original pulp and therefore, the DVB/CAR/PDMS fiber was selected to characterize the volatile compounds. A total of 109 volatile compounds were detected, 92 of them were positively identified (Table 1). The major chemical families were aldehydes, alcohols, terpenes, acids and ketones, but it is interesting to note the high number of unsaturated aldehydes, alcohols and acids, which is not common in other tropical fruits (Pino, 2014; Yilmaztekin, 2014; Steingass et al., 2015; Bonneau et al., 2016; Lasekan and Yap, 2018). HS-SPME-GC-O by frequency analysis was performed to find odorant zones in the olfactograms and then identify the aroma compounds potentially responsible for these odors. The odors detected by the experts, together with the compounds identified as responsible for those odor impressions are given in Table 2. A total of 21 odorants, included many

Table 1. Volatile compounds identified in pechiche fruit

Compound	LRI	Area% ± std. dev.	Compound	LRI	Area% ± std. dev.
Acetic acid	645	0.1 ± 0.01	Methyl octanoate	1127	0.2 ± 0.02
3-Methylbutanal	654	0.1 ± 0.01	(*E,Z*)-2,6-Nonadienal	1155	tr
2-Methylbutanal	658	0.1 ± 0.01	(*E*)-2-Nonenal	1162	0.2 ± 0.01
Pentanal	706	tr	Methyl (*E*)-2-octenoate[a]	1165	tr
Methyl butanoate	729	tr	Nonan-1-ol	1169	0.1 ± 0.01
3-Methylbutan-1-ol	741	tr	Octanoic acid	1183	0.2 ± 0.02
2-Methylbutan-1-ol	742	tr	Methyl salicylate	1192	tr
Hexan-2,3-dione	786	tr	(*E*)-4-Decenal	1195	34.0 ± 0.8
Hexan-2-one	792	tr	Decanal	1202	3.6 ± 0.3
Hexan-3-ol[a]	797	tr	(*Z*)-2-Octenoic acid[a]	1230	0.2 ± 0.01
Hexanal	802	0.4 ± 0.03	Neral	1238	tr
Ethyl butanoate	805	0.4 ± 0.02	(*Z*)-4-Decen-1-ol	1259	5.7 ± 0.4
(*E*)-2-Hexenal	856	0.4 ± 0.03	(*E*)-2-Decenal	1264	0.9 ± 0.05
(*Z*)-3-Hexen-1-ol	859	0.2 ± 0.01	Geranial	1267	tr
Hexan-1-ol	871	0.2 ± 0.01	Decan-1-ol	1270	0.2 ± 0.01
3-Methylbutyl acetate	881	tr	Ethyl salicylate	1270	tr
2-Methylbutyl acetate	884	tr	(*Z,Z*)-2,4-Decadienal	1284	tr
Heptan-2-one	892	0.1 ± 0.01	Undecanal	1307	tr
Heptanal	902	0.1 ± 0.01	Methyl (*E*)-4-decenoate[a]	1310	0.1 ± 0.01
Anisole	918	tr	(*E,E*)-2,4-Decadienal	1317	0.1 ± 0.01
Methyl hexanoate	927	0.2 ± 0.01	(*Z*)-2-Decenoic acid[a]	1368	4.0 ± 0.3
α-Thujene	930	tr	Methyl decadienoate[a]	1375	tr
α-Pinene	939	0.1 ± 0.01	Ethyl (*E*)-4-decenoate[a]	1380	0.4 ± 0.03
2-Ethylhexanal[a]	951	tr	(*Z*)-3-Decenoic acid[a]	1385	0.1 ± 0.01
(*E*)-2-Heptenal	955	tr	(*E*)-5-Dodecenal[a]	1388	tr
Benzaldehyde	960	0.9 ± 0.07	β-Elemene	1391	0.1 ± 0.01
Heptan-1-ol	967	tr	Ethyl decanoate	1396	tr
Sabinene	975	tr	Dodecanal	1409	tr
1-Octen-3-one	980	1.7 ± 0.07	3-Methylbutyl octanoate	1451	tr
1-Octen-3-ol	982	11.5 ± 0.5	Geranyl acetone	1455	tr
Octan-3-one	986	0.3 ± 0.02	Undecanoic acid	1465	tr
Octan-3-ol	991	1.3 ± 0.06	γ-Decalactone	1467	tr
Myrcene[a]	994	0.2 ± 0.01	(*Z*)-2-Pentenyl hexanoate[a]	1469	tr
Octanal	999	3.1 ± 0.07	Dodecan-1-ol	1472	tr
Hexanoic acid	1000	1.3 ± 0.05	β-Selinene	1490	0.1 ± 0.01
p-Cymene	1025	0.4 ± 0.02	α-Selinene	1498	0.1 ± 0.01
Limonene	1029	7.3 ± 0.4	Methyl dodecadienoate[a]	1502	tr
1,8-Cineole	1031	0.1 ± 0.01	(*E*)-Nerolidol	1563	tr
2-Ethylhexan-1-ol	1033	tr	Dodecanoic acid	1566	0.2 ± 0.01
(*E*)-2-Hexenoic acid[a]	1036	tr	3-Octen-2-yl hexanoate[a]	1568	0.4 ± 0.02
Phenylacetaldehyde	1042	0.4 ± 0.02	Octyl hexanoate	1570	0.2 ± 0.01
Salicylaldehyde	1045	tr	2-Phenylethyl hexanoate	1642	tr
(*E*)-2-Octenal	1057	14.5 ± 0.4	Selin-11-en-4-α-ol	1660	tr
Acetophenone	1064	tr	Tetradecan-1-ol	1673	tr
(*E*)-2-Octen-1-ol	1067	0.2 ± 0.01	(*Z*)-4-Decen-1-yl angelate[a]	1675	0.6 ± 0.03
Octan-1-ol	1069	0.8 ± 0.06	Tetradecanoic acid	1769	0.3 ± 0.02
Terpinolene	1089	0.1 ± 0.01	Oct-3-en-2-yl octanoate[a]	1775	tr
Nonan-2-one	1092	0.5 ± 0.04	Octyl octanoate	1780	tr
Heptanoic acid	1094	0.4 ± 0.04	Ethyl tetradecanoate	1796	tr
Linalool	1097	tr	2-Methylpropyl tetradecanoate	1830	tr
Ethyl heptanoate	1099	tr	2-Phenylethyl octanoate	1847	tr
Nonanal	1101	0.2 ± 0.02	Pentadecanoic acid	1853	tr
2-Phenylethanol	1107	0.1 ± 0.01	(*Z*)-9-Hexadecenoic acid	1958	tr
(*E,E*)-2,4-Octadienal	1110	0.2 ± 0.01	Hexadecanoic acid	1962	0.1 ± 0.01

[a] *Tentative identification (only by matching LRI and mass spectra from libraries). tr: < 0.1%.*

aldehydes, were detected as potentially contributing to the overall pechiche fruit aroma, due to their detection frequencies were in the range 4 to 6.

The HS-SPME-GC-O strategy do not allow to look at the effect of the food matrix on volatile compound binding as it happen when is evaluated the overall flavor of the food. Hence, to examine the impact of volatiles to pechiche fruit aroma, they were quantified in the pulp and the data was used to calculate the OAVs (Table 2). The same 21 odorants found by HS-SPME-GC-O had OAVs ≥ 2 and therefore, they were considered as potentially odor-active compounds, from which *(E)*-4-decenal, *(E)*-2-octenal, octanal, decanal, *(E)*-2-decenal, *(E)*-2-nonenal, 1-octen-3-one and *(E,Z)*-2,6-nonadienal were the most potentially to the overall pechiche fruit aroma.

The definitive role played by the odorants will have to be finally assessed using different reconstitution techniques and sensory evaluation.

4. CONCLUSIONS

A total of 109 volatiles were detected in pechiche fruit, including a high number of unsaturated aldehydes, alcohols and acids. Twenty-one volatiles were considered as potentially odor-active compounds, from which *(E)*-4-decenal, *(E)*-2-octenal, octanal, decanal, *(E)*-2-decenal, *(E)*-2-nonenal, 1-octen-3-one and *(E,Z)*-2,6-nonadienal were the most odor-active compounds.

Table 2. Aroma active compounds of pechiche fruit

Compound[a]	Descriptor	Content ± std dev (µg/kg)	Odor threshold (µg/kg)	DF[b]	OAV[c]
(*E*)-4-Decenal	Orange-like	2267 ± 26	0.8[d]	6	2833
(*E*)-2-Octenal	Green-leafy	967 ± 20	3[d]	6	322
Octanal	Citrus	206 ± 12	0.7[e]	6	294
Decanal	Citrus	240 ± 8	1[e]	6	240
(*E*)-2-Decenal	Waxy	57 ± 5	0.4[f]	6	142
(*E*)-2-Nonenal	Fatty	11 ± 1	0.08[d]	6	138
1-Octen-3-one	Mushroom	116 ± 4	1[f]	6	116
(*E,Z*)-2,6-Nonadienal	Cucumber-like	1 ± 0.1	0.01[f]	6	100
Limonene	Lemon-like	488 ± 18	10[e]	5	49
Ethyl butanoate	Fruity	25 ± 2	1[e]	5	25
3-Methylbutanal	Fruity	8 ± 0.5	0.5[g]	5	16
Nonanal	Citrus	15 ± 1	1[e]	5	15
Hexanal	Green	25 ± 2	2.4[g]	5	10
2-Methylbutanal	Pungent	10 ± 1	1.5[g]	4	7
1,8-Cineole	Camphoraceous	8 ± 0.4	1.3[d]	4	6
Phenylacetaldehyde	Floral, honey	24 ± 2	4[d]	5	6
Nonan-2-one	Green, fruity	30 ± 2	5[f]	4	6
Octan-3-ol	Sweet	86 ± 3	18[d]	4	5
Heptanal	Fatty	10 ± 1	3[f]	4	3
(*E*)-2-Hexenal	Fruity	26 ± 2	17[e]	4	2
p-Cymene	Terpeny	25 ± 2	11.4[d]	4	2

[a]Identification by comparison of LRI and mass spectra; [b]GC-O detection frequency; [c]Odor activity values calculated by concentration/odor detection threshold ratio; [d]from Pino and Mesa (2006); [e]Leffingwell & Assoc. (2011); [f]Van Gemert (2011); [g]Czerny et al. (2008).

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