Quim. Nova, Vol. 42, No. 6, 607-610, 2019 http://dx.doi.org/10.21577/0100-4042.20170369

Artigo

IDENTIFICATION OF AROMA-ACTIVE VOLATILE COMPOUNDS IN *Pouteria sapota* FRUIT BY AROMA EXTRACTION DILUTION ANALYSES (AEDA)

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Recebido em 01/03/2019; aceito em 02/05/2019; publicado na web em 23/05/2019

Pouteria sapota is a tropical fruit that is commonly found in central and north part of South America where is widely consumed by its biofunctional properties. The aroma active volatile compounds of *Pouteria sapota* fruit were studied by using the molecular sensory approach, which includes a SAFE (Solvent Assisted Flavor Evaporation) distillation to get a volatile extract that resembles the fruit aroma, and the combination of GC-O (Gas Chromatography coupled to Olfactometry) with GC-MS analyses to identify odour-active compounds. By AEDA (Aroma Extract Dilution Analysis), β–damascenone, furaneol, linalool, (*Z*)-3-hexenal, and benzaldehyde, were identified as key aroma compounds in this fruit.

Keywords: SAFE; AEDA; gas chromatography-olfactometry; tropical fruit.

INTRODUCTION

"Sapote mamey" or "sapote costeño" (*Pouteria sapota*, Sapotaceae) is a plant that grows naturally from Southern Mexico to Northern South America. The intense orange-red fruit pulp (Figure 1) is a good source of nutrients, vitamins, and notably rich in carotenoids.[1-3] This fruit is consumed fresh or used to prepare sherbets, and its demand has been increased considerably in Australia, Europe, and the United States.[1,4] Its soft and fruity aroma is a significant attribute of *Pouteria sapota* fruit, that has not been studied before. A couple of works have reported the volatile composition of this fruit. Pino *et al.*[5] reported the volatile composition of *P. sapota* fruit extract obtained by SDE (Simultaneous steam Distillation-solvent Extraction) with dichloromethane, concluding that benzaldehyde, hexanal, and hexadecanoic acid were the major constituents. Recently, Rodríguez *et al.*[6] reported cedrol, azulene, β-ionone, naphthalene, α-pinene, and benzaldehyde as the major constituents of *P. sapota* fruit by using an optimised HS-SPME method.

One important step on flavor analyses is the selection of the extraction method so that, the aroma extract resembles the original sample one; additionally, losing of volatile compounds or generation of new ones by thermal treatment must be avoided.[7] Nowadays, SAFE (Solvent-Assisted Flavor Evaporation)[8] is the most suitable method to obtain aroma extracts because it is a solvent-assisted high vacuum distillation technique that allows to perform a gentle and efficient volatile extraction without the presence of fatty acids or artifact formation. Then, the odour-active volatiles or those that are able to interact with human olfactory receptors[9] are recognised from the odourless volatile compounds by GC-olfactometry on serial dilutions of aroma extract (Aroma Extract Dilution Analysis, AEDA).[10]

The aim of this work was to chemically study the aroma-active compounds from *Pouteria sapota* fruit, by using the molecular sensory approach,[11] that correlates instrumental (GC-FID and GC-MS) and sensory analyses (GC-O). AEDA was used to determine the contribution of each aroma-active compound to the whole aroma of this fruit.

EXPERIMENTAL

Plant material

Samples of ripe red *Pouteria sapota* fruits were acquired in local markets from Barranquilla, Atlántico, Colombia and processed immediately upon arrival at the laboratory. The pH of fruit pulp was determined by using a Jenway pH meter (model 370, Essex, England). Total soluble solids were determined with an Atago refractometer (HRS-500, Tokyo, Japan) and the results were expressed as °Brix.

Chemicals

Dichloromethane, pentane, sodium sulphate (anhydrous), 2-heptanol, 2-methyl-1-propanol, and *n*-alkane mix (C_9–C_{25}) were of analytical grade and acquired from Merck (Darmstadt, Germany). All solvents were freshly distilled prior to use. The other reference standards of (*E*)-2-hexenal, (Z)-3-hexenal, acetic acid, and *trans*-linalool oxide furanoid were purchased from Sigma–Aldrich (St. Louis, MO); and linalool from Alfa Aesar (Heysham, UK). β-Myrcene, β-ocimene, α-copaene, cedrol, benzaldehyde, 3-methyl butanoic acid, β-damascenone, 2-phenylethanol, and 4-hydroxy-2,5-dimethyl-3(*2H*)-furanone were generously supplied by DISAROMAS S.A. (Bogotá, Colombia).

Isolation of volatile compound extract by SAFE distillation

Aroma extract of *P. sapota* fruits was obtained by solvent extraction followed by Solvent-Assisted Flavour Evaporation (SAFE distillation).[8] The fruit pulp (300 g, pH 5.51, °Brix = 5.8) was homogenised by using a commercial stainless-steel blender, and then mixed with portions of anhydrous sodium sulfate (100 g) to remove water and improve the extraction efficiency. The homogenate was extracted with a dichloromethane (300 mL), and after SAFE, the organic phase was dried over anhydrous sodium sulfate and concentrated to 1 mL using a Vigreux column (50 × 1 cm).

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Analyses of odour-active volatiles by GC-O (Gas Chromatography-olfactometry) and Aroma Extract Dilution Analysis (AEDA)

GC-O Analyses were performed in a gas chromatograph HP 5890 Series II (Hewlett-Packard, USA) equipped with FID and operated in split mode (1:10, injected volume, 1 µL). The injection port was set at 230 °C and Helium was used as carrier gas at 1.0 mL min^{-1}. Two capillary columns, HP-FFAP and HP-5 (HP, 50 m x 0.25 mm i.d., df = 0.25 µm, and Agilent, 30 m × 0.32 mm i.d., 0.25 µm film thickness, respectively) were used for the volatile analyses. The column oven was programmed at 40 °C for 4 min, then the temperature was increased at 6 °C min^{-1} until 180 °C, then at 12 °C min^{-1} until 230 °C for the HP-FFAP and 300 °C for the HP-5, and finally the columns were maintained for 10 min at the maximum temperatures. The end of the capillaries was connected to a deactivated Y-shaped glass splitter (Chromatographie Handel Mueller, Fridolfing, Germany), which divides the effluent into two equal parts, one for FID (230 °C) and the other for heated sniffing port (230 °C) by using deactivated fused silica capillaries of the same length (50 cm x 0.32 mm i.d.). Sniffing port consisted of a self-made elbow-shaped aluminium tube (80 x 5 mm i.d).

Three panelists located the odour-active zones of the SAFE extract by GC-O and described the odour notes perceived at the sniffing port. Then, the aroma extract was stepwise diluted to obtain 2^n dilutions, and each solution was analysed by GC-O in the same conditions above-mentioned for HP-FFAP column. The odour activity of each compound (FD value) was determined as the greatest dilution at which that compound was still detected by comparing all of the runs.[12]

Aroma active volatile identification and quantitation

Linear retention indexes (LRI) of the aroma active compounds were calculated by using a mixture of normal paraffin (C_9-C_{25}) as external references. The identification of volatile compounds was completed by comparison of their retention indexes (in the two columns), mass spectra, and odour notes, with those exhibited by standard solutions of volatiles (50 µg mL^{-1}), when they were available.

Quantitative analyses of odour-active volatiles (those exhibiting dilution factors higher than 8) was done by using the internal standard (IS) method. 2-Heptanol was used as internal standard, dissolved in the extraction solvent (500 µg mL^{-1}), and added to the fruit puree (1.67 mg kg^{-1} fruit) before extraction by SAFE. To determine the response factor for each volatile compound, calibration curves were constructed using a series of solutions of varying nominal concentrations containing each analyte (IS:analyte from 1:5 to 5:1), where the slope was assumed as the response factor. An identical amount of the internal standard was added to each solution.[13] The concentration of each analyte was calculated by comparison of GC-FID signals in FFAP column with those of standards, and the relative response factor was including, according to the following equation:

$$[\ ]_X = \frac{A_X}{A_{istd}} \frac{\mu g\ IS}{kg\ fruit} RF^{-1}$$

where, $[\ ]_x$ is the analyte concentration in µg kg^{-1} fruit, A_X is the analyte area, A_{istd} is the internal standard area, and RF is the response factor. Key-aroma compounds were determined based on their OAV (odour activity value = concentration divided by odour threshold).[12]

Gas chromatography-mass spectrometry (GC-MS) analyses

GC-MS (EIMS) analyses of aroma active compounds were carried out on a GC Agilent 7890B gas chromatograph coupled to a mass spectrometry 5977A (Agilent Technologies Inc. Wilmington, DE, USA). MS data were recorded between 40-350 u, with an electron energy of 70 eV and processed by Mass Hunter software. Chromatographic conditions were the same that those above-mentioned for GC-FID analyses for FFAP column. Data acquisition was carried out with Qualitative Analysis Software (database NIST/EPA/NIH Mass Spectral Library 2014 (2.2)).

RESULTS AND DISCUSSION

In flavour chemistry, the correlation between sensory and instrumental data enable the identification of the aroma active compounds and their contribution to the overall aroma.[14] Thus, a total of 18 aroma active regions were detected in the *Pouteria sapota* fruit aroma by GC-O analyses of SAFE extract, showing sweet, fruity, floral, green, almond-like, and fermented odour notes. The volatile compounds with the highest FD values (Figure 1) were: 3-methyl-butanal, (Z)-3-hexenal, benzaldehyde, 3-methyl butanoic acid, 4-hydroxy-2,5-dimethyl-3(*2H*)-furanone (furaneol), *trans*-linalool furanoid oxide, and linalool. But, after calculation of OAVs values that involves the quantitation data of aroma compounds, β-damascenone, furaneol, linalool, (*Z*)-3-hexenal, benzaldehyde, and 3-methyl butanal were identified as the key aroma compounds in this fruit (Table 1). Great differences in odour potency determined by FD and OAV were found for *trans*-linalool oxide furanoid, 3-methyl butanoic acid, and β-damascenone. This happens because the odour threshold values, which are relatively high for *trans*-linalool oxide furanoid and 3-methyl butanoic acid, and too low for β-damascenone.

3-Methyl-butanal has been reported as aroma-active volatile in *Lucuma hypoglauca* Standley,[15] and *Diospyros digyna* Jacq.;[16] linalool and β-ocimene in *Diospyros digyna* Jacq.;[16] and (*E*)-2-hexenal in *Manikara zapota* L[17] fruits, all belonging to sapotaceae family as well as *Pouteria sapota*.

The presence of linalool, its furanoid and pyranoid oxides, as well as β-myrcene, and β-ocimene, as aroma active compounds, suggests that terpene reactions and rearrangements are activated during the ripening of this fruit. It has been reported that many of the terpenols are stored in plants as glycosides, and they are released when the glycosidic bond is cleaved by effect either glycosidases or acid pH.[18] Fisher and Scott[19] proposed that linalool is released from linalool glycosides in acid pH, then the C_6-C_7 double bond is oxidised to produce the linalool epoxide, which is the precursor of the diasteroisomeric linalool oxides, furanoids or pyranoids, after the OH of C3 nucleophilically substitutes the C6 or C7 of epoxide, respectively. The presence of β-myrcene and β-ocimene could be explained as a rearrangement produced by dehydration of linalool at C3, involving the formation of a double bond with C10 or C4, respectively. Other biogenetic routes that could be activated in this fruit are: the enzymatic oxidative degradation of linolenic acid to produce (*Z*)-3-hexenal and by further rearrangements, (*E*)-2-hexenal, and (Z)-3-hexenol, as well as, of linoleic acid to produce hexanal and hexanol;[19] the oxidative degradation of carotenoids to produce β-damascenone; and the enzymatic conversion of the aminoacid leucine into 3-methyl-1-butanal, which by further oxidation, produces the corresponding 3-methyl butanoic acid.

It is well-known that the volatile profiles can be affected by the extraction method used.[20] Pino *et al.*[5] extracted the volatile components of *P. sapota* fruits by SDE with dichloromethane as solvent, finding benzaldehyde, hexanal, and hexadecanoic acid as major constituents. SDE extraction significantly changes the composition of volatile extract due to the effect of temperature; for example, labile aroma active volatiles such as, 3-methyl-1-butanal, 2-methyl-1-propanol, (*Z*)-3-hexenal, linalool, and β-damascenone,

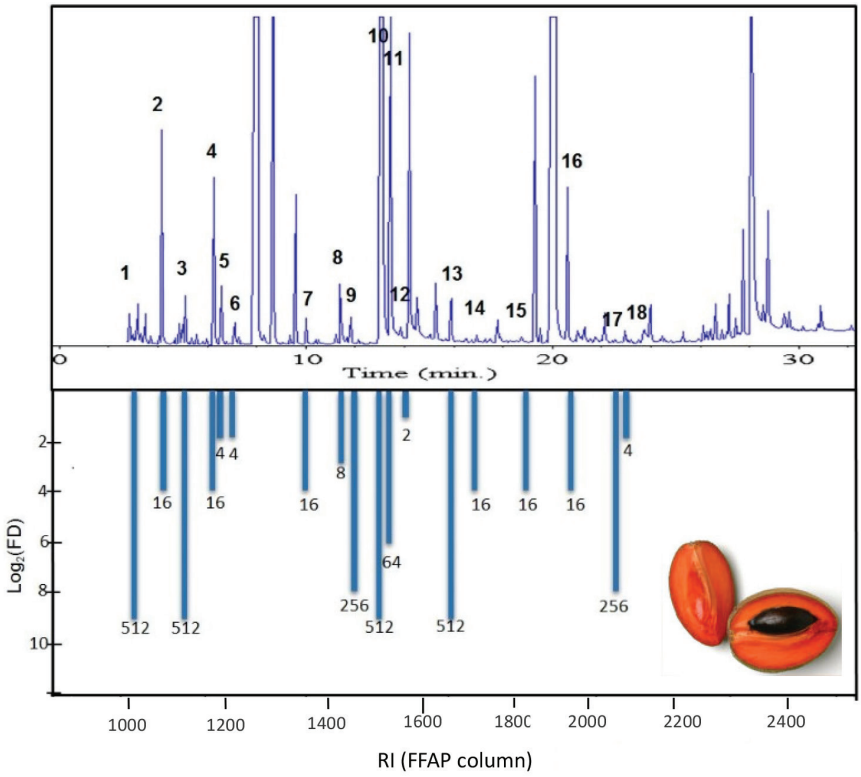

Figure 1. *GC and AEDA analyses on FFAP column of aroma active compounds from Pouteria sapota fruit obtained by SAFE distillation. Numbers correspond to Table 1 and FD values are placed on the figure.*

Table 1. Aroma active volatile compounds in SAFE extract of *Pouteria sapota* fruit

No[a]	Odorant[b]	Odour description[c]	RI		Flavor dilution Factor (FD)	Amount (μg kg^{-1} fruit)[d]	Odour threshold (μg kg^{-1} water)	*OAV*[e]
			FFAP	HP-5				
1	3-Methyl butanal[f]	Sweet, caramel, fruity	<1000	<900	512	25	0.9[g]	27.8
2	2-Methyl-1-propanol	Sweet, moldy	1085	<900	16	324	40000[h]	0.0081
3	(*Z*)-3-Hexenal	Green, herbal	1149	<900	512	34	0.25[i]	136.0
4	β–Myrcene	Metallic, spicy	1190	993	16	126	13[i]	9.7
5	(*E*)-2-Hexenal	Green, herbal	1229	<900	4	-	17[i]	-
6	(Z)-β-Ocimene	Sweet, herbal, citrus	1261	1046	4	-	-	-
7	4-Hydroxy-4-methyl-2-pentanone[f]	Fruity, fermented	1379	<900	16	51	64000[h]	0.0008
8	Acetic acid	Vinegar	1460	<900	8	-	22000[i]	-
9	*trans*-Linalool oxide furanoid	Creamy, earthy	1468	1075	256	71	3000[j]	0.02
10	Benzaldehyde	Almond	1528	969	512	22617	350[i]	64.6
11	Linalool	Floral, citrus	1547	1101	64	1174	6[i]	195.7
12	α-Copaene	Woody, earthy	1564	1380	2	-	-	-
13	3-Methyl butanoic acid	Rancid, sweaty	1670	<900	512	96	120[i]	0.8
14	*trans*-Linalool oxide pyranoid[f]	Fruity, floral	1762	1173	16	64	-	-
15	(E)–β-Damascenone	Fruity, honey	1825	1412	16	8	0.002[i]	4000
16	2-Phenylethanol	Floral	1926	1112	16	324	750[i]	0.4
17	4-Hydroxy-2,5-dimethyl-3(*2H*)-furanone (furaneol)	Sweet, caramel	2067	1090	256	10	0.03[k]	333.3
18	Cedrol	Fruity, putrid	2083	1604	4	-	-	-

[a]Odorants were consecutively numbering according to their retention time on the FFAP column (Figure 1). [b]Odorants were identified by comparing their retention indices (RI) on HP-FFAP and HP-5 columns, their mass spectra obtained by GC-MS, and their odour quality with respective data reference compounds. [c]Odour quality as perceived at the sniffing port during GC-O analyses. [d]Based on the amount of internal standard 2-heptanol. - = not determined. [e]*OAV* = odour activity value, concentration divided by odour threshold. [f]The identification was performed because their mass spectrum and retention indexes agreed with literature data (database NIST/EPA/NIH Mass Spectral Library 2014 (2.2)). [g]ref. 21. [h]ref. 22. [i]ref. 23. [j]ref. 24. [k]ref. 25.

among others were not found in these conditions. In contrast, oxidised (*i. e*. α-pinene oxide, and *trans*-sabinene hydrate) or with high-molecular weight compounds (*i. e*. tetradecanoic acid, hexadecanol, methyl hexadecanoate, and hexadecanoic acid) were detected in the SDE extract. This can be explained because during SDE extraction, the *P. sapota* fruit was boiled under water steam, being this a higher temperature than it is used during SAFE distillation.

In a recent report, the volatile components of *P. sapota* fruits were extracted by HS-SPME, maintained the sample 50 min at 65 °C with salting out.[6] Several terpenes and fatty acid esters were found in this extract, and among aroma active compounds (Table 1), only benzaldehyde and cedrol were detected. Likely, because the long-extraction times tends to provide poor recovery and can lead to the desorption of the more volatile compounds from the fibre. Additionally, those HS-SPME conditions allow to extract low polar high-molecular weight compounds, that are detected by GC-MS analyses but they do not contribute to the aroma of this fruit.

CONCLUSIONS

In this work, the odour-active volatiles of *P. sapota* were identified by using the molecular sensory approach that combines sensory and instrumental GC analyses. SAFE extraction showed to be an appropriate technique to get a volatile extract that resembles the aroma of the original material. This knowledge will be useful for the quality control of added-value products developed from this tropical fruit.

ACKNOWLEDGMENTS

Authors greatly acknowledge the financial support provided by Fondo Nacional de Financiamiento para la Ciencia, la Tecnología y la Innovación, Francisco José de Caldas, Contract No. 0459-2013, Red Nacional para la Bioprospección de Frutas Tropicales-RIFRUTBIO. The authors thanks Disaromas S. A (Bogota, Colombia for generously supplied the volatile standards, and Professor Roger Valle from U. Atlantico for supplying the *P. sapota* fruits.

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