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Short communication

Discovering a volatile organic compound fingerprinting of *Pouteria lucuma* fruits

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Summary

Introduction – **The unfamiliarity of European consumers with fresh tropical fruits has restricted their introduction on the international markets. Several tropical species, such as *Pouteria lucuma*, have instead a great economic potential for the European markets, producing edible fruits known for their nutritional and healing properties. Currently, fruit aroma and sensory characteristics are one of the primary factors determining the fresh fruit quality and consumers' choice and purchasing intention. Therefore, in the last years great attention has been shown towards the characterization of Volatile Organic Compounds (VOCs) which contribute to characterize odors and flavors of a wide variety of tropical fruits and that can attract the interest of consumers.** *Materials and methods* – **In this work, proton-transfer-reaction time-of-flight mass spectrometry (PTR-MS-TOF) measurements were carried out to assess for the first time, the VOCs profile of lucuma fruit, which is much common and widely consumed in Peru and Chile.** *Results and discussion* – **More than 50 aromatic compounds were determined in ripe fruits and the most abundant signals observed were m/z = 27.022, 33.033, 45.033 and 47.049, which were tentatively identified as acetylene, methanol, acetaldehyde and ethanol, respectively. Interestingly, many of the compounds that usually contribute to the flavor of several tropical fruits, such as mono-, hemi- and sesqui-terpens, were not present in the aroma profile of the lucuma fruit.** *Conclusion* – **The entire dataset obtained shows that the lucuma fruit aroma is due in large part to the presence of short-chain alcohols (methanol, acetaldehyde, ethanol) but also of volatile esters, aldehydes and hydrocarbons that contributes to the complex mixture of volatile aroma.**

Keywords

tropical fruits, *Pouteria lucuma*, aromatic compounds, proton-transfer-reaction time-of-flight mass spectrometry (PTR-MS-TOF), underutilized species, nutritional value

Résumé

Découverte de l'empreinte biochimique en composés organiques volatils des fruits de *Pouteria lucuma*.

Significance of this study

What is already known on this subject?

- Demand for fresh tropical fruits is increasing in Europe, as consumers are becoming more aware of their nutritional value and role in disease prevention.

What are the new findings?

- This paper is aimed to assess the main VOCs of *Pouteria lucuma* fruit, trying to fill a scientific gap due to the lack of information about flavor compounds of *P. lucuma.*

What is the expected impact on horticulture?

- Our findings could contribute to increase the consumption of unknown tropical fruits in Europe, increasing their production in the countries of origin.

Introduction – **Les fruits tropicaux sont peu connus des consommateurs européens, ce qui limite leur introduction sur les marchés internationaux. Plusieurs espèces tropicales, telles que *Pouteria lucuma*, ont pourtant un fort potentiel économique pour le marché européen, avec des fruits comestibles réputés pour leurs propriétés nutritionnelles et curatives. Actuellement, l'arôme des fruits et les caractéristiques sensorielles sont parmi les principaux facteurs déterminant la qualité des fruits frais, le choix des consommateurs et leur intention d'achat. Ces dernières années, une attention particulière est portée à la caractérisation des composés organiques volatils (COV) qui contribuent à caractériser les odeurs et les arômes d'une grande variété de fruits tropicaux et qui peuvent attirer l'intérêt des consommateurs.** *Matériel et méthodes* – **Au cours de ce travail, des mesures de spectrométrie de masse (MS) à temps de vol (TOF) et ionisation par transfert de proton (PTR) ont été effectuées pour évaluer pour la première fois le profil COV des fruits du lucuma, très répandu et largement consommé au Pérou et au Chili.** *Résultats et discussion* – **Plus de 50 composés aromatiques ont été déterminés à partir des fruits mûrs et les signaux les plus abondants ont été observés aux rapports masse/charge (m/z) de 27,022, 33,033, 45,033 et 47,049 provisoirement identifiés comme l'acétylène, le méthanol, l'acétaldéhyde et l'éthanol, respectivement. Il est intéressant de constater que de nombreux composés qui contribuent habituellement à la saveur de plusieurs fruits tropicaux, tels les mono-, hémi- et ses-**

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qui-terpènes, n'étaient pas présents dans le profil aromatique des fruits du lucuma. *Conclusion* **– L'ensemble des données obtenues montre que l'arôme des fruits du lucuma est dû en grande partie à la présence d'alcools à chaîne courte (méthanol, acétaldéhyde, éthanol) mais aussi d'esters volatils, d'aldéhydes et d'hydrocarbures qui contribuent au mélange complexe d'arômes volatils.**

Mots-clés
fruits exotiques, *Pouteria lucuma*, composes aromatiques, spectrométrie de masse à temps de vol et ionisation par transfert de proton (PTR-MS-TOF), espèce sous-utilisée, valeur nutritionnelle

Introduction

Demand for fresh tropical fruits is steadily increasing in Europe (Sabbe *et al*., 2009), as consumers are becoming more aware of their nutritional value and role in disease prevention (Song and Forney, 2008). Moreover, this increase can be associated also to other factors, such as: the improvement of storage technologies and transportation systems, the increase of international travels and higher incomes, the growing search for new flavors and innovative products (Sabbe *et al*., 2009). Currently, consumers show more attention to the fruit quality and some parameters, as flavor and dietary value, have become very important. The quality of fresh fruits depends indeed on many aspects, among which appearance, color, texture, flavor and nutritional benefit (Song and Forney, 2008). Furthermore, it has been proven that sensory characteristics are one of the primary factors for consumers' choice and purchasing intention (Verbeke, 2006; Enneking *et al*., 2007).

In 2002 Tuorila and Cardello (2002) in a study performed to analyze consumer responses to an off-flavor in juice in the presence of specific health claims, highlighted the importance of sensory liking as the principal predictor of consumption. In practice, if the first impression of taste is poor, the consumer is prevented from trying the product a second time and consuming it in the future.

Therefore, improvements in fruit flavor are needed to satisfy consumer demands (Kader, 2004). The great diversity of exotic fruits, especially tropical ones, represents a promising area for studying aromas with unusual sensory properties that can attract the interest of consumers. For this reason, in the last years great attention has been shown towards the characterization of the volatile profile of a wide variety of exotic fruits (Lasekan and Abbas, 2012). Fruit aromas consist of a complex mixture of Volatile Organic Compounds (VOCs) whose composition is specific to each fruit type and variety (Schwab *et al*., 2008). All fruits produce and emit a wide variety of VOCs, such as esters, terpenes, lactones and derivatives of amino acids, fatty-acids and phenolic compounds, which contribute to characterize and differentiate their odors and flavors (Baietto and Wilson, 2015). Each type of fruit, although sharing some aromatic characteristics, has a distinctive sensory characteristic that depends on the specific combination of all VOCs present in the aroma mixture (Tucker, 1993). Therefore, VOCs can be used for the characterization of agro-industrial products, including fruits, and for food evaluation to determine their quality and consumer acceptance (Baietto and Wilson, 2015; Taiti *et al*., 2015a). Moreover, fruit aroma is the key to determine consumer acceptance based on individual preference (Baietto and Wilson, 2015). A large number of VOCs has been identified in many exotic or temperate fruits, but more studies are needed to characterize each fruits, such as: aroma fingerprinting, the threshold of perception and the interactions with other compounds. Actually, PTRMS TOF represents a simple and fast technology which allows a rapid and real-time detection of the headspace composition of VOCs, and could be used for compounds identifications and their monitoring during the "from farm to table" production chain to meet the satisfaction of the consumer demands. Indeed, PTRMS TOF has been extensively used to assess and monitoring the flavor compounds of fruits, for example spices (Taiti *et al*., 2015b), apples (Farneti *et al*., 2015) and tropical fruits (Taiti *et al*., 2015a).

Pouteria lucuma Ruiz & Pav. is a subtropical perennial tree producing an important edible fruit known for its nutritional and healing properties. This species belongs to the *Sapotaceae* family and is native to the high Andean valleys of Chile, Ecuador and Peru (Gutierrez, 1980). Although some species belonging to the genus *Pouteria*, such as "lucuma" or "sapote", have been cultivated for centuries by the native populations and were used in the traditional diet and medicine (Fuentealba, 2016), they are almost unknown on the international trade market, thus representing a strong economic potential for native peoples. About 88% lucuma fruit world production is concentrated in Peru. In this country lucuma is a staple food, generally much common for domestic consumption and for export to other countries, such as Chile, US and Canada (AMPEX, 2010) that are wide consumers (Quilter *et al*., 1991). Both on the national and international trade market, lucuma is usually consumed as fresh fruit and juices or can be used for the preparation of ice-creams and desserts as flour and frozen or dehydrated pulp (Diaz, 1987).

Lucuma is a climacteric fruit and the ripening process is associated by color changes and increase of soluble solids (Lizana *et al*., 1986). It is composed by an outer fleshy edible part and a hard inedible inner seed (nut), known for its healing properties since, as tough other plants such as soybean, corn and canola oils, it represents a rich natural source of fatty acids (Simopoulos, 2004; Rojo *et al*., 2010). Moreover, lucuma fruit is a good source of fiber, minerals, phenolic compounds, carotene and other compounds (Yahia, 2011) and it is used as natural alternative to sucrose used as sweeteners (Belščak-Cvitanović *et al*., 2015). Only few information is available in literature about metabolites present in lucuma fruit (Fuentealba *et al*., 2016) while no studies are known on the aromatic properties of fruit.

The aim of this work was to assess the main VOCs that characterize lucuma fruit, helping to fill a scientific gap due to the lack of information about flavor compounds of *Pouteria lucuma*. Therefore, some conventional measurements (SSC and color) were performed to evaluate the ripening stage of the fruit, while non-conventional VOC analysis by PTRMS TOF were carried out to assess, for the first time, the volatile compound profile of lucuma fruit.

Materials and methods

Experimental materials

A collection of 12 homogeneous fresh lucuma fruits (unknown variety) were purchased as "ready to eat" by "Shangrilamarketplace", a local importer of exotic fruits in the city of Florence (Tuscany, Italy). Fruit selection was based on the

Table 1. Soluble solid content (SSC) and color at various ripening stages (1–5) of *Pouteria lucuma* fruit samples. GY: Green-yellow; DY: Dark-yellow; OY: Orange-yellow. SSC values are means of 3 refractometric measurements on the juice freshly extracted from each fruit.

	Fruit samples											
	1	2	3	4	5	6	7	8	9	10	11	12
Ripening stage	4	5	5	4	4	4	4	5	4	4	4	4
SSC (°Brix)	26.3±0.76	25.5±0.53	26.0±0.84	24.5±1.22	23.5±0.80	25.5±1.10	20.5±0.50	26.5±0.76	20.5±0.25	22.0±0.96	20.3±0.45	22.3±1.24
Peel color	GY	GY	GY	GY	GY	GY	GY	GY	GY	GY	GY	GY
Pulp color	DY	OY	OY	DY	DY	DY	DY	OY	DY	DY	DY	DY

absence of external defects and on size homogeneity. All the samples have been imported (by air) from a local market in Peru (Lima) and were maintained at room temperature (23 ± 3 °C; 45–55% relative humidity) for two days before the analysis. For each fruit sample all the measurements were conducted in triplicate.

Color evaluation and soluble solid content (SSC)

The ripening stage of the fruit samples was assessed following the classification developed according to peel and pulp color ripening scale by Lizana (1980). The peel can range from green to yellowish-green color while the pulp from light yellow to orange-yellow (Lizana, 1980).

Solid soluble content (SSC, %) was measured with an N1 Atago refractometer (Atago Co., Japan) and values were expressed as °Brix, following the procedure reported by Lizana *et al.* (1986). Briefly, pulp from ripe fruit has been disrupted by mechanical means and diluted with distilled water (1:5). Finally the homogenate was analyzed by refractometer.

PTRMS-TOF analysis of lucuma fresh fruit

The proton-transfer-reaction time-of-flight mass spectrometry (PTR-MS-TOF) has been defined as a valuable instrument for VOCs fingerprinting and quality evaluation of fresh or processed fruits (Taiti *et al.*, 2015a; Farneti *et al.*, 2015; Mayr *et al.*, 2002). Measurements were performed with a commercial PTR TOF 8000 model, from Ionicon Analytik GmbH (Innsbruck, Austria) in its standard configuration (V mode) and using H_3O^+ as reagent ion for the proton-transfer reaction. The ionization conditions for all measurements in the drift tube were the following: 110 °C drift tube temperature, 2.30 mbar drift pressure and 550 V drift voltage and E/N value of 135 Td. Data acquisition was carried out at 1 spectrum per second. All mass spectra (m/z) have been sampled ranging between 25 and 210, and subsequently the signal at m/z = 37 has been deleted since it is related to water cluster and not to VOCs. For the sample preparation, the procedure previously used by Taiti *et al.* (2015a) has been followed. A 5-cm^3 portion of fruit was cut from each sample, weighed and put immediately in a 50-mL glass jar, topped with a special glass cap which allows the connection between the inlet of the PTRMS-TOF, the headspace collecting and the zero-air-generating.

For each of the 12 lucuma fruits, VOC measurements were collected on three different portions and, in the end, the average value and standard deviation of the three replicates were calculated. Before the measurement, each fruit sample was incubated at room temperature for five minutes. To avoid possible systematic errors, the apparatus was flushed with clean air for 5 min from one measurement of lucuma sample to the next one. Humidity and temperature were kept constant during the measurements, since chemical reactions are sensitive to changes of these parameters (Mancuso *et al.*, 2015). Compounds of exactly known m/z, such as trichlorobenzene (m/z =180.937), were continuously added to the sample inlet system and together with other known low mass ions were used for a precise conversion of "time-of-flight" into "mass-to-charge" ratio (m/z). To assign the exact mass and the chemical formula of all ions detected during VOC analysis, m/z = 29.997 (NO^+), m/z = 59.049 ($C_2H_5O_2^+$) and m/z = 180.937 ($C_6H_4Cl_3^+$) were used for internal calibration. This operation was performed off-line. Considering the lack of information on aromatic compounds of lucuma fruit, the tentative identification of VOCs provided by the tool was compared, when possible, with published VOCs emitted from tropical fruit species.

Results and discussion

Color and SSC parameters

All the fruit samples showed a ripening stage of 4 or 5 following the classification suggested by Lizana (1980) comprising five stages of maturity. The soluble solid values varied from 20.3 to 27.0 °Brix, revealing a high sugar content in the pulp of the tested fruits (Table 1). Since lucuma is a climacteric and highly perishable fruit, the ripening process is accompanied by increasing sugar contents and changes in skin and pulp color (Yahia, 2004). The parameters commonly used to assess a ripening stage are: change of peel and pulp color and solid soluble content (Lizana *et al.*, 1986). Moreover, immature fruits often lack flavor because of the close relationship between maturity and volatile biosynthesis (Kader, 2004); it is therefore really important to define the ripening stage in order to properly evaluate the lucuma fruit aroma. Following the classification suggested by Lizana (1980) the lucuma fruit samples used in this paper showed a 4 or 5 stage of ripening.

Rapid detection of volatile compounds using PTRMS-TOF

The volatile profile of lucuma fruits was analyzed via PTRMS-TOF. Peak extraction showed the detection of 50 tentatively identified compounds in the range of measured masses (25 < m/z < 210), derived from the protonation of various VOCs (Table 2). About 75% of the identified compounds were detected within the range 25–100 while only the 25% were placed between 100 and 210. Moreover, the intensity of signal found in the range 25–100 describes more than 99% of total intensity recorded. Figure 1 shows the snapshot of the mass spectra obtained in the range 25–210 for one lucuma fruit sample. All compounds identified for each analyzed fruit and their signal intensity are listed in Table 2, together with their m/z ratio, chemical name, molecular formula, chemical and functional grouping and related

Table 2. Compounds identified through PTR-Analysis. Values are means of three replicates of each fruit ± standard deviations. [a] Protonated measured *m/z*; [b] Putative identifications; [c] Compound's Chemical formula (H^+ added by protonation); [d] Compound classification based on their chemical and biochemical properties: H hydrocarbon, T terpene, O others; [e] VOCs headspace intensity (ncps) for each fruit sample; [f] Abundant signals expressed as (%); [g] Compounds related to their bibliography: ^ Tropical fruits articles where molecule was reported; * PTR-TOF-MS articles where molecule was reported.

Protonated measured m/z [a]	Tentative identification [b]	Protonated chemical formula [c]	Chemical and functional grouping [d]	Fruit samples: VOCs headspace intensity (ncps)[e]												Compound (%)[f] on the total	References[g]
				1	2	3	4	5	6	7	8	9	10	11	12		
27.022	Acetylene	$C_2H_3^+$	H1	3586.67 ±546.24	3717.40 ±397.69	3397.55 ±409.45	2927.55 ±322.54	4617.55 ±222.45	3967.50 ±187.76	3157.55 ±454.58	4107.55 ±454.24	2387.55 ±340.44	2747.55 ±578.54	3817.34 ±290.43	2527.44 ±445.33	8.47	Taiti *et al.*, 2016*
31.018	Formaldehyde	CH_3O^+	O1	3021.15 ±333.65	4449.10 ±450.75	5119.15 ±432.45	4933.15 ±334.34	3779.10 ±545.45	3718.10 ±298.23	2681.15 ±256.65	2586.15 ±400.57	4804.16 ±418.56	4540.35 ±321.34	2802.66 ±225.66	2673.75 ±276.50	9.33	Mancuso *et al.*, 2015*
31.042	Methylamide	CH_5N^+	N1	67.13 ±11.12	59.07 ±10.22	76.98 ±12.11	54.66 ±7.98	47.11 ±10.22	39.41 ±5.43	40.41 ±8.56	49.33 ±10.34	68.99 ±11.12	57.23 ±8.45	56.12 ±13.40	45.32 ±10.32	0.14	
33.033	Methanol	CH_5O^+	O2	3690.55 ±333.87	3540.00 ±440.20	4280.05 ±209.40	5190.40 ±432.32	3040.10 ±130.30	4290.05 ±134.55	3370.05 ±309.50	3850.44 ±221.34	3830.05 ±406.45	5030.10 ±210.34	3330.05 ±455.43	3190.35 ±345.5	9.64	Mancuso *et al.*, 2015*
39.022	Isoprene fragment	$C_3H_3^+$	H2	560.80 ±44.30	562.70 ±55.90	587.80 ±111.25	579.90 ±90.70	520.40 ±60.40	512.60 ±30.80	489.90 ±104.50	475.60 ±45.50	579.50 ±112.30	561.30 ±40.90	499.50 ±33.50	486.50 ±25.90	1.33	Taiti *et al.*, 2016*
41.038	Alkyl fragment	$C_3H_5^+$	H3	6597.50 ±989.45	6688. 46 ±1090.34	7367.8 ±1545.34	7224.8 ±887.45	6558.8 ±767.54	6445.8 ±1023.43	7340.8 ±1545.50	7220.8 ±976.44	7978.48 ±1667.47	7623.8 ±878.54	7569.80 ±2090.45	7392.80 ±1220.45	17.79	Taiti *et al.*, 2015a*
43.018	Alkyl fragment	$C_2H_3O^+$	O3	2967.50 ±300.34	2377.50 ±709.50	2067.55 ±209.30	3747.20 ±670.90	1987.50 ±320.90	1787.500 ±320.75	1210.35 ±130.43	1035.40 ±120.20	1067.50 ±135.70	737.50 ±200.34	990.00 ±110.00	964.00 ±110.40	4.12	
43.054	Alkyl fragment (Propene)	$C_3H_7^+$	H4	125.10 ±	335.12 ±40.35	78.55 ±10.40	106.35 ±20.80	125.50 ±30.70	110.5 ±35.90	327.75 ±80.50	127.35 ±44.60	138.55 ±45.60	129.75 ±33.50	144.35 ±39.50	141.15 ±26.40	0.39	Taiti *et al.*, 2016*
45.033	Acetaldheyde	$C_2H_5O^+$	O4	2346.50 ±444.50	3545.4 ±402.45	8876.4 ±1190.56	7035.20 ±1255.60	3096.4 ±460.70	2889.45 ±338.70	2986.34 ±280.80	3424.34 ±409.50	3457.4 ±502.50	4096.25 ±690.60	4041.45 ±477.50	4757.3 ±680.90	10.45	Mancuso *et al.*, 2015*, Laohakunjit *et al.*, 2007^
47.049	Ethanol	$C_2H_7O^+$	O5	7202.40 ±1560.55	8058.85 ±1720.50	9291.60 ±2055.70	8361.55 ±1677.32	4351.80 ±640.30	3951.22 ±550.35	8931.82 ±1676.40	12561.8 ±2660.33	7981.8 ±860.56	7171.8 ±766.54	3461.8 ±350.56	4351.8 ±378.90	17.72	Mancuso *et al.*, 2015*, Alves and Franco, 2003^
51.042	Methanol hydrate	$CH_7O_2^+$	O6	15.50 ±3.20	16.12 ±1.22	16.48 ±4.05	15.73 ±5.80	15.51 ±3.20	15.73 ±2.50	15.34 ±3.35	15.65 ±3.90	16.84 ±4.60	17.07 ±5.20	16.96 ±3.40	16.95 ±2.40	0.04	
53.039	Cyclobutadiene	$C_4H_5^+$	H5	332.14 ±39.40	237.01 ±55.60	257.60 ±30.80	152.61 ±22.66	248.25 ±19.20	243.31 ±45.77	380.20 ±77.80	275.01 ±55.63	282.65 ±80.55	172.54 ±33.37	203.20 ±42.35	275.90 ±55.50	0.63	Taiti *et al.*, 2016*
55.054	C4 aldehydes fragment	$C_4H_7^+$	H6	369.45 ±40.60	375.35 ±55.75	411.40 ±60.50	501.25 ±101.11	264.75 ±51.77	273.65 ±51.35	285.05 ±61.56	291.40 ±32.35	425.12 ±101.35	402.55 ±95.39	323.30 ±57.80	319.65 ±67.66	0.88	Taiti *et al.*, 2016*
57.033	2-propenal/2E-Hexenal fragment	$C_3H_5O^+$	O7	66.36 ±10.50	105.36 ±16.50	48.96 ±8.70	141.16 ±45.26	92.76 ±25.55	99.06 ±15.26	130.06 ±21.35	39.56 ±5.65	46.36 ±6.65	40.66 ±11.60	98.06 ±12.22	49.16 ±8.65	0.25	
57.070	Alkyl fragment (Hexanol/Hexyl acetate)	$C_4H_9^+$	H7	202.55 ±33.40	105.45 ±18.40	117.65 ±28.20	155.25 ±46.78	78.24 ±10.68	180.26 ±41.55	129.35 ±32.35	135.75 ±35.33	128.92 ±31.64	121.45 ±27.80	208.95 ±32.40	150.35 ±20.55	0.35	Taiti *et al.*, 2016*
59.049	Propanal	$C_3H_7O^+$	O8	2359.24 ±400.60	3366.25 ±450.55	1918.25 ±343.30	875.25 ±77.50	1221.25 ±210.30	2073.26 ±340.56	913.25 ±87.65	1017.25 ±110.35	834.25 ±117.80	1057.25 ±101.35	923.25 ±280.55	1213.25 ±221.35	3.68	Mancuso *et al.*, 2015*
61.028	Acetates	$C_2H_5O_2^+$	O9	1748.44 ±320.35	3818.20 ±960.75	1045.10 ±323.44	899.15 ±22.20	1420.30 ±195.20	1200.13 ±260.90	960.15 ±85.45	810.20 ±55.80	1061.85 ±123.40	912.35 ±45.50	610.00 ±81.00	830.00 ±204.00	3.17	Mancuso *et al.*, 2015*, Alves and Franco, 2003^
65.038	Alkyl fragment	$C_5H_5^+$	H8	5.36 ±2.20	5.63 ±1.23	5.93 ±2.33	5.82 ±2.11	5.28 ±1.09	4.96 ±0.67	5.02 ±1.40	5.09 ±2.05	6.27 ±1.47	6.65 ±2.45	5.33 ±1.11	5.05 ±1.55	0.01	Taiti *et al.*, 2016*
67.054	Alkyl fragment	$C_5H_7^+$	H9	15.55 ±3.40	16.83 ±4.45	18.22 ±3.35	18.10 ±1.10	13.33 ±1.35	13.96 ±1.55	15.52 ±2.50	16.72 ±1.73	21.95 ±5.60	21.66 ±1.55	19.11 ±2.52	19.23 ±2.53	0.04	Taiti *et al.*, 2016*
69.036	Furan	$C_4H_5O^+$	T1	105.85 ±30.30	110.35 ±20.45	114.85 ±23.55	114.24 ±39.70	106.05 ±28.65	106.55 ±32.35	97.05 ±26.40	92.72 ±17.66	109.75 ±23.35	108.10 ±25.60	93.83 ±8.63	89.65 ±18.15	0.26	Taiti *et al.*, 2016*
69.069	Isoprene/1,3-Pentadiene	$C_5H_9^+$	T2	73.94 ±17.40	75.51 ±8.80	84.29 ±10.63	82.15 ±14.67	51.59 ±15.65	53.78 ±8.65	103.35 ±18.15	107.70 ±18.75	89.22 ±5.33	83.99 ±15.13	120.50 ±16.70	120.50 ±25.35	0.22	Taiti *et al.*, 2015c*, Laohakunjit *et al.*, 2007^
71.049	2-butenal/2,3-Dihydrofuran	$C_4H_7O^+$	O10	5220.22 ±900.60	4090.24 ±711.30	3260.23 ±340.52	6120.22 ±1550.90	3920.24 ±365.67	2990.20 ±455.60	3310.24 ±516.32	2520.24 ±336.30	1630.64 ±165.55	1160.66 ±146.44	2720.55 ±406.67	1680.24 ±220.80	7.99	Taiti *et al.*, 2015b*
73.028	Alkyl fragment	$C_3H_5O_2^+$	O11	11.47 ±3.20	15.23 ±3.40	10.56 ±2.22	16.23 ±6.13	8.40 ±2.44	6.67 ±2.45	11.46 ±3.79	17.20 ±5.44	11.23 ±2.50	9.56 ±3.67	8.77 ±2.60	11.09 ±2.22	0.03	

73.064	Isobutanal/Butanone/ Methyl ethyl ketone	$C_4H_9O^+$	O12	95.65 ±10.50	103.25 ±25.60	115.15 ±20.87	115.25 ±35.33	81.47 ±22.27	85.59 ±6.45	76.77 ±11.65	79.55 ±19.66	125.05 ±35.60	121.35 ±22.35	86.49 ±11.45	87.61 ±8.05	0.24	Junker and Blüthgen, 2010*
75.044	Propanoates/ Methyl acetate	$C_3H_7O_2^+$	O13	307.82 ±60.70	313.92 ±75.50	638.62 ±44.83	329.02 ±61.45	202.32 ±32.67	208.82 ±27.60	120.42 ±12.76	122.62 ±17.80	326.32 ±55.66	311.52 ±62.55	436.62 ±92.35	133.12 ±27.22	0.71	Taiti *et al.*, 2015a*, Laohakunjit *et al.*, 2007^
77.038	Alkyl fragment	$C_3H_9O_2^+$	H10	2.82 ±0.50	4.00 ±1.10	3.17 ±0.35	5.59 ±1.20	4.51 ±2.25	2.61 ±0.70	3.93 ±1.25	4.18 ±1.05	5.68 ±1.50	3.40 ±1.11	4.45 ±0.95	5.55 ±1.25	0.01	Taiti *et al.*, 2016*
79.054	Benzene	$C_6H_9^+$	H11	17.16 ±2.10	16.86 ±3.45	18.02 ±4.11	17.66 ±2.56	15.33 ±6.67	15.47 ±3.40	15.01 ±3.20	14.61 ±4.44	20.37 ±5.63	19.31 ±2.50	16.21 ±6.44	15.41 ±5.77	0.04	Alves and Franco, 2003^
81.069	Alkyl fragment (Hexenal/Hexenol/ Terpene fragments)	$C_6H_9^+$	H12	8.56 ±2.50	8.40 ±1.40	8.79 ±0.89	8.98 ±2.67	8.76 ±2.90	9.09 ±0.95	21.94 ±5.95	22.33 ±6.77	14.51 ±3.40	13.53 ±3.26	22.12 ±4.77	22.14 ±7.25	0.03	Taiti *et al.*, 2015b*
83.049	2-Methylfuran	$C_5H_7O^+$	T3	4.36 ±1.10	5.11 ±2.10	5.40 ±1.44	5.62 ±1.95	4.09 ±0.40	4.63 ±1.49	13.43 ±2.50	15.04 ±4.90	8.02 ±1.95	7.73 ±1.26	14.41 ±3.20	15.79 ±2.90	0.02	Mancuso *et al.*, 2015*
83.085	C6 compounds/ Hexenal fragment	$C_6H_{11}^+$	H13	7.41 ±2.25	7.87 ±1.15	9.08 ±0.87	8.68 ±1.11	5.02 ±0.47	5.48 ±1.05	28.66 ±6.95	31.81 ±5.65	9.76 ±2.30	9.29 ±2.45	33.87 ±16.20	34.67 ±7.20	0.04	Taiti *et al.*, 2015c*
85.065	Methyl-butenal	$C_5H_9O^+$	T4	19.76 ±5.60	21.50 ±4.70	25.25 ±6.73	25.28 ±7.26	16.89 ±3.95	17.97 ±6.44	35.66 ±6.32	38.53 ±10.35	32.03 ±7.50	30.17 ±11.26	39.48 ±10.55	40.53 ±7.66	0.07	Taiti *et al.*, 2016*
87.044	γ-Butyrolactone/ 2,3-Butandione	$C_4H_7O_2^+$	O14	168.85 ±30.35	193.95 ±33.50	212.15 ±45.70	340.25 ±70.33	104.95 ±37.50	115.85 ±26.95	73.55 ±10.95	157.95 ±33.20	361.65 ±110.45	260.75 ±57.20	150.65 ±32.35	100.95 ±40.20	0.46	Taiti *et al.*, 2015b*
87.080	2,3-Methylbutanal/ 2-Pentanone	$C_5H_{11}O^+$	O15	117.60 ±20.50	125.05 ±30.60	45.85 ±10.33	145.50 ±57.26	98.41 ±17.80	103.65 ±33.36	69.58 ±13.20	95.05 ±16.32	144.65 ±37.26	78.05 ±18.55	110.45 ±47.50	96.44 ±21.52	0.25	Taiti *et al.*, 2015a*, Alves and Franco, 2003^
89.059	Ethyl acetate/Butanoic acid	$C_4H_9O_2^+$	O16	578.15 ±140.55	401.10 ±55.60	336.12 ±54.92	891.15 ±177.32	464.15 ±167.52	369.15 ±77.66	731.15 ±101.35	597.15 ±73.20	484.15 ±160.30	294.15 ±70.40	417.15 ±65.70	555.11 ±90.85	1.30	Junker and Blüthgen, 2010*, Alves and Franco, 2003^
91.070	2-3-Butanediol/ 1-Methoxy-2-propanol	$C_4H_{11}O_2^+$	O17	13.16 ±3.90	7.02 ±1.20	14.31 ±5.57	8.49 ±2.65	12.74 ±3.47	20.34 ±5.56	14.8 ±2.26	15.27 ±5.26	18.1 ±2.50	5.82 ±1.11	26.03 ±8.44	10.42 ±3.32	0.03	Laohakunjit *et al.*, 2007^
93.069	Alkyl fragment (Terpene fragments)	$C_7H_9^+$	H14	4.41 ±1.45	4.33 ±0.55	4.05 ±0.60	3.93 ±1.83	3.40 ±1.44	3.27 ±0.80	2.88 ±0.84	3.09 ±1.55	3.69 ±0.68	3.48 ±1.44	3.14 ±1.11	3.25 ±0.80	0.01	Taiti *et al.*, 2016*
95.085	Alkyl fragment (Terpene fragments)	$C_7H_{11}^+$	H15	3.98 ±1.10	4.20 ±0.40	3.22 ±1.20	3.98 ±1.05	4.99 ±0.93	3.31 ±0.47	1.87 ±0.11	2.11 ±1.05	4.09 ±1.55	2.12 ±0.26	3.88 ±1.49	3.10 ±0.78	0.01	Junker and Blüthgen, 2010*
99.080	Hexenal/ Methyl-pentenone	$C_6H_{11}O^+$	O18	2.33 ±0.55	2.28 ±0.30	2.59 ±0.44	6.58 ±0.60	2.05 ±0.45	2.28 ±0.40	10.34 ±2.50	4.55 ±1.44	3.38 ±1.11	3.15 ±1.05	4.75 ±0.87	4.92 ±1.60	0.01	Taiti *et al.*, 2015b*, Pino *et al.*, 2005^
101.059	2,3-Pentanedione	$C_5H_9O_2^+$	O19	8.88 ±2.25	9.75 ±2.50	11.27 ±2.80	11.53 ±3.20	8.20 ±2.22	8.72 ±3.50	11.85 ±3.40	12.84 ±4.57	16.63 ±4.46	16.07 ±2.26	15.8 ±3.25	16.86 ±4.74	0.03	Pino *et al.*, 2005^
101.096	Hexanal/2-Hexenol/ 3-Hexen-1-ol	$C_6H_{13}O^+$	O20	5.16 ±1.20	5.60 ±2.40	6.40 ±0.60	6.47 ±0.80	4.66 ±1.56	5.09 ±1.05	6.57 ±2.33	7.27 ±2.23	8.44 ±0.95	8.26 ±2.76	8.18 ±2.90	8.78 ±0.80	0.02	Taiti *et al.*, 2015b*, Alves and Franco, 2003^
103.075	2-Methylbutanoic acid/ Methyl butanoate/Valeric acid	$C_5H_{11}O_2^+$	O21	20.14 ±4.30	10.49 ±3.45	12.31 ±3.35	22.35 ±3.45	10.46 ±4.66	13.56 ±5.60	24.62 ±10.44	30.87 ±9.50	16.63 ±4.43	21.35 ±6.30	25.87 ±6.67	27.16 ±3.32	0.05	Alves and Franco, 2003^
107.049	Benzaldehyde	$C_7H_7O^+$	O22	7.78 ±2.90	7.96 ±2.25	8.41 ±0.55	8.22 ±0.65	6.87 ±0.95	6.93 ±1.91	5.95 ±2.10	5.92 ±1.15	9.58 ±3.44	9.16 ±2.26	6.59 ±1.87	6.61 ±2.30	0.02	Taiti *et al.*, 2015b*, Alves and Franco, 2003^
109.076	2,5-Dimethylpyrazine	CHN^+	H16	1.72 ±0.55	1.88 ±0.35	2.05 ±0.25	1.99 ±0.80	1.51 ±0.15	1.56 ±0.20	3.35 ±1.05	3.75 ±0.50	2.39 ±1.03	2.45 ±0.75	4.09 ±0.80	4.23 ±1.25	0.01	Taiti *et al.*, 2015c*, Pino *et al.*, 2005^
111.082	2-Propylfuran	$C_7H_{11}O^+$	O23	1.59 ±0.40	1.70 ±0.45	1.77 ±0.65	1.73 ±0.43	1.51 ±0.44	1.59 ±0.50	2.63 ±0.40	2.94 ±1.10	2.31 ±0.55	2.17 ±0.35	3.01 ±0.47	3.16 ±0.53	0.01	Taiti *et al.*, 2016*
121.065	Phenylacetaldehyde/ Acetophenone	$C_8H_9O^+$	O24	2.80 ±0.50	1.50 ±0.30	2.20 ±0.40	2.03 ±0.55	1.75 ±0.30	1.84 ±0.44	1.99 ±0.60	1.93 ±0.55	2.33 ±0.45	2.25 ±0.45	2.14 ±0.30	1.68 ±0.50	0.01	Taiti *et al.*, 2015c*, Alves and Franco, 2003^
127.040	Methyl-2-Furoate/ 3-hydroxy-2-pyranone	$C_6H_7O_3^+$	T5	2.16 ±0.30	1.20 ±0.25	1.33 ±0.30	1.26 ±0.35	2.34 ±0.80	1.10 ±0.20	2.27 ±0.40	2.40 ±0.55	1.33 ±0.20	1.33 ±0.20	2.63 ±0.65	2.71 ±0.50	0.00	Taiti *et al.*, 2015c*, Pino *et al.*, 2005^
131.110	C7 ester/ Isopentyl acetate	$C_7H_{15}O_2^+$	O25	2.38 ±0.30	1.60 ±0.25	1.71 ±0.30	3.81 ±1.10	1.59 ±0.30	1.71 ±0.65	2.23 ±0.80	2.38 ±1.10	2.25 ±0.40	2.19 ±0.15	2.61 ±0.45	2.65 ±0.50	0.01	Mancuso *et al.*, 2015*, Alves and Franco, 2003^
135.117	p-Cymene	$C_{10}H_{15}^+$	T6	0	1.09 ±0.20	0	0	0	0	0	1.11 ±0.10	0	0	0	2.16 ±0.40	0.00	Taiti *et al.*, 2015c*, Pino *et al.*, 2005^
137.137	Monoterpenes	$C_{10}H_{17}^+$	T7	2.12 ±0.12	1.33 ±0.20	1.22 ±0.10	0	1.10 ±0.05	1.12 ±0.11	2.00 ±0.25	1.24 ±0.20	1.22 ±0.15	1.50 ±0.10	1.33 ±0.25	1.35 ±0.05	0.00	Mancuso *et al.*, 2015*, Baietto and Wilson, 2015^
143.107	2,5-Dimethyl-4-methoxy-3-furanone	$C_7H_{11}O_3^+$	O26	0	1.13 ±0.05	0	0	0	0	0	1.50 ±0.10	0	0	2.30 ±0.25	1.25 ±0.11	0.00	Taiti *et al.*, 2015c*, Pino et al., 2005^
153.126	Terpenoid-like compound	$C_{10}H_{17}O^+$	T8	1.55 ±0.34	1.35 ±0.54	1.45 ±0.15	0	1.32 ±0.20	0	1.37 ±0.25	1.49 ±0.30	1.21 ±0.11	0	0	1.54 ±0.14	0.00	Taiti *et al.*, 2015c*

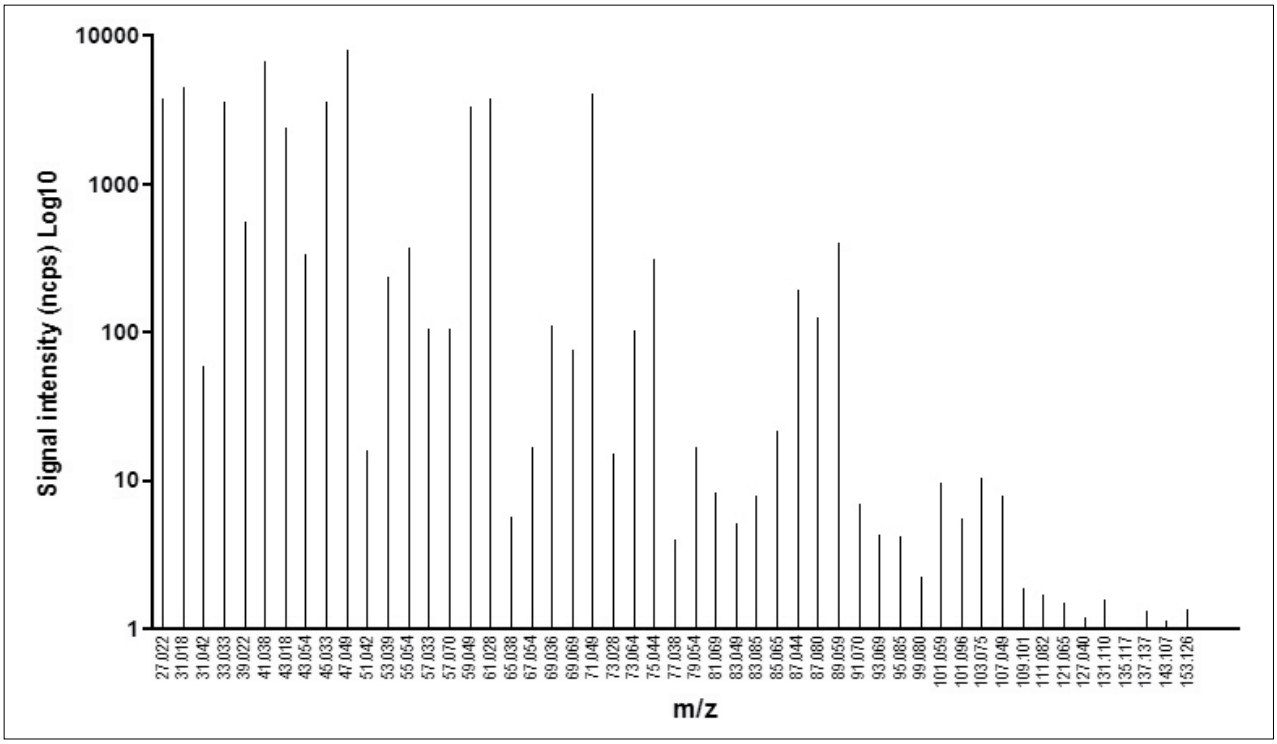

Figure 1. Example of a typical PTR-TOFMS mass spectrum obtained by volatile analysis of one lucuma fruit.

literature. The most recent version of PTRMS-TOF allows a higher mass resolution (Taiti *et al*., 2015a) whereby, for all signals identified, all the m/z were tentatively assigned to the mass formulas reported and after compared with previous knowledge of the VOCs emitted by other fruits or detected in other PTR studies.

The most abundant signals (>1% of the total) found in lucuma fruits were: 27.022 (Tentative identification: Acetylene), 31.018 (TI: Formaldehyde), 33.033 (Methanol), 39.022 (TI: Isoprene fragments), 41.038 (TI: Alkyl fragment), 43.018 (TI: Alkyl fragment), 45.033 (TI: Acetaldehyde), 47.049 (TI: Ethanol), 59.049 (TI: Propanal), 61.028 (TI: Acetates), 71.049 (TI: 2-Butanal) and 89.059 (TI: Ethyl acetate). Other identified compounds, instead, showed a signal intensity between 0.1 and 0.9% of the total, such as: 31.042 (TI: Methylamide), 43.052 (TI: Alkyl fragment), 53.026 (Cyclobutadiene), 55.054 (TI: C_4 aldehydes fragment), 57.033 (TI: 2-Propenal), 57.070 (TI: Alkyl fragment), 69.033 (TI: Furan), 69.069 (TI: Isoprene), 73.064 (TI: Butanone), 75.044 (TI: Methylacetate), 87.044 (TI: γ-Butyrolactone) and 87.080 (TI: 2-Pentanone).

By our experience lucuma fruit does not have easily identifiable flavor impact compounds, but all the compounds listed above may play an important role on the overall aroma of this fruit. VOCs are generally biosynthesized from amino acids, membrane lipids and carbohydrates (Sanz *et al*., 1996) and the availability of primary precursor substrates regulates the amount and composition of VOCs production (El Hadi *et al*., 2013). Fatty acids and amino acids are the most precursors of aroma volatiles in most of the fruits (Sanz *et al*., 1996). Moreover, the fatty acid-derived straight chain alcohols, aldehydes, ketones, acids, esters and lactones are important aroma compounds that are responsible for fresh fruit flavors (El Hadi *et al*., 2013). Considering the high presence of fatty acids in lucuma (Rojo *et al*., 2010), it could be assumed that many of the identified compounds (Table 2) were generated through this pathway.

Comparing the lucuma fruit spectrum obtained by PTRMS TOF tool with other tropical fruits, such as mango, avocado, banana and mangosteen (Taiti *et al*., 2015a; Mayr *et al*., 2002; White *et al*., 2016), it was possible to observe the two following points: 1) the significantly reduced or almost absent signals of some compounds commonly identified in several fruits, among which monoterpenes (m/z = 137), terpenoids (153) and sesquiterpenes (205); 2) the low signal intensity over 100 and the high signal intensity <100 as for acetylene (m/z = 27), formaldehyde (31), methanol (33), acetaldehyde (45) and ethanol (47).

Missing volatile compounds

Interestingly, lucuma fruit did not show emission of hemiterpenes (C5), monoterpenes (C10) and sesquiterpenes (C15) whereas it is known that in many fruits (*i.e.*, mango, strawberry, citrus, etc.) these compounds are the most representative type of volatiles in the aroma profile and are also the key compounds determining the characteristic aroma (El Hadi *et al*., 2013). In fact, fruits are generally rich in terpenes that determine their specific bouquet and can attract or repel the favorable or antagonist animals respectively (Junker and Blüthgen, 2010). For example, in mango fruits terpenes are considered to be important contributors to the flavor (Taiti *et al*., 2015a). On the contrary, in other fruits the flavor notes are due to other compounds, even those not present in lucuma, such as: isoamyl acetate, isobutyl acetate, (E)-2-hexenal, hexanal in banana (El Hadi *et al*., 2013); octenoic acid, methyl ester, hexanoic acid, octanoic acid and ethyl ester in pineapple (Zhang *et al*., 2009); methyl and ethyl butanoate, (E)-2-hexenal, hexanal, 3-hexenol, and methyl benzoate in kiwifruit (Carcia *et al*., 2013); ethanol, (Z)-3-hexanol and (E)-2-hexenal in avocado (El Hadi *et al*., 2013); (Z)-3-hexenal and hexanal together with monoterpenes compounds in guava fruit (Egea *et al*., 2014).

Low signal intensity compounds

The low intensity of peaks detected at m/z > 100 can be explained by the limited presence of molecules with high molecular weight, such as the above mentioned monoterpenes (m/z = 137), terpenoids (153), sesquiterpenes (205), isopentyl acetate (131) etc., which are generally high in several fruits (Taiti *et al*., 2015a; El Hadi *et al*., 2013; Junker and Blüthgen, 2010). On the other hand, some esters and alcohol detected at m/z < 100 (Table 1) probably represent important molecules for the aroma determination of lucuma fruit. These molecules can be the key contributors to the fruity aroma, as already observed in some apple or fig fruits (Rodríguez *et al*., 2013). Volatile esters, mainly acetate derived compounds as methyl acetate (m/z = 75.044), ethyl acetate (89.059) and 2-pentanone (87.080), represent more than 2% of the total VOCs amount detected, while the short-chain alcohols, as methanol and ethanol, represent 10 and 16%, respectively (Table 2). Methyl acetate, ethyl acetate and 2-pentanone give respectively the fermented, fruity and ethereal flavor notes (Rodríguez *et al*., 2013); meanwhile the high intensity of signals recorded at m/z = 33.033 (methanol), 45.033 (acetaldehyde), 47.049 (ethanol), at least partially, can be related to the ripening process (Taiti *et al*., 2015a). During the normal ripening process acetaldehyde, methanol and ethanol are produced as a result of aerobic respiration; the final result of the respiratory metabolism is the production of pyruvate which is firstly decarboxylated to acetaldehyde and finally converted into ethanol (El Hadi *et al*., 2013). The obtained values are in line with the ripening level of the fruit evaluated by colorimetric observation. In addition, this is also confirmed by the low values (Table 2) obtained for some alcohols as hexanal (m/z = 101.096), hexenal (99.080) and benzaldehyde (107.049). Alcohols detected at m/z = 101 and 99 are important contributors to the green-leafy topnote in fresh fruit flavors; *i.e.*, trans-2-Hexenol has a more desirable sweet, fruity flavor (Bugaud and Alter, 2016), while benzaldehyde is known for its characteristic almond flavor note (Yahia, 2004). Despite the various ripening stages (4 or 5, see Table 1) of the fruits, the VOC composition and quantification did not show any difference between the analyzed samples.

Conclusion

Fruit aroma is known as an important indicator that reflects the quality of fruit flavor and determines its acceptance by the consumer. Before this study, no in-depth research on the role of VOCs on the perception of lucuma flavor had been conducted. In this research we used the great potential of PTRMS TOF for the rapid and non-destructive monitoring of volatile compounds emitted by fresh lucuma fruits. More than 50 aromatic compounds were found in "ready to eat" fruits of lucuma. The entire dataset obtained seems to show that the aroma of lucuma fruit is due in large part to the presence of short-chain alcohols (methanol, acetaldehyde, ethanol) but also of volatile esters, aldehydes and hydrocarbons that compose the complex mixture of volatile aroma.

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