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ORIGINAL ARTICLE

Identification of volatile compounds in cured Mexican vanilla (*Vanilla planifolia* G. Jackson) beans using headspace solid-phase microextraction with gas chromatography-mass spectrometry

Sabik Hassan[1], Pérez-Silva Araceli[2,*], Bélanger Denis[1], Vivar-Vera María de los Ángeles[2], Nicolás-García Mayra[2] and Reyes-López Delfino[3]

[1] Food Research and Development Centre, Agriculture and Agri-Food Canada, Saint-Hyacinthe, Quebec J2S 8E3, Canada
[2] Instituto Tecnológico de Tuxtepec, Av. Dr. Víctor Bravo Ahuja s/n, Col. 5 de Mayo, C.P. 68350, Tuxtepec, Oaxaca, Mexico
[3] Escuela de Ingeniería Agrohidráulica, Benemérita Universidad Autónoma de Puebla. Domicilio conocido, San Juan Acateno, Tezuitlán, Puebla Pue, Mexico

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Abstract – Introduction. Headspace solid-phase microextraction combined with gas chromatography–mass spectrometry (HS–SPME–GC/MS) was applied to identify volatile compounds in cured vanilla (*Vanilla planifolia* G. Jackson) beans originating from the state of Veracruz, Mexico. **Materials and methods.** A 6 kg batch from the 2012 production year was used in the study. Carboxen/polydimethylsiloxane (85 μm) fibre was selected because it had shown a high capacity to extract detected volatiles. **Results and discussion.** A total of 81 volatile compounds were detected, of which 77 were identified by comparing their mass spectra and retention times, as well as their Kovats retention indices, with those of injected standards and/or by searching the National Institute of Standards and Technology's Mass Spectral Library database. Of those compounds, 21 shikimate derivatives, 14 terpenes/cadinenes, 12 furan derivatives, 6 esters, 7 acids, 4 ketones, 5 aldehydes, 4 hydrocarbons, 3 alcohols and 1 pyrrole were identified. **Conclusion.** A total of 31 volatiles have already been reported as aroma-active compounds in cured beans of *Vanilla planifolia* and *Vanilla tahitensis*. However, to our knowledge, 11 aromatic compounds found in this study have not previously been detected in vanilla beans.

Keywords: Mexico / vanilla / *Vanilla planifolia* / aroma / volatile compounds

Résumé – Identification des composés volatils des gousses de vanille mexicaine séchées (*Vanilla planifolia* G. Jackson) par HS-SPME–GC/MS. Introduction. La micro-extraction par surface de tête en phase solide couplée avec chromatographie en phase gazeuse et spectrométrie de masse (HS–SPME–GC/MS) a été appliquée pour identifier les composés volatils des gousses de vanille séchées (*Vanilla planifolia* G. Jackson) provenant de l'État de Veracruz, au Mexique. **Matériel et méthodes.** Un lot de 6 kg issu de la production de l'année 2012 a été utilisé dans l'étude. Une fibre en carboxy-polydiméthylsiloxane (85 μm) a été choisie en raison de sa grande capacité d'extraction des composés volatils détectés. **Résultats et discussion.** Un total de 81 composés volatils ont été détectés, dont 77 ont été identifiés en comparant leurs spectres de masse et leurs temps de rétention, ainsi que leurs indices de rétention Kovats, avec ceux des standards injectés et/ou en recherchant dans la base de données de la bibliothèque de l'Institut National des Références et de Technologie en Masse Spectrale. Parmi ces composés, ont été identifiés 21 dérivés shikimate, 14 terpènes/cadinènes, 12 dérivés du furanne, 6 esters, 7 acides, 4 cétones, 5 aldéhydes, 4 hydrocarbures, 3 alcools et 1 pyrrole. **Conclusion.** Un total de 31 volatils avaient déjà été rapportés comme composés aromatiques actifs dans les gousses séchées de *Vanilla planifolia* et *Vanilla tahitensis*. Cependant, à notre connaissance, 11 composés aromatiques présents dans cette étude n'avaient jamais encore été détectés dans les gousses de vanille.

Mots clés : Mexique / vanille / *Vanilla planifolia* / composé d'arôme / composé volatil

* Corresponding author: apsilva30@hotmail.com

1 Introduction

The fruit of the orchid *Vanilla planifolia* was first used in Mexico by the Aztecs to scent their temples and flavor their drinks. Historically, the Aztecs protected the secrets of the production of domesticated vanilla to flavor chocolate; it was also used to pay tribute to the emperor and the gods. The history of vanilla is full of traditions and it has been valued throughout the centuries for culinary, medicinal and religious reasons. It is currently the second most expensive spice after saffron [1]. The use of vanilla became familiar during the conquest of Mexico by the Spaniards; the plant was spread throughout the world and is now grown in tropical regions such as Madagascar, Papua New Guinea, Uganda, Indonesia, India, Tahiti, the Comoro Islands, Mayotte, Reunion Island and Mexico [2]. Although Mexico is no longer the leading producer of vanilla, it is its ancestral home and one of the most important centers of genetic diversity of this appreciated orchid [3]. Its cultivation persists to date as a source of income for approximately 4,000 rural families, and is part of the culture and history of the Totonacs [4–6]. Global demand for cured vanilla currently stands at about 4,000 tons per year, of which only half is produced; this shortage has resulted in an increase in price. In 2015, the cost of quality gourmet vanilla beans reached $ 250 kg^{-1} [7]. Madagascar and Indonesia together supply about 80% [8] while Mexico ranks seventh, with 4% of the world production.

The aroma "vanilla" is one of the most commonly used flavors in the food industry [9], particularly preferred by consumers of dairy products, confectionery products and pharmaceutical formulations [10, 11]. However, only 1% of consumer products are flavored with natural vanilla [9, 12]; other aromatized "vanilla" products are flavored with synthetic vanillin [13], *Vanilla planifolia* being the species with the highest content of vanillin. However, the exquisite natural aroma of vanilla is very complex and is due to a complex mixture of different volatile compounds, not only to the presence of vanillin [14–19]. On the other hand, the aroma of cured vanilla beans may vary depending on various factors such as the species [20], maturity of the pods [12, 21], place and production conditions [13, 22], curing process [13, 15, 23, 24], and method of analysis [25, 26]. In this latter aspect, the complex aroma of vanilla has been studied mainly by analyzing alcoholic extracts and cured vanilla beans using different extraction and analytical methods, including thin-layer chromatography [27], high-performance liquid chromatography [28], liquid chromatography-mass spectrometry [29], gas chromatography-mass spectrometry [30], micellar electrokinetic chromatography [31] and nuclear magnetic resonance [32]. Most of these methods were extensively described by Sinha *et al.* [26]. Various studies of flavors have led to the identification of more than 450 volatile compounds in samples of vanilla (*Vanilla planifolia*) from different parts of the world [14, 17, 18, 27, 30, 33–40]. Phenolic volatiles, namely vanillin, vanillic acid, *p*-hydroxybenzaldehyde and *p*-hydroxybenzoic acid, are the major components in *V. planifolia*. However, several studies have recently demonstrated the importance of other flavors in cured vanilla beans using gas chromatography coupled to a sniffing port (gas chromatography-olfactometry, (GC-O)). Pérez-Silva *et al.* [14] first reported the presence of 26 odor-active compounds in an organic extract (pentane/diethyl ether, 1:1) prepared from 500 mg of *V. planifolia* powder from Mexico. This work highlighted the contribution of other compounds present at low levels to the key aroma of vanilla. Subsequently, Zhang and Mueller [17] identified 78 flavors in an organic extract (dichloromethane) of vanilla from Madagascar and Uganda. Takahashi *et al.* [18] reported 17 flavors in an organic extract (diethyl ether) using 30 g of vanilla powder from Madagascar, whereas Brunschwig *et al.* [16] reported 38 compounds in extracts obtained by micro-simultaneous distillation-extraction (micro-SDE) from three different cultivars of *V. tahitensis*, which is the second most commercially important species of vanilla. Another study conducted by Takahashi *et al.* [19] reported the detection of 20 Tahitian vanilla flavors in organic extracts.

Most studies on the identification of volatiles in vanilla extracts used mainly large quantities of organic solvents as well as laborious extraction techniques. Moreover, their recoveries in solvent extracts were not always exhaustive, as not all volatiles were extracted, particularly at low levels. The best extraction and analytical techniques for food flavor analysis should not adulterate samples in any way. The solid-phase microextraction (SPME) technique fits the criteria for food industry flavor analysis, as SPME is solvent-free, low cost, easy to use and relatively fast, yet it is sensitive enough for quality control purposes and does not adulterate samples at suitable extraction temperatures [41–47]. A few studies have analyzed vanilla flavors using SPME combined with GC/MS (SPME–GC/MS), particularly for the determination of the quality and authenticity of the alcoholic extracts of vanilla [25, 29, 48] and the evaluation of vanilla essential oils [49], but no studies have focused on the identification of flavors in cured vanilla bean pods. Our laboratory has developed methods to optimize the analysis of volatile compounds by headspace SPME (HS–SPME) combined with GC-MS (HS–SPME–GC/MS) that will be adapted to cured vanilla beans.

The objective of the present study was to establish a HS–SPME–GC/MS method as well as to identify volatile components in cured vanilla (*V. planifolia*) bean pods originating from the state of Veracruz, Mexico.

2 Materials and methods

2.1 Chemicals and reagents

Reagents and 26 pure commercial standards (benzaldehyde, *p*-cresol, guaiacol, *p*-creosol, salicylic acid methyl ester, *p*-anisaldehyde, *p*-anisyl alcohol, (*Z*)-cinnamic acid methyl ester, *p*-hydroxybenzaldehyde, vanillin, *p*-cymene, d-limonene, α-terpinene, furfural, γ-butyrolactone, 5-methylfurfural, 2-pentylfuran, hexanal, heptanal, safranal, 3-methylbutanoic acid, pentanoic acid, hexanoic acid, hexanoic acid methyl ester, nonanoic acid methyl ester and 1-pentanol) were purchased from Sigma-Aldrich (Oakville, ON, Canada). Methanol was purchased from Burdick & Jackson and supplied by VWR International (Toronto, ON, Canada).

2.2 Preparation of standards

Individual stock solutions of each commercial standard were prepared in 15-mL screw-top amber vials (Supelco, Oakville, ON, Canada) as follows. Pre-sterilized tips for use with Rainin Instrument POSD pipettes (Oakland, CA, USA) were used for distributing the standards. A 10-μL amount of each commercial standard was individually diluted in 10 mL of methanol (Solution 1). A 1-mL amount of Solution 1 was then diluted ten-fold individually and as a group in methanol (Solutions 2 and 3, respectively). A 10-μL amount of Solutions 2 and 3 were separately diluted in 1-mL of NaCl solution (6 M) and then analyzed by HS–SPME–GC/MS.

2.3 Sampling and sample preparation

A 6-kg batch of cured vanilla (*V. planifolia* G. Jackson) bean pods was selected from Gutiérrez Zamora, in the state of Veracruz, Mexico. The batch was from the 2012 production year. The moisture percentage and vanillin content of vanilla samples were 34% and 1.9% dry weight, respectively.

Then, the cured vanilla beans (6 kg) were cut lengthwise and crosswise, using scissors, into pieces of about 0.5 cm (430 g). The samples were stored in the dark at 4 °C until analysis. A 1-g sample of homogenized cured vanilla beans was weighed into a 10-mL screw-top headspace amber vial, and the vial was sealed with a stainless steel (magnetic) screw cap containing a polytetrafluoroethylene (PTFE)/silicone septum (Varian, Mississauga, ON, Canada). Powder samples were prepared by first freezing cut vanilla in liquid nitrogen and then slowly adding the frozen cut vanilla to a centrifugal grinding mill (Retsch LB-49; Newtown, PA, USA) operated at 10,000 rpm for 5 min using a 0.5- or 1.0-mm sieve in order to obtain powders of the desired particle sizes.

2.4 HS–SPME–GC/MS analyses

The HS–SPME sampling was carried out using an automated multipurpose sampler (MPS2; Gerstel, Baltimore, MD, USA). Three fibres, namely Carboxen/polydimethylsiloxane (CAR/PDMS, 85 μm), Carbowax/divinylbenzene (CW/DVB, 65 μm) and polyacrylate (PA, 85 μm), purchased from Supelco, were tested at different extraction temperatures (40, 50 or 60 °C) and times (20, 30 or 40 min).

The analyses were performed using a Varian model 3800 GC system (Palo Alto, CA, USA) fitted with a 1078/1079 split/splitless injector (SPME glass insert, 0.8 mm ID; Varian, Mississauga, ON, Canada) suitable for HS–SPME analysis, along with a Saturn 2000 mass spectrometry (MS) system (Varian, Palo Alto, CA, USA). Helium was used as the carrier gas, with a constant flow rate of 1 mL min^{-1}. The components were separated on a VF-5MS capillary column (5% phenyl/ 95% dimethylpolysiloxane, equivalent to a DB-5MS; Varian, Mississauga, ON, Canada) measuring 30 m × 0.25 mm with a film thickness of 0.25 μm. Targeted compounds were desorbed for 9 min by directly inserting the fibre into the injection port of the gas chromatography (GC) unit, which was operated in splitless mode for 3 min at 300 °C. The oven temperature program began with 3 min at 35 °C, followed by a 6 °C/min increase to 280 °C, and then 5 min at 280 °C. Detection was carried out by MS on the total ion current obtained by electron impact at 70 eV. The mass range acquisition was m/z 30–400. The best analytical conditions were determined using real cured vanilla bean samples. However, 25 standards were injected to determine their Kovats retention indices. Volatiles were identified on the basis of their retention times when standards were available, as well as by means of searches of the 2005 version of the National Institute of Standards and Technology's Mass Spectral Library database. Otherwise, they were identified on the basis of their linear retention indices as well as electron ionization mass spectra from the literature or from reference compounds. The linear retention index was calculated using *n*-alkanes (C5–C20) as a reference.

3 Results and discussion

3.1 Extraction and HS–SPME–GC/MS conditions

The concentration of analytes in the headspace depends in general on several factors: (1) the concentration in the original sample; (2) the volatility of the compound; (3) the solubility of that compound in the sample matrix; (4) the temperature of the sample; and (5) a combination of the size of the vial and the time that the sample has been in it [50].

To establish the HS–SPME–GC/MS method, the following steps were performed: (1) a choice was made between cut and ground vanilla samples; and (2) the fibre and the HS–SPME–GC/MS parameters were selected.

3.1.1 Selection of the sample preparation method

Three different samples were prepared (1 g each, in triplicate) as follows: the first sample was cut vanilla, and the second and third samples were ground vanilla prepared using 0.5-mm and 1.0-mm sieves, respectively. The powdered vanilla samples were analyzed by themselves and also with the addition of 1 mL water or 1 mL of NaCl solution (6 N). All trials were done using CAR/PDMS fibre under the following conditions: 1 g sample, extraction temperature of 50 °C, extraction time of 30 min and desorption time of 3 min.

The results did not show any significant differences among the sample preparation methods in terms of sensitivity and selectivity for detected volatiles (results not shown), and thus cut vanilla samples were chosen because of their ease of preparation.

3.1.2 Selection of SPME fibre

Based on their capacities to show broad retention over a wide range of polarities, three types of fibres were selected for study: CAR/PDMS (85 μm), CW/DVB (65 μm) and PA (85 μm). All trials were done using the following conditions: 1 g of cut cured vanilla beans, extraction temperature of 50 °C,

extraction time of 30 min and desorption time of 3 min. Because of the very high percentage of vanillin (85%) in relation to the total amount (surface area) of volatiles detected in cured vanilla beans from Papantla, Veracruz, Mexico [14], the total number of volatiles detected was used instead of their total amount (surface area) to select the SPME fibre. Thus, 81, 70 and 78 volatile compounds were detected when using CAR/PDMS, CW/DVB and PA fibres, respectively. The majority of compounds detected using CW/DVB and PA fibres were also detected using CAR/PDMS fibre; however, the surface areas of their related peaks were different for each fibre. In terms of sensitivity and selectivity, CAR/PDMS fibre provided better results than the others for volatile components and was thus selected for the present study. This finding concurred with those of other studies on foodstuffs [51–53]. In fact, this fibre showed the greatest capacity to extract chemical compounds with a broad spectrum of polarities and molar masses [53].

3.1.3 Selection of HS–SPME–GC/MS parameters

The temperature of extraction and the time of extraction and desorption were reported previously to be the most significant factors in HS–SPME–GC/MS analysis of flavor compounds. Factors modifying the matrix can also influence the sensitivity of the fibre extraction. The addition of a salt such as NaCl improves the effectiveness of the extraction by decreasing the solubility of the analytes (the phenomenon of salting out) in an aqueous sample [54]. The pH can also modify the matrix; for example, the use of a 0.1 M phosphate buffer, with a pH lower than the pK_a of the acids involved, decreases the solubility of the acids and renders them more volatile. Finally, sample agitation reduces the extraction time and generally improves extraction efficiency [55]. These parameters were all considered in a previous study [43] and were taken into account in the present one as well. Extraction temperatures of 40, 50 and 60 °C and extraction times of 20, 30 and 40 min were tested in all combinations using CAR/PDMS fibres. A low extraction temperature (*e.g.*, 40 °C) promotes the detection of less polar compounds, which come out of the column before 10 min. In contrast, a high extraction temperature (*e.g.*, 60 °C) promotes the detection of more polar compounds, which come out of the column after 10 min, specifically vanillin. *Figure 1* shows the extraction percentages of vanillin using CAR/PDMS fibre at different temperatures and extraction times. The lowest percentage was obtained at 40 °C 20 min^{-1} (10%), and the highest percentage was obtained at 60 °C 40 min^{-1} (100% of the scale).

A high percentage of vanillin saturates the detector [14, 17]. Of all the extraction conditions tested, the best were 40 °C for 20 min^{-1} using CAR/PDMS fibre (see *figure 2*). This choice was a compromise between the more volatile (less polar) products that come out of the column before 10 min and the more polar products, especially vanillin, that come out after 10 min. Indeed, increases in the time and temperature of extraction will help increase the intensity of vanillin. This increase in vanillin intensity has two drawbacks if one is interested in detecting as many volatile components as possible: less polar components that are present in vanilla in very small percentages may not be detected, and the high level of vanillin could contaminate the system and thus would still be detected in the subsequent blank samples (the carry-over phenomenon).

Matrix preparation and sample volume/weight can also strongly influence the adsorption of analytes onto the SPME fibre. At higher values for each of these parameters, reverse diffusion of analytes could occur from the fibre to the sample, resulting in a reduction of the fibre's capacity to adsorb the analytes [56]. A series of tests were carried out on the selected fibre in cut cured vanilla beans with and without the addition of 1 mL of MilliQ water or NaCl solution (6 M). These treatments evaluated the influence of the addition of water or salt on the migration of the analytes from the matrix to the headspace. Neither agitation nor the addition of MilliQ water or NaCl solution (6 M) produced any improvement in percentage recoveries for the majority of volatiles (results not shown). A 1-g mass of cured vanilla beans was sufficient to allow detection of targeted compounds; no significant increase in sensitivity was observed when the sample weight was increased to 2 or even 3 g. The desorption time was set at 9 min, as a shorter time was not sufficient to completely desorb some analytes, particularly vanillin. All trials conducted for method development and validation used 1 g cut cured vanilla beans, CAR/PDMS fibre (85 μm), an extraction temperature of 40 °C, an extraction time of 20 min, and a desorption time of 9 min. The detection limit (DL) was assumed to be less than or equal to three times the signal/noise (S/N) ratio (DL ≤ 3 S/N). The quantification limit (QL) was assumed to be less than or equal to 10 times the S/N (QL ≤ 10 S/N).

The repeatability of extraction by CAR/PDMS fibre was measured with six samples of cured vanilla beans (1 g cut vanilla) under the conditions established above. Coefficients of variation (CV) ranged from 2.2% to 21.9% (see *table I*).

3.2 Identification of volatile components

The analysis of cured vanilla bean samples using HS–SPME–GC/MS detected 81 volatiles, of which 77 were identified. Of those compounds, 21 were shikimate derivatives, 14 were terpenes/cadinenes, 12 were furan derivatives, 6 were esters, 7 were acids, 4 were ketones, 5 were aldehydes, 4 were hydrocarbons, 3 were alcohols and 1 was a pyrrole. A total of 31 volatiles have already been reported as aroma-active compounds in cured beans of *V. planifolia* and *V. tahitensis* (*table I*). However, to our knowledge, 11 aromatic compounds found in this study have not previously been detected in vanilla beans.

3.2.1 Shikimate derivatives (21)

Shikimate derivatives were found to be the most abundant family and their total relative surface area was over 50%, 48% of which was related to vanillin. This family comprised aldehydes (benzaldehyde, *p*-anisaldehyde, *p*-hydroxybenzaldehyde and vanillin), alcohols (benzyl alcohol, *p*-cresol, guaiacol, *p*-creosol, *p*-anisyl alcohol and eugenol), esters (salicylic acid methyl ester, (*Z*)-cinnamic acid methyl ester, (*E*)-cinnamic acid methyl ester, vanillic acid methyl ester and benzoic acid phenylmethyl ester), ethers (veratrole,

Table I. Volatile compounds identified in vanilla beans by HS–SPME–GC/MS at 40 °C 20 min^{-1} using CAR/PDMS fibre.

Compounds[a]	RSA (%)[b]	CV[c]	Kovats RI[d] **(Exp.) (Lit.)**	ID[e]	Odor descriptors[f] and references
Shikimate derivatives [21]					
o-Cresol methyl ether[g]	0.10	13.7	(946) (983)	MS, RI	
Benzaldehyde	0.63	13.3	(956) (956)	MS, RI, STD	Sweet aromatic, spicy, bitter almond and dark, cherry-like [3]
Benzyl alcohol	0.33	17.6	(1026) (1021)	MS, RI	Chemical, fruity with balsamic nuances [3]
p-Cresol methyl ether	0.24	16.1	(1035) (1032)	MS, RI	Plastic, ether [2]
p−Cresol	0.78	5.7	(1071) (1075)	MS, RI, STD	Balsamic, wood, spicy, animal, phenolic [1–3]
Guaiacol	3.00	3.9	(1087) (1089)	MS, RI, STD	Chemical, sweet, smoky, aromatic, phenolic, medicinal [1–4]
1,2-Dimethoxybenzene (*veratrole*)	0.14	4.1	(1145) (1150)	MS, RI	Aromatic, somewhat phenolic, medicinal, slightly [3]
p-Creosol	0.50	4.6	(1189) (1202)	MS, RI, STD	Smoky, powerful cresylic [2, 3]
Salicylic acid methyl ester	0.91	4.8	(1193) (1190)	MS, RI, STD	Chalk, medicinal, phenolic, sweet, characteristic wintergreen [1, 3]
p-Anisaldehyde	0.08	7.7	(1257) (1251)	MS, RI, STD	Anise-like, almond, sweet, herbaceous-spicy, creamy, raspberry-like [2, 3, 5]
p-Anisyl alcohol	0.04	2.2	(1302)	MS, STD	Herbal, anise-like, sweet aromatic, balsamic, caramel, nutty, floral [1–3, 5]
(*Z*)-Cinnamic acid methyl ester	0.09	20.0	(1309) (1312)	MS, RI, STD	Sweet, fruity, balsamic, strawberry-like, cinnamon-like [1, 3, 4]
Eugenol	0.05	10.0	(1354) (1357)	MS, RI	Clove-live, spicy [3–5]
(*E*)-Cinnamic acid methyl ester	0.09	12.0	(1390) (1379)	MS, RI	Fruity, balsamic, strawberry-like, cinnamon-like [2–5]
p-Hydroxybenzaldehyde[h]	0.03	9.3	(1391)	MS, STD	Vanilla-like, biscuit [1]
Vanillin	48.02	7.1	(1406) (1393)	MS, RI, STD	Vanilla, sweet, creamy [1, 3–5]
Isovanillin	0.12	12.7	(1440) (1392)	MS, RI	Phenol, medicinal [5]
Acetovanillone	0.01	15.1	(1494) (1439)	MS, RI	Vanilla, sweet, honey [1, 3]
Vanillic acid methyl ester	0.15	11.6	(1515) (1526)	MS, RI	Sweet aromatic, spicy, slightly vanilla [3]
Vanillic acid[h]	0.04	20.6	(1567) (1560)	MS, RI	Sweet aromatic, somewhat vanilla; creamy, milky [3]
Benzoic acid phenylmethyl ester[h]	0.04	4.62	(1772) (1775)	MS, RI	Balsamic, oil, herb [6]
Terpenes/cadinenes [14]					
m−Menth-1-ene[g]	1.87	8.2	(971) (987)	MS, RI	
p−Cymene	0.25	13.2	(1008) (1025)	MS, RI, STD	Fresh, solvent, citrus [8]
d-Limonene	2.48	3.5	(1014) (1030)	MS, RI, STD	Citrus-like, fresh [6, 8]
α−Terpinene	0.18	10.9	(1054) (1018)	MS, RI, STD	Lemony, citrusy [8]
α−Bergamotene	0.36	6.0	(1434) (1431)	MS, RI	Wood, warm, tea [6]
α−Curcumene	0.14	10.9	(1480) (1480)	MS, RI	Herb [6]
Calamenene	0.12	12.7	(1527) (1524)	MS, RI	Herb, spice [6]
α−Calacorene	0.07	8.7	(1548) (1550)	MS, RI	Wood [6]
Cadalene	0.02	11.5	(1680) (1675)	MS, RI	Herbal, savory [3]
α−Cubebene[g]	0.04	7.9	(1348) (1348)	MS, RI	Herb, wax [6]
α−Copaene	0.29	4.7	(1378) (1377)	MS, RI	Wood, spice [6]
γ−Muurolene	0.04	6.0	(1478) (1475)	MS, RI	Herb, wood, spice [6]
α−Muurolene	0.05	8.3	(1500) (1502)	MS, RI	Wood [6]
β−Cadinene	0.16	3.3	(1520) (1519)	MS, RI	
Furan derivatives [12]					
2-Ethylfuran	0.96	13.4	(698) (702)	MS, RI	

Table I. Contineud.

Compounds[a]	RSA (%)[b]	CV[c]	Kovats RI[d] **(Exp.) (Lit.)**	ID[e]	Odor descriptors[f] and references
Furfural	4.08	4.2	(835) (830)	MS, RI, STD	Sweet caramel-like, nutty, baked bread, almond [3]
2-Furyl methyl ketone (*2-acetylfuran*)	0.27	11.5	(882) (893)	MS, RI	Balsamic [3]
2-Butylfuran	1.01	18.9	(888) (893)	MS, RI	
γ−Butyrolactone	0.03	12.1	(944) (927)	MS, RI, STD	Caramel, sweet [6]
5-Methylfurfural	0.67	4.3	(952) (978)	MS, RI, STD	Almond, caramel, burnt [6]
2-Pentylfuran	7.94	4.8	(976) (980)	MS, RI, STD	Green bean, butter [6]
2-[(2*E*)-2-Pentenyl]furan[g]	0.43	6.9	(983) (1000)	MS, RI	
2-[(1*E*)-1-Pentenyl]furan[g]	0.25	15.9	(1042) (1048)	MS, RI	
6-(5-Methyl-furan-2-yl)-hexan-2-one[g]	0.03	12.4	(1276)	MS	
Dihydro-5-pentyl-2(3H)-furanone (*γ-nonanolactone*)	0.05	10.0	(1361) (1360)	MS, RI	Creamy-fatty, coconut- and apricot-like [3,4]
Dihydroactinidiolide	0.02	9.9	(1539) (1536)	MS, RI	
Esters [6]					
Hexanoic acid methyl ester	0.44	15.6	(919) (884)	MS, RI, STD	Fruity, fresh, sweet [6]
Heptanoic acid methyl ester	0.08	10.1	(1005) (1006)	MS, RI	
2-Methyl-2-nonenoic acid methyl ester[g]	0.35	10.8	(1110)	MS	
Octanoic acid methyl ester	0.21	18.6	(1120) (1112)	MS, RI	Fruity, fatty [3]
Nonanoic acid methyl ester	0.19	10.3	(1219) (1224)	MS, RI, STD	Oily, fatty, slightly fruity [3]
Hexadecanoic acid methyl ester[h]	0.83	12.7	(1838) (1870)	MS, RI	
Acids [7]					
2-Methylpropanoic acid (*isobutyric acid*)	0.12	16.7	(769) (765)	MS, RI	Buttery[1]
3-Methylbutanoic acid (*isovaleric acid*)	0.29	20.0	(858) (848)	MS, RI, STD	Buttery, oily, acid, cheese, unpleasant [1–5]
2-Methylbutanoic acid	0.47	18.8	(867) (863)	MS, RI	Cheese, fruity, animal, acid, sweaty, buttery [2–5]
Pentanoic acid (*valeric acid*)	0.07	16.0	(892) (913)	MS, RI, STD	Cheese, strongly acidic, caprylic [1,3]
Hexanoic acid	0.75	6.0	(986) (990)	MS, RI, STD	
2-Heptenoic acid	0.39	11.4	(1115)	MS	
Nonanoic acid	0.08	10.0	(1263) (1272)	MS, RI	Oily, fatty, caprylic, cheesy [3]
Ketones [4]					
Pentan-2-one	7.65	20.1	(682) (679)	MS, RI	Ether, fruit [6]
1-(2-Methyl-1-cyclopenten-1-yl)-ethanone[g]	1.18	12.1	(850) (996)	MS, RI	
3-Octen-2-one	0.55	13.7	(1024) (1034)	MS, RI	Nut, crushed bug, fatty [6]
Hexahydrofarnesyl acetone[h]	0.14	20.8	(1811) (1843)	MS, RI	Fat [6]
Aldehydes [5]					
Propanal	0.28	18.4	(506) (506)	MS, RI	Solvent, pungent, plastic [6,7]
2-Methylpropanal	0.31	19.7	(550) (554)	MS, RI	Green, pungent, burnt, malty [7]
Hexanal	1.74	12.6	(799) (803)	MS, RI, STD	Green, fruity [2]
Heptanal	0.61	10.9	(900) (904)	MS, RI, STD	Soap, fat, almond oily, rancid, powerful [6,8]
Safranal	0.11	4.8	(1200) (1210)	MS, RI, STD	Herb, sweet [6]
Hydrocarbons [4]					
2,4-Dimethylhexane[g]	2.31	7.8	(797) (736)		
(*Z*)-2-Octene[g]	0.59	14.6	(803) (808)	MS, RI	

Table I. Contineud.

Compounds[a]	RSA (%)[b]	CV[c]	Kovats RI[d] (Exp.) (Lit.)	ID[e]	Odor descriptors[f] and references
(*E*)-2-Octene[g]	0.69	21.9	(811) (810)	MS, RI	
(3*E*)-1,3-Octadiene	0.89	13.9	(823) (827)	MS, RI	
Alcohols [3]					
1-Pentanol	0.29	17.9	(773) (771)	MS, RI, STD	Green, grassy, powerful [6, 8]
1-Octen-3-ol	0.75	13.4	(969) (969)	MS, RI	Mushroom, earthy [8]
(*Z*)-3-Nonen-1-ol	0.37	19.4	(1102) (1126)	MS, RI	
Others [5]					
Unknown	0.15	8.6	(1002)		
Unknown	0.23	12.3	(1125)		
3-Ethyl-4-methyl-1H-pyrrole-2,5-dione	0.08	4.1	(1231)	MS	
Unknown	0.06	12.3	(1361)		
Unknown	0.03	18.8	(1489)		

[a] Compounds are listed on the basis of their chemical family and Kovats retention index (RI) values.
[b] RSA: Relative surface area (%)
[c] CV: coefficient of variation for six replicates;
[d] Kovats RI: retention index on a VF-5MS column (equivalent to DB-5MS) using C5–C20 alkanes; Exp.: experimental; Lit.: literature.
[e] ID: identification. The reliability of the identification proposal is indicated by the following: MS: mass spectrum in agreement with the mass spectral database; RI: Kovats RI in agreement with the literature; STD: mass spectrum and Kovats RI in agreement with standards.
[f] [1]: Pérez-Silva *et al.* [2, 14]: Brunschwig *et al.* [16]; [3] Zhang and Mueller [4, 17]: Takahashi *et al.* [18]; (http://www.flavornet.org [7, 65]: http://www.pherobase.com [66]; [8]: http://www.crec.ifas.ufl.edu [67]. Descriptors in bold have been reported in the genus *Vanilla*.
[g] Compound detected for the first time in *Vanilla*.
[h] Identification conditions: 60 °C 20 min^{-1}.

o-cresol methyl ether and *p*-cresol methyl ether), one ketone (acetovanillone), and one acid (vanillic acid). The majority of these shikimate derivatives have previously been reported as aromatic compounds in the genus *Vanilla* [14, 16–19, 57], with the exception of *o*-cresol methyl ether. Clearly, the shikimate derivatives have a marked influence on the overall aroma of cured vanilla beans, but this is the first time that *o*-cresol methyl ether has been detected in vanilla. *p*-hydroxybenzaldehyde, vanillic acid (major compounds in vanilla) and benzoic acid phenylmethyl ester were detected only at an extraction temperature of 60 °C during the method development process. The most common odor qualities reported for this family were 'balsamic', 'spicy', 'sweet', 'wood', 'phenolic', 'smoky' and 'vanilla', due largely to the following phenolic compounds: vanillin, *p*-hydroxybenzaldehyde, *o*-cresol methyl ether, *p*-cresol methyl ether, *p*-creosol, eugenol, isovanillin and guaiacol. Guaiacol has a negative effect on the key aroma of vanilla [48], especially when the guaiacol content increases and the vanillin content decreases [18].

The descriptors 'anise-like', 'almond', 'raspberry-like' and 'floral' were due in particular to *p*-anisaldehyde and *p*-anisyl alcohol [16, 19]. Anisaldehyde was identified as an aromatic compound in *V. planifolia* from Mexico [14, 38], as well as in Bourbon and Ugandan vanilla beans [17]. These compounds together with anisic ethers and anisic acid are relatively more abundant in *V. tahitensis* and thus have a strong impact on the characteristic aroma of this species [16,19,20,58,59], as shown in studies using AEDA (aroma extract dilution analysis) dilution factors of 1 953 125, 390 625 and 15 625 for anisaldehyde, anisyl alcohol and anisyl acetate, respectively [19]. Brunschwig *et al.* [16] reported that Tahitian vanilla extract was characterized by two families of descriptors (odors), 'anise spicy' and 'phenolic', that respectively accounted for 35% and 32% of the overall flavor as analyzed by CHARM (combined hedonic aroma response measurement). These classes were sensory fingerprints of Tahitian vanilla flavors. The 'aldehyde' notes were rather important in Tahitian vanilla flavors, as they contributed to 17% of the overall aroma.

In previous studies [37, 38, 57, 60], 10 compounds were identified in a glycosylated form in the green pods of *V. planifolia*: benzyl alcohol, *p*-cresol, *p*-creosol, salicylic acid methyl ester, *p*-anisyl alcohol, *p*-hydroxybenzaldehyde, vanillin, acetovanillone, vanillic acid methyl ester and vanillic acid. Although guaiacol was reported in glycosylated form in green pods [57], the study of the evolution of odor-active compounds in vanilla (*V. planifolia* G. Jackson) beans during traditional curing showed that guaiacol was formed in the second stage of curing, after the first heat treatment of green pods, due to thermal decarboxylation of vanillic acid [38]. Although vanillic acid has a glycosidic origin, it can also be formed by the oxidation of vanillin by peroxidases [61] or during heat treatment [11].

3.2.2 Terpenes/cadinenes (14)

Terpene compounds consisting of isoprene units are an important part of essential oils. With the exception of

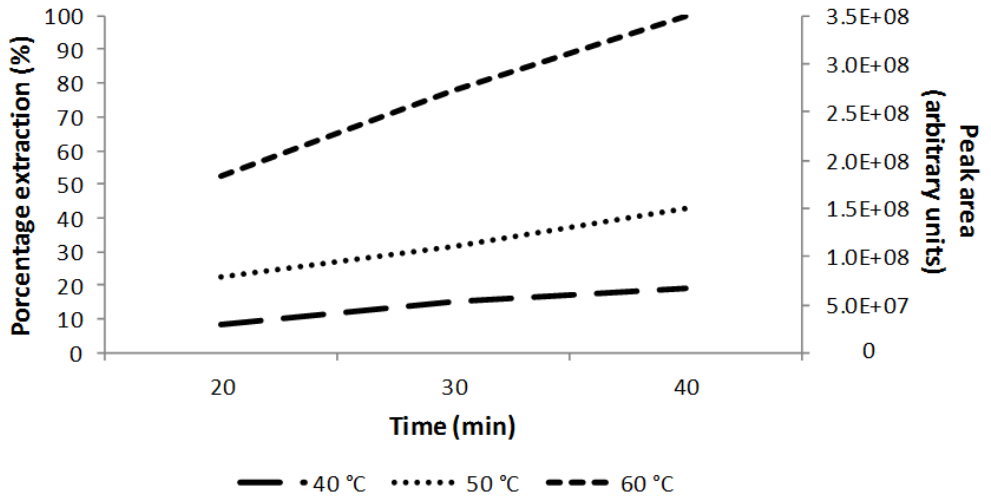

Figure 1. Vanillin HS–SPME–GC/MS extraction using CAR/PDMS fibre (extraction temperature: 40, 50 or 60 °C; extraction time: 20, 30 or 40 min).

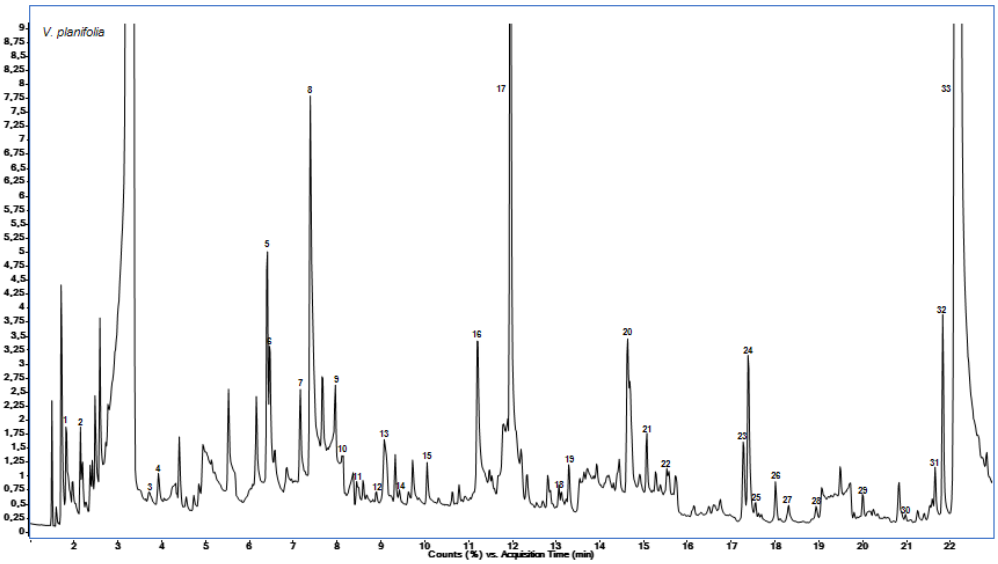

Figure 2. Representative GC chromatogram of the volatiles in cured vanilla (*V. planifolia* G. Jackson), produced in Veracruz, Mexico. Peak numbers: 1: propanal; 2: Propanal, 2-methyl-; 3: 2-Pentanone; 4: Furan, 2-ethyl-; 5: Hexane, 2,4-dimethyl-; 6: Hexanal; 2,4-dimethyl; 7: 1,3-Octadiene; 8: Furfural; 9: Ethanone, 1-(2-methyl-1-cyclopenten-1-yl)-; 10: Butanoic acid, 3-methyl-; 11: Butanoic acid, 2-methyl-; 12: 1-Propanone, 1-(2-furanyl)-; 13: Furan, 2-butyl-; 14: Heptanal; 15: Hexanoic acid, methyl ester; 16: benzaldehyde; 17: Furan, 2-pentyl-; 18: D-Limonene; 19: 3-Octen-2-one; 20: Phenol, 4-methoxy-; 21: 3-Nonen-1-ol, (Z)-; 22: Octanoic acid, methyl ester; 23: Phenol, 2-methoxy-4-methyl-; 24: Salicylic acid methyl ester; 25: Safranal; 26: Nonanoic acid, methyl ester; 27: 1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl; 28: Benzaldehyde, 4-methoxy-; 29: Cinnamic acid, methyl ester; 30: α-Cubebene; 31: Copaene; 32: Cinnamic acid, methyl ester, (E)-; 33: Vanillin.

m-menth-1-ene, all terpenes, namely monoterpenes (*p*-cymene, d-limonene and α-terpinene) and sesquiterpenes (α-bergamotene and α-curcumene), identified and analyzed in the studied pods have already been reported in previous studies [17, 36]. In addition to the above terpenes, eight cadinenes were identified in vanilla, namely calamenene, α-calacorene, cadalene, α-cubebene, α-copaene, γ-muurolene, α-muurolene and β-cadinene. α-Cubebene is reported for the first time in *V. planifolia*. Some compounds of this family have been identified in vanilla samples with mainly the following flavor attributes: 'fresh', 'citrus-like', 'lemony', 'herb' and 'wood'. Cadelene was identified in *V. planifolia* samples from Bourbon and Uganda as an aromatic compound with the descriptors 'herbal' and 'savory', as reported by Zhang and Mueller [17].

3.2.3 Furan derivatives (12)

Twelve furans were identified, of which 2-[(2*E*)-2-pentenyl] furan, 2-[(1*E*)-1-pentenyl] furan and 6-(5-methyl-furan-2-yl)-hexan-2-one have not previously been reported

in vanilla. Dihydroactinidiolide is also reported here for the first time in *V. planifolia*, as this furan has been reported only in *V. tahitensis*, by Da Costa and Pantini [59]. The aromatic compounds of this family reported in *V. planifolia* include 2-furyl methyl ketone, 2-butylfuran and γ-nonanolactone [17,18]. The flavor attributes described for these compounds were 'sweet', 'caramel-like', 'nutty', 'baked bread', 'almond', 'balsamic', 'creamy-fatty', 'coconut' and 'apricot-like'. According to the technical details provided by the flavor and fragrance manufacturer Givaudan, dihydroactinidiolide will confer a 'ripe, apricot, red fruit and wood' organoleptic character. Furans such as pyran (3-ethyl-4-methyl-1H-pyrrole-2,5-dione) can be formed by thermal degradation of sugars during pod curing, as in coffee and cocoa [62].

3.2.4 Esters (6), acids (7) and other compounds

The aliphatic esters group reported in this study was the fourth most abundant family of compounds identified in the analyzed samples; the 2-methyl-2-nonenoic acid methyl ester is reported for the first time. In a study by Sostaric *et al.* [25] of alcoholic vanilla extract, PA fibre, which is more polar, gave better results than PDMS and CW/DVB for the extraction of vanillin and the esters ethyl benzoate, ethyl octanoate, ethyl nonanoate and methyl 3-phenyl-2-propenoate (cinnamic acid methyl ester). Cinnamic acid methyl ester was also identified in this study and was grouped in the shikimate derivatives section along with seven other esters. Zhang and Mueller [17] identified octanoic acid methyl ester ('fruity', 'fatty') and nonanoic acid methyl ester ('oily', 'fatty', 'winey', 'slightly fruity') as aromatic compounds in *V. planifolia* extracts.

The acids grouped in this family corresponded to aliphatic acids with three to nine carbons and have been previously reported in *V. planifolia* and *V. tahitensis*. Among the aromatic compounds reported were 2-methylpropanoic acid, 3-methylbutanoic acid, 2-methylbutanoic acid, pentanoic acid and nonanoic acid, with mainly attributes such as 'buttery, cheese, oily and fatty' [14, 16–19]. Studies on the evaluation of volatiles in coffee showed that CAR/PDMS fibre extracts more aliphatic acids with four and five carbons, whereas acetic acid and propionic acid were extracted better with CW/DVB fibre [63].

Among the four ketones identified, 1-(2-methyl-1-cyclopenten-1-yl)-ethanone is reported for the first time in *V. planifolia*. Hexahydrofarnesyl acetone could be detected only at 60 °C for 20 min.

Four aliphatic aldehydes (propanal, 2-methlypropanal, hexanal and heptanal) and one cyclic aldehyde (safranal) were identified. Although all these compounds have already been reported in vanilla, only hexanal was reported as an aromatic in *V. tahitensis*, with the generation of 'green and fruity' attributes [16]. The poor levels of these compounds may be due to the extraction competition between them and vanillin. In fact, they all have similar chemistry, and vanillin is present at high concentrations (1.4%), in comparison with the very low concentrations of these aldehydes in vanilla. Studying the aromatic profile of vanilla pods from organic solvent extraction allowed a greater number of compounds of this family to be identified [14, 16, 17, 38].

3.2.5 Hydrocarbons (4) and alcohols (3)

Only four hydrocarbons were identified in the present study, which are reported for the first time in *V. planifolia*: isomers (*Z*) and (*E*) of 2-octene, (3*E*)-1,3-Octadiene and 2,4-dimethylhexane. However, various aliphatic hydrocarbons have already been reported in the literature [14, 17, 33, 36, 38]. Thus, Ramaroson-Raonizafinimanana *et al.* [27] identified 25 *n*-alkanes, 17 branched alkanes and 12 alkenes in three vanilla bean species: *V. fragrans*, *V. madagascariensis* and *V. tahitensis*. Because of the chemical nature of hydrocarbons, a non-polar fibre such as PDMS would be more suitable for their extraction [54, 63, 64].

Three aliphatic alcohols were identified, including 2,4-dimethylpentan-3-ol, which is reported for the first time in vanilla. Few volatiles belonging to this family were extracted in comparison with the other groups, perhaps because of the type of fibre used (CAR/PDMS). According to Kataoka *et al.* [54], PA fibre is more effective for alcohols and phenols. Indeed, in the preliminary trials for fibre selection (data not shown), PA fibre also allowed the identification of volatile phenols listed in the shikimate derivatives section (vanillin and guaiacol, among others) as well as alcohols (benzyl alcohol and *p*-anisyl alcohol). However, mixed-coating fibres increase holding capacity because each coating has the effect of enhancing adsorption and distribution in the stationary phase; hence PDMS/DVB and CAR/DVB fibres can be used for the extraction of more polar volatiles with low molecular weight [54]. Because of the sensitivity and selectivity of CAR/PDMS fibre, it was selected for the analysis of the studied vanilla samples to extract shikimate derivatives, which include some phenols and alcohols [63].

4 Conclusion

A headspace solid-phase microextraction technique combined with gas chromatography-mass spectrometry was successfully applied to detect 81 volatiles, of which 77 were identified, in cured vanilla (*Vanilla planifolia* G. Jackson) beans originating from the state of Veracruz, Mexico. Of those compounds, 21 were shikimate derivatives, 14 were terpenes/cadinenes, 12 were furan derivatives, 6 were esters, 7 were acids, 4 were ketones, 5 were aldehydes, 4 were hydrocarbons, 3 were alcohols and 1 was a pyrrole. A total of 31 volatiles have already been reported as aroma-active compounds in cured beans of *V. planifolia* and *V. tahitensis*. However, to our knowledge, 11 of these aromatic compounds have not previously been detected in vanilla beans. The proposed semi-quantitative HS–SPME–GC/MS method has the potential to be used in routine analysis of volatiles in vanilla beans, in order to evaluate rapidly the different profiles that may have been produced in cured vanilla beans depending on their origin and curing process. This method allows detection of the major volatile compounds which have already been recognized in previous work as aromatic compounds.

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Resumen – Introducción. La microextracción en el espacio de cabeza en fase sólida acoplada a la cromatográfia de gases-espectrometría de masas (HS-SPME-CG/MS) fue usada para la identificación de compuestos volátiles en vainas de vainilla beneficiada (*Vanilla planifolia* G. Jackson) originarias del estado de Veracruz, México. **Materiales y métodos.** Un lote de 6 kg de la producción del año 2012 fue estudiado. Una fibra de Carboxen/polydimethylsiloxane (85 μm) fue seleccionada porque mostró una alta capacidad en la extracción de compuestos volátiles detectados. **Resultados y discusión.** Un total de 81 compuestos volátiles fueron detectados, de los cuales 77 fueron identificados comparando sus espectros de masas y sus tiempos de retención con los de los compuestos puros empleados como patrones de referencia inyectados y/o buscados en la Base de Datos de la Biblioteca del Instituto Nacional de Referencias y de Tecnología en Espectros de Masas. Entre los compuestos dentificados, 21 fueron derivados shikímicos, 14 terpenos/cadinenos, 12 derivados furanos, 6 ésteres, 7 ácidos, 4 cetonas, 5 aldehídos, 4 hidrocarburos, 3 alcoholes y 1 pirrol. **Conclusión.** Un total de 31 volátiles han sido reportados previamente como compuestos activos olfativamente en el aroma de las vainas curadas de *V. planifolia* and *V. tahitensis.* Sin embargo, 11 compuestos volátiles se reportan por primera vez en este estudio.

Palabras clave: México / vainilla / *Vanilla planifolia* / compuesto aroma / compuesto volátil

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