Chemical Characterization of *Vanilla pompona* Sheide, Part I

V. pompona is resistant to climate change and diseases, and therefore is preferred for hybridization with *V. planifolia*. Its chemical characterization was carried out in a series of studies, described here in the first of four parts.

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Editor's note: *This article is the first in a series of four describing the chemical characterization of* V. pompona*. GC-MS, DTD-GC-MS and GC-O/MS were used to indicate its quality as a potential source of aromatic compounds for fragrance applications. Here, existing literature on* V. pompona *is reviewed and compared with* V. tahitensis *and* V. planifolia*. Test protocols for GC-MS and DTD-GC-MS are described, and some results are discussed. Part II will continue with results from these tests and a discussion. Part III will set up the GC-O/MS protocol and discuss some results, while Part IV will conclude the results and describe how the chemical distribution of* V. pompona *impacts its odor profile.*

Vanilla is one of the most widely used flavor ingredients, and while there are 110-130 species of vanilla, only three are cultivated and have significant economic importance. This is mainly due to their history of being successfully introduced in Madagascar and Indonesia for commercialization; and their acceptance by the U.S. standard of identity for food and beverages—a big market driver. The three species include: *Vanilla planifolia* Andrews, *Vanilla tahitensis* and *Vanilla pompona*.

The chemical and aroma compositions of *V. planifolia* and *V. tahitensis* have extensively been studied, and their attributes and applications are well-known. Likewise, *V. pompona* exhibits strong aromatic and flavorful properties;[1, 2] its aroma has been reported as strong vanillin-like, perfumy, floral/heliotropin-like, anise-like, creamy and sweet.[3-6]

Considering the lengthy and labor-intensive cultivation and curing process required to develop the flavor of vanilla beans, the fact that vanilla vines are vulnerable to multiple factors affecting their yield is troublesome. Climate change and plant diseases are difficult to prevent and control, and historically have been responsible for fluctuating vanilla prices. Therefore, robustness is a desirable attribute in cultivated vanilla, and is held only by wild vanilla species.

In relation, among the cultivated species, *V. pompona* is uniquely regarded as resistant to climate change and diseases, including the lethal *Fusarium* or root rot disease.[7] As a result, *V. pompona* species has been preferred for hybridization programs with *V. planifolia* to produce a more resistant aromatic hybrid.[8] Such hybrids have been successfully introduced in several countries such as Costa Rica. Here, existing literature on *V. pompona* is reviewed and compared with *V. tahitensis* and *V. planifolia*, and a sample of cured *V. pompona* beans from the region of Veracruz, Mexico, was characterized by GC-MS and direct thermal desorption-gas chromatography-mass spectrometry (DTD-GC-MS).

Literature Review

Concerning the chemical composition of *V. pompona*, *The Handbook of Vanilla Science* edited by Havkin-Frenkel and Belanger[9] compiled the volatiles and semivolatiles found in *V. pompona* from studies published by Gnadinger (1925), Simony (1953), Klimes and Lamparsky (1976), Shiota and Itoga (1975), and Lee (2006). In addition, the species *V. pompona* has been introduced in literature as a "lower quality" vanilla,[3, 10] mainly due to reports that this species contains the lowest concentration of vanillin, compared with the two other main species.[4]

However, other studies show different results. For instance, Nakazawa[5] performed GC analysis on several vanilla species from different geographical origins, including *V. pompona* (Guadalupe vanilla) from the West Indies, and compared its composition with *V. planifolia* and *V. tahitensis*. The vanillin content of cured *V. pompona* beans was comparable to the commercially cultivated vanilla species, and even higher than the sample of Tahitian vanilla.

The most recent publication[1] on *V. pompona* reported on the concentration of glucosides and aglycones of the eight main compounds in uncured *V. pompona* beans from a Peruvian forest. High concentrations of vanillin, 4-hydroxybenzyl alcohol and anisyl alcohol were found by GC-MS. In fact, the concentrations of vanillin and other main aromatic compounds in Peruvian *V. pompona* after nine months of fruit development were comparable to the levels found in commercial *V. tahitensis* and *V. planifolia*. Similarly, Lee[6] evaluated the characteristic compounds of several vanilla species using DTD-GC-MS, and determined the vanillin content in *V. pompona* from Madagascar was higher (2.33%) than in *V. tahitensis* (1.04%) and *V. planifolia* (1.92%).

Giving the different physiology of glucosides development of *V. pompona* compared to *V. planifolia* found by Maruenda et al.,[1]—whereby the levels of glucovanillin spiked after eight months instead of gradually developing, as in *V. planifolia*—the different concentrations of vanillin in the different studies could be explained by the unknown origin of the fruits used. Therefore, it is reasonable to include harvesting time as a parameter to control and document for a consistent and accurate comparison of aroma quality of this species, particularly when comparing its quality with the other extensively cultivated species.

Despite its novel attributes and potential source of aromatic compounds including vanillin, *V. pompona* remains locally cultivated. This species is scarcely traded in the global market, and particularly absent in the United States. Old myths of low vanilla quality, and former issues with adulteration in the early twentieth century could be the root causes of its exclusion from the standard of identity for vanilla extract used in food in the United States. However, the lack of presence of *V. pompona* in the fragrance industry seems more related to the inaccessibility of cured beans and lack of scientific information on its aroma properties. Further knowledge of the chemical and aroma composition of this novel vanilla would therefore shed light on its potential as a valuable raw material for more widespread commercialization.

The present study was undertaken to generate information about the chemical composition of *V. pompona* Schiede, and to provide indicators of its quality. This characterization, combined with the recognized endurance of the plant during cultivation, could assist in validating this species as a valuable source of aromatic compounds for fragrance applications.

Materials and Methods

A sample of cured beans of *V. pompona* Schiede was obtained[a] from the region of Veracruz, Mexico. The beans were grown and cured using the traditional Mexican process of oven/sun drying. The green beans were scalded using a typical oven at 60°C for 48 hr. The resulting cured pods were characteristically dark, and had a mellow odor described as nutty, roasted, earthy, sweet and cherry. The pods were curved, thicker and shorter than the usual spike-like *V. planifolia* pods.

The volatiles and semi-volatiles were identified by GC-MS with a flame ionization detector (FID)[b], in both a 60 m x 0.32 mm ID x 1.00 µm DF column[c], and a 0.32 mm x 60 m x 1 µm df column[d], for confirmation. Analytical characterization also was performed using a DTD-GC-MS device[e], located at the injection port of the GC and directly injected into the GC-MS. The GC[f] had a 30 m x 0.32 mm ID x 0.25 µm -60/325 (350)°C capillary column, and the detector[g] was a high-resolution, double focusing magnetic sector MS.

V. pompona Test Protocol

V. pompona extract (3×) was weighed (5 g) in a centrifuge tube. Water (20 mL) and dichloromethane (DCM) (20 mL) were added, then 100 µL of chlorocyclohexane (CCH) was measured with an analytical pipette and added as an internal standard; i.e., 0.5% CCH in methanol. The sample was vortexed for 5 min and centrifuged for 5 min at 1,800 rpm. The upper layer phase was discarded using suction equipment and the bottom DCM phase layer was kept. The sample was filtered and dried by pouring it onto paper filter filled with anhydrous magnesium sulfate (Mg_2SO_4), previously prepared. Next, DCM (20 mL) was poured gently on top of the paper filter. The filtrate was concentrated[h] down to a final volume of 0.5 mL.

GC/MS: The *V. pompona* extract (1 µL) was injected in the GC-MS setup described[b] in a 0.32 mm x 60 m x 1 µm df column[d]. Helium was used as carrier gas at a constant flow rate of 2.6 mL/min. The temperature of GC injectors was 250°C. The oven temperature program consisted of: 40°C, held at 5 min; increased to 300°C at 4°C/min; and held at 300°C for 20 min., for a total run time of 80 min. The split ratio was 10:1. The mass spectrometer was operated in electron ionization (EI) mode and 70 eV. The temperatures of quadrupole and ion source were 150°C and 230°C, respectively. The MSD transfer line temperature was 280°C. The mass scan range was m/z 15 to m/z 450. The olfactory port transfer line temperature was 240°C.

DTD-GC/MS: A 9-mg sample of vanilla beans was weighed into a desorption tube and plugged with silanized glass wool. The tube was spiked with 10 µg of 2,6-dimethoxyphenol[j] internal standard by injecting 1.0 µL of a methanol stock solution (10 mg/mL) using a solvent flush technique. Next, the spiked desorption tube was connected into a DTD-GC-MS device[e]. This unit thermally desorbed volatiles from a sample of cured *V. pompona* beans and directly injected it into the GC column. Desorption tubes[k] 3.0 mm ID x 10 cm were previously conditioned and used for this purpose.

The thermal desorption conditions were: 10 sec helium purge, 30 sec injection, and desorption at 220°C for 5 min. The end of the capillary column was inserted into the mass spectrometer. The heat transfer line was maintained at 280°C from the GC to the mass spectrometer. The temperature was programmed starting at -20°C, which was held for 5 min during the thermal desorption interval; raised to 40°C at the rate of 10°C/min; then increased to 320°C at 4°C/min and held for 20 min. The injector temperature was 250°C and detector temperature was 325°C. Helium was used as a carrier gas with a flow rate of 1.0 mL/min at a split ratio of 100:1. The mass spectrometer was operated in EI mode, scan masses were 35-350, scan time was 0.6 sec, and interscan time was 0.8 sec.

The resulting DTD-GC-MS chromatograms were processed using an automated mass spectral deconvolution and identification system (AMDIS) program in simple mode for the identification of volatiles. Semi-quantification of compounds was made by relative GC-MS area counts of analyte compared to internal standard using a response factor.

The GC/MS was analyzed using the AMDIS software, which identifies compounds by means of calibrated linear retention indices, and using in-house and National Institute of Standards and Technology (NIST) spectral libraries. The software was used in retention index mode for GC/MS and in DTD-GC/MS in simple mode.

Results: Chemical Characterization

One hundred and twenty three volatile and semi-volatile compounds were identified by GC-MS in the *V. pompona* extract. The highest peaks in the Total Ion Chromatogram (TIC) (see

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[a] Desarrollo Agroindustrial Gaya, S.A. de C.V.

[b] Agilent 6890 GC with MSDS 5973, Agilent

[c] Rtx-Wax column and [d] Rxi-1ms GC column, Restex

[e] Short Path Thermal Desorber, Scientific Instrument Services, Inc.

[f] 3400 GC, Varian

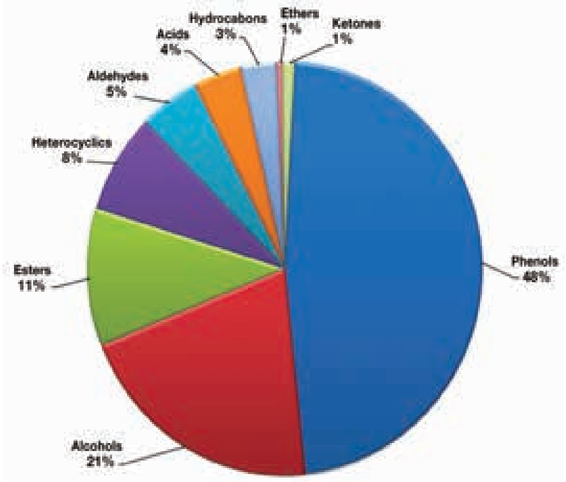
[g] Mat 8230, Finnigan

[h] TurboVap II, Biotage

[j] 2,6-dimethoxyphenol, 99% pure, Aldrich Chemical

[k] Silanized, glass lined stainless steel, Scientific Instrument Services Inc.

F-1. One hundred and twenty three volatile and semi-volatile compounds were identified by GC-MS in the *V. pompona* extract



F-1) reflected the most abundant compounds: vanillin, anisyl alcohol, 5-(hydroxymethyl)-2-furfural, p-anisaldehyde, p-vinyl guaiacol, anisyl acetate, methyl linoleate, methyl linolelaidate and tricosane (see **F-2** on Page 26). The remaining volatiles were present at levels less than 1% of the total abundance, which represents relative concentrations up to 10 ppm in the threefold extract.

This volatile profile of *V. pompona* extract was found to be typical of the vanilla species. In fact, Klimes and Lamparsky[11] and Perez-Silva et al.[12] stressed the importance of abundant trace volatiles in the typical vanilla aroma profile, which make the quality of the aroma complex and difficult to emulate solely by vanillin or a blend of chemically synthetized compounds.

In terms of chemical groups, the volatile profile of the *V. pompona* extract was comprised of nine chemical classes: hydrocarbons (24), phenols (11), alcohols (20), esters (18), heterocyclic compounds (11), aldehydes (12), ketones (13), ether (1) and aliphatic acids (13), and the specific chemical composition of *V. pompona* extract is presented in **T-1**. This installment of the article series discusses hydrocarbons and phenolic compositions in more detail. Part II will cover the remaining classes and provide an overview of results.

Interestingly, in spite of the long history of research in vanilla, advances in technology continuously enable the discovery of new compounds, which in this case was facilitated by AMDIS. Interestingly, twelve compounds were found that had not been previously identified in vanilla, to the best of these authors' knowledge. In all cases, these compounds can be found in natural materials; the list of these compounds and their corresponding natural sources are presented in **T-2** on Page 26.

Hydrocarbons

In this study, hydrocarbons contained the most numerous compounds (24) but only represented 3.30% of the total extract (see **F-2** on Page 26). Among them, seven terpenoids and three sesquiterpenes were present in the *V. pompona* extract, at trace concentrations ≤ 0.1%. This number of hydrocarbons contrasts with Perez-Silva et al.,[12] who only detected two hydrocarbons—tricosane and pentacosane—using GC-MS in an extract of pentane/ether (P/E) *V. planifolia* Jackson.

The compound *cis* 9-tricosene, also called muscalure, was identified. Interestingly, this is a known pheromone of the common female housefly, and bees. Cadalene also was confirmed by identification in both the polar and nonpolar GC columns. These two compounds were recently reported by Zhang and Mueller[13] in *V. planifolia* using GC-MS analysis with the AMDIS deconvolution program.

The formation of terpenes arises from carbohydrate and lipid metabolism in the plant. The presence of these hydrocarbons may not be considered significant in terms of aroma contribution and potency of odors; however, terpenes (mono- and sesquiterpenes) are considered important to the aroma of certain fruits. The presence of volatile terpenoids can also be associated to woody background odors. The compound benzophenone present in this extract at 0.02% was previously reported at the

T-1. Volatile compounds in ethanolic-water-dichloromethane extract from cured *V. pompona* beans using a polar and non-polar column

No.	RT (min)	Compound Name	CAS #	Abundance (Area %)	RI (RI$_{lib}$) Δ Polar column	RI (RI$_{lib}$) Δ Non-Polar column	ID Criteria
Chemical group: Phenols; Number of compounds = 11; Area percentage = 48.47%							
1	41.96	vanillin	121-33-5	44.84	2592 (2586)	1363(1357)	MS,RI
2	32.75	4-vinyl guaiacol		2.22	2219 (2206)	1288 (1287)	MS,RI
3	44.48	acetovanillone	498-02-2	0.52	2653 (2640)	1448 (1446)	MS,RI*
4	29.46	creosol	93-51-6	0.28	1981(1988)	1169 (1173)	MS,RI
5	28.32	2-methoxyphenol = guaiacol	90-05-1	0.20	1883 (1893)	1063 (1062)	MS,RI
6	36.15	*p*-vinylphenol	2628-17-3	0.15	2395 (2390)	1184 (1185)	MS,RI
7	30.79	*p*-cresol	106-44-5	0.12	2087 (2084)	1046 (1047)	MS,RI
8	36.27	4-methoxyphenol (*p*-hydroxyanisole)	150-76-5	0.06	2400 (1866)	1179 (1182)	MS,RI*
9	29.81	phenol	108-95-2	0.05	2010 (1974)	954 (958)	MS,RI
10	27.56	methyl creosol	494-99-5	0.02	1818 (1807)	1206 (1212)	MS,RI
11	29.12	4-hydroxybenzyl alcohol	623-05-2	0.02	1952	-1337	MS^{T}
Chemical group: Alcohols; Number of compounds = 20; Area percentage = 20.61%							
12	34.16	anisyl alcohol	105-13-5	18.85	2301 (2313)	1251 (1249)	MS,RI*
13	43.58	veratryl alcohol	93-03-8	0.52	2632	**	MS^{T}
14	28.43	benzyl alcohol	100-51-6	0.34	1893 (1904)	1005 (1008)	MS,RI*
15	39.54	5-acetyl-2-furfurol	55087-82-6	0.28	2519	**	MS,RI
16	11.12	*tert*-amyl alcohol	75-85-4	0.15	1009 (1007)	623 (625)	MS,RI
17	23.28	2,3-butanediol I	513-85-9	0.06	1535 (1529)	761 (761)	MS,RI
18	33.70	T-muurolol	19912-62-0	0.06	2274 (2190)	1654 (1656)	MS,RI*
19	35.73	1-hexadecanol	36653-82-4	0.05	2375 (2386)	-1872	MS,RI
20	32.89	alpha-cadinol	481-34-5	0.05	2228 (2221)	1654 (1644)	MS,RI
21	30.9	viridiflorol	552-02-3	0.04	2095 (2091)	1613 (1604)	MS,RI
22	23.84	2,3-butanediol II		0.04	1567 (1563)	751 (752)	MS,RI
23	28.91	phenethanol	60-12-8	0.04	1934 (1915)	1086 (1088)	MS,RI*
24	32.62	T-cadinol	11/1/5937	0.03	2212 (2167)	1641 (1640)	MS,RI
25	15.56	*trans*-3-penten-2-ol	3899-34-1	0.02	1169 (1171)	**	MS,RI
26	21.70	1-octen-3-ol	3391-86-4	0.02	1447 (1445)	963 (966)	MS,RI
27	17.56	1-pentanol = amyl alcohol	71-41-0	0.02	1250 (1254)	750 (755)	MS,RI
28	29.26	1-dodecanol	112-53-8	0.02	1964 (1965)	-1472	MS,RI
29	11.89	2-methyl-3-buten-2-ol	115-18-4	0.01	1036 (1034)	**	MS,RI
30	25.33	2-furfurol	98-00-0	0.01	1658 (1663)	**	MS,RI
31	19.83	1-hexanol	111-27-3	0.01	1353 (1355)	**	MS,RI
Chemical group: Heterocyclics (Furans, pyrans, pyrazines,lactones); Number of compounds = 11; Area percentage = 7.62%							
32	39.06	5-(hydroxymethyl)-2-furfural	67-47-0	6.63	2505 (2505)	1176 (1174)	MS,RI
33	22.27	2-furfural	98-01-1	0.22	1478 (1467)	799 (803)	MS,RI
34	17.42	2-pentylfuran	3777-69-3	0.13	1244 (1238)	980 (984)	MS,RI
35	37.09	dihydroactinidiolide = 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-2(4H)-benzofuranone	15356-74-8	0.10	2431 (2351)	1506 (1509)	MS,RI
36	34.27	3,5-dihydroxy-2-methylpyran-4-one = 5-hydroxymaltol	1073-96-7	0.10	2306 (2238)	1155 (1155)	MS,RI
37	29.66	maltol = corps praline = 3- hydroxy-2-methyl-4-pyranone	118-71-8	0.07	1999 (1995)	1076 (1076)	MS,RI
38	24.31	5-methyl-2-furfural	620-02-0	0.04	1595 (1565)	929 (929)	MS,RI
39	29.62	2-acetylpyrrole	1072-83-9	0.04	1994 (1970)	**	MS,RI
40	15.41	2-ethylpyrazine	13925-00-3	0.03	1163 (1334)	**	MS,RI
41	27.25	2(5H)-furanone	497-23-4	0.02	1793 (1795)	**	MS,RI
42	23.12	2-acetylfuran	1192-62-7	0.01	1525 (1513)	880 (883)	MS,RI
Chemical group: Carbonyls, aldehydes; Number of compounds = 12; Area percentage = 4.54%							
43	30.65	*p*-anisaldehyde	123-11-5	3.26	2076 (2064)	1220 (1220)	MS,RI
44	13.47	hexanal	66-25-1	0.52	1092 (1090)	776 (778)	MS,RI

(Cont. ...)

T-1. Volatile compounds in ethanolic-water-dichloromethane extract from cured *V. pompona* beans using a polar and non-polar column (Cont.)

No.	RT (min)	Compound Name	CAS #	Abundance (Area %)	RI (RI$_{lib}$) Δ Polar column	RI (RI$_{lib}$) Δ Non-Polar column	ID Criteria
45	27.83	E2,E4-decadienal	25152-84-5	0.27	1841 (1818)	1292 (1296)	MS, RI
46	36.59	veratraldehyde	120-14-9	0.22	2413 (2393)	1430 (1435)	MS,RI
47	19.66	*trans*-2-heptenal	18829-55-5	0.19	1344 (1325)	930 (930)	MS,RI
48	27.19	E2,Z4-decadienal	25152-83-4	0.18	1789 (1793)	1270 (1274)	MS,RI
49	21.78	*trans*-2-octenal	2548-87-0	0.08	-1419	1033 (1031)	MS,RI
50	21.79	*cis*-3-nonenal	31823-43-5	0.08	1345 (1452)	**	MS
51	23.73	*trans*-2-nonenal	18829-56-6	0.05	1561 (1560)	1124 (1137)	MS
52	23.67	benzaldehyde	100-52-7	0.02	1557 (1527)	931 (931)	MS,RI
53	16.30	heptanal	111-71-7	0.01	1197 (1189)	879 (877)	MS,RI
54	12.24	crotonaldehyde = 2-butenal	123-73-9	0.003	1048 (1047)	**	MS,RI
Chemical group: Esters; Number of compounds = 18; Area percentage = 10.85%							
55	40.3	ethyl linoleate	544-35-4	3.79	2542 (2524)	2143 (2142)	MS,RI
56	39.29	methyl linoleate	112-63-0	2.69	2512 (2478)	2074 (2073)	MS,RI
57	32.13	anisyl acetate	104-21-2	1.22	2181 (2149)	1382 (1388)	MS,RI
58	31.59	anisyl formate	122-91-8	0.69	2144 (2108)	1298 (1317)	MS,RI
59	33.47	ethyl palmitate	628-97-7	0.67	2261 (2254)	1975 (1977)	MS,RI
60	47.09	dibutyl phthalate	84-74-2	0.44	2715	1923 (1922)	MS
61	32.87	methyl palmitate = methyl hexadecanoate	112-39-0	0.43	2227 (2206)	1906 (1909)	MS,RI
62	38.72	ethyl oleate	111-62-6	0.33	2493 (2477)	2150 (2149)	MS,RI
63	31.27	methyl *trans*-cinnamate	19713-73-6	0.20	2122 (2113)	**	MS
64	37.82	methyl *cis*-12-octadecenoate	2733-86-0	0.18	2459 (2462)	**	MS,RI
65	42.63	ethyl linolenate	1191-41-9	0.17	2610 (2566)	**	MS
66	43.00	methyl vanillate	3943-74-6	0.02	2618 (2598)	1480 (1482)	MS,RI
67	19.04	methyl acetate	79-20-9	0.01	1315	**	MS^T
68	21.66	ethyl caprylate	106-32-1	0.01	1445 (1439)	**	MS,RI
69	25.18	ethyl caprate	110-38-3	0.01	1649 (1645)	**	MS,RI
70	30.18	isopropyl myristate	110-27-0	0.004	2040 (2048)	**	MS,RI
71	26.20	benzyl formate	104-57-4	0.003	1715 (1688)	1049 (1049)	MS,RI
72	14.53	*n*-amyl formate	638-49-3	0.001	1130 (1123)		MS,RI
Chemical group: Hydrocarbons; Number of compounds = 24; Area percentage = 3.30%							
73	34.10	tricosane	638-67-5	1.10	2298 (2300)	2298 (2300)	MS,RI
74	36.24	tetracosane	646-31-1	0.50	2399 (2400)	2397 (2400)	MS,RI
75	38.91	pentacosane	629-99-2	0.37	2500 (2500)	2497 (2500)	MS,RI
76	32.38	docosane	629-97-0	0.32	2198 (2200)	2198 (2200)	MS,RI
77	33.88	cadalene	483-78-3	0.20	2285 (2262)	1670 (1672)	MS
78	28.34	calamenene	6617-49-8	0.17	1885 (1845)	1523 (1528)	MS,RI
79	29.40	alpha-calacorene	021391-99-1	0.11	1976 (1941)	1543 (1550)	MS,RI
80	33.08	alpha-d-curcumene	000644-30-4	0.10	2239	1477 (1480)	MS,RI
81	27.36	delta-cadinene	000483-76-1	0.09	1801 (1768)	**	MS,RI
82	34.59	*cis*-9-tricosene	027519-02-4	0.08	2321	2272 (2276)	MS,RI
83	30.93	heneicosane	000629-94-7	0.06	2098 (2100)	2098 (2100)	MS,RI
84	46.84	heptacosane	000593-49-7	0.03	2708 (2700)	**	MS
85	27.33	octadecane	000593-45-3	0.03	1799 (1800)	**	MS,RI
86	24.38	hexadecane	000544-76-3	0.03	1599 (1600)	**	MS,RI
87	25.97	heptadecane	000629-78-7	0.02	1699 (1700)	**	MS,RI
88	20.79	tetradecane	000629-59-4	0.02	1399 (1400)	**	MS,RI
89	16.36	dodecane	000112-40-3	0.02	1199 (1200)	**	MS,RI
90	18.71	tridecane	000629-50-5	0.01	1299 (1300)	**	MS,RI
91	13.67	undecane	001120-21-4	0.01	1099 (1100)	**	MS
92	26.50	gamma-muurolene	030021-74-0/317819-80-0	0.01	1737 (1690)	**	MS^T
93	22.67	pentadecane	000629-62-9	0.01	1499 (1500)	**	MS,RI
94	18.43	bromocyclohexane	000108-85-0	0.01	1287	957 (958)	MS,RI
95	16.85	limonene	000138-86-3	0.005	1220 (1200)	**	MS
96	18.18	vinylbenzene = styrene	000100-42-5	0.004	1276 (1255)		MS

(Cont. ...)

T-1. Volatile compounds in ethanolic-water-dichloromethane extract from cured *V. pompona* beans using a polar and non-polar column (Cont.)

No.	RT (min)	Compound Name	CAS #	Abundance (Area %)	RI (RI_{lib}) Δ Polar column	RI (RI_{lib}) Δ Non-Polar column	ID Criteria
Chemical group: Aliphatic acids; Number of compounds = 13; Area percentage = 3.65%							
97	27.79	caproic (hexanoic) acid	142-62-1	0.83	1838 (1847)	955 (957)	MS,RI
98	31.72	pelargonic (nonanoic) acid	112-05-0	0.79	2153 (2156)	1241 (1244)	MS,RI
99	21.61	acetic acid	64-19-7	0.77	1443 (1434)	570 (579)	MS,RI
100	22.64	formic acid	64-18-6	0.33	1498 (1484)	**	MS,RI
101	30.28	caprylic (octanoic) acid	124-07-2	0.31	2047 (2058)	1144 (1157)	MS,RI
102	38.12	lauric (dodecanoic) acid	143-07-7	0.21	2470 (2477)	1536 (1541)	MS,RI
103	29.00	oenanthic (heptanoic) acid	111-14-8	0.18	1942 (1956)	1048 (1061)	MS,RI
104	26.41	valeric (pentanoic) acid	109-52-4	0.11	1731 (1737)	859 (860)	MS,RI
105	25.42	isovaleric acid	503-74-2	0.04	1664 (1672)	**	MS,RI
106	23.21	propionic acid	79-09-4	0.02	1531 (1534)	**	MS,RI
107	25.45	2-methylbutyric acid = N293	116-53-0	0.02	1666 (1668)	**	MS,RI
108	24.73	butyric (butanoic) acid	107-92-6	0.02	1621 (1618)	**	MS,RI
109	23.74	isobutyric acid	79-31-2	0.02	1561 (1557)	735 (738)	MS,RI
Chemical group: Carbonyls, ketones; Number of compounds = 13; Area percentage = 0.55%							
110	31.48	6,10,14-trimethyl-2-pentadecanone (Hexahydrofarnesyl acetone)	000502-69-2	0.250	2136 (2118)	**	MS
111	24.60	4-cyclopentene-1,3-dione	000930-60-9	0.090	1612 (1625)	**	MS,RI
112	20.99	nonanal	000124-19-6	0.060	1409 (1399)	**	MS,RI
113	45.13	methyl vanillyl ketone	002503-46-0	0.040	2668 (2702)	1490 (1492)	MS,RI
114	20.33	1-hydroxy-2-pentanone	064502-89-2	0.030	1377	814 (824)	MS,RI
115	22.85	3-hydroxy-2-pentanone	003142-66-3	0.020	1510		MS^{T}
116	40.12	benzophenone	000119-61-9	0.020	2536 (2484)	1603 (1606)	MS,RI
117	21.31	3-octen-2-one	001669-44-9	0.020	1427 (1420)	**	MS,RI
118	19.33	2,3-octanedione = acetyl caproyl	000585-25-1	0.010	1329 (1331)	**	MS,RI
119	16.22	2-heptanone = methyl amyl ketone	000110-43-0	0.010	1194 (1184)	**	MS,RI
120	26.85	propiophenone = phenyl ethyl ketone	000093-55-0	0.004	1763	1139 (1143)	MS,RI
121	20.88	2-nonanone = methyl heptyl ketone	000821-55-6	0.002	1404 (1392)	**	MS,RI
122	17.98	3-octanone	000106-68-3	0.001	1268 (1260)	**	MS,RI
Chemical group: Ethers; Number of compounds = 1; Area percentage = 0.03%							
123	22	*p*-methylanisole = *p*-cresyl methyl ether	000104-93-8	0.03	1463	1001 (1000)	MS,RI

**Blank spaces indicate the retention indices values were not available in the library (for reference) and/or were not detected by the GC column.

Δ Retention Indices (RI_{lib}) in this table are sourced from olfactory databases (vcf-online and Firmenich internal database) and/or published papers (Perez Silva et al., 2006; Brunschwig et al., 2012; Zhang and Mueller, 2013).

^{T}Compound is considered tentative.

same level in cured beans of Bourbon vanilla.[14] Interestingly, benzophenones are considered active molecules and are used to treat various pathological conditions.[15-17]

Traces of styrene (no. 96 in **T-1**) can occur naturally in some plants and foods, such as cinnamon, coffee beans and peanuts. It also was reported in *V. fragans* by Klimes and Lamparsky;[11] however, it is likely that the presence of trace levels of styrene as well as bromocyclohexane (no. 94 in **T-1**) in *V. pompona* extract came from plastic packaging. The compounds gamma curcumene (no. 80 in **T-1**) and germacrene were co-eluting and therefore tentatively identified; the similarity of both spectra at trace concentrations in a highly crowded background limited their clear identification, despite the use of the powerful AMDIS program. Both compounds were previously reported in *V. planifolia* species.[13]

Hydrocarbons have previously been identified in the cured beans and extracts of *V. planifolia* and *V. tahitensis*.[6,13,18,19] Here, a copious presence of hydrocarbons, including terpenoids and sesquiterpenes, in *V. pompona* extract was confirmed for the first time.

Phenolic Compositions

The phenolic composition of *V. pompona* extract consisted of 11 compounds. In this extract, the compounds grouped in the phenolic class were not the most numerous (see **F-2**) but were the most abundant chemical class in terms of composition, comprising at least 48.4%. Vanillin was by far the most abundant phenol, followed by anisyl alcohol. The concentrations of vanillin and anisyl alcohol were so high that they saturated the MS detector.

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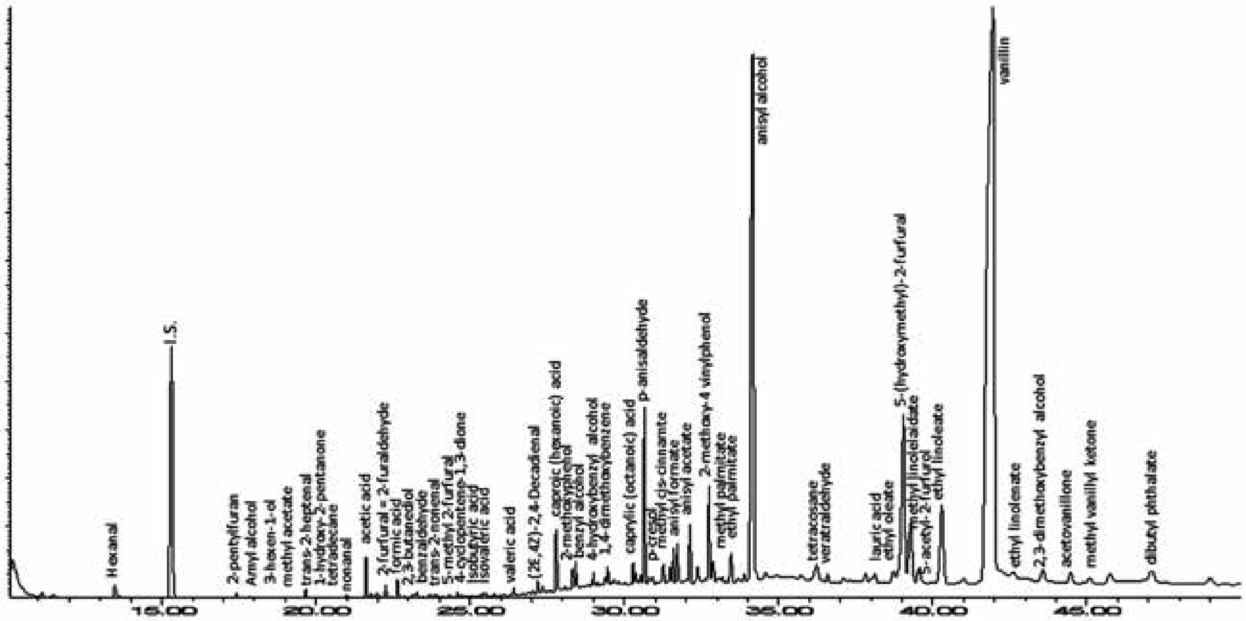
(Continued from Page 22)

F-2. Percent area distributions of volatile compounds in ethanolic-water-dichloromethane extract 3x from cured *V. pompona* beans using 60 m x 0.32 mm id x 1.00 µm df capillary column



The most abundant phenol in this species was vanillin, representing near 45% of the total volatiles profile. This is similar to the range reported in the two other commercial species—i.e., between 50% and 85%.[12] After vanillin, high concentrations of anisyl alcohol (18.85%, see no. 12 in **T-1**) and *p*-anisaldehyde (3.26%, see no. 43 in **T-1**) were found, which is in agreement with their prominence in *V. pompona* (Vanillon), as reported.[4, 5] Other phenol compounds such as *p*-vinyl guaiacol at 2.22%, acetovanillone at 0.52%, 4-methyl guaiacol at 0.28% and guaiacol at 0.20% also were found, but in far less quantities. These findings match those reported by Lee[6] in a study of *V. pompona* from Madagascar.

All phenol compounds found have been reported previously in vanilla, with the exception of 4-methoxyphenol, an isomer of guaiacol, which also is present in honey, licorice, sesame seed and *Alpinia* species. Interestingly, important phenolic compounds such as p-hydroxybenzoic acid and vanillic acid, known as indicators of quality and possibly the origin of commercial vanilla species, were not detected in this *V. pompona* extract using the GC polar column, under the given analytical

T-2. Compounds present in *V. pompona* extract that are newly identified in vanilla and naturally occurring

Compound	Selected natural sources*
trans-2-nonenal	Artichoke, asparagus, bread, carrot, coffee, ginger, grape, melon, olive, peas, peach, strawberry, sesame seed and tea
cis-3-nonenal	Cucumber, watermelon
trans-2-octenal	Acerola, apple, avocado, buckwheat, chamomile, cardamom, cherry, cocoa, citrus fruits, grape, berries, potato, rice, tomato, tea and walnut
2-butenal	Apple, celery, citrus fruits, cocoa, carrot, cabbage, malt, date, guava, olive, peach, peanut, strawberry, peanut, potato and tomato
4-methoxyphenol	Honey, licorice, sesame seed and *Alpinia* species
2-methyl-3-buten-2-ol	Bilberry, cardamom, cherimoya, coffee, cranberry, mango, lemon, lavender oil, black currant and hops
trans-3-penten-2-ol	Calvados, cognac, *Marasmius alliaceus*
amyl formate	Strawberry, honey, strawberry, prickly pear and tomato
p-cresyl methyl ether	Blue cheese, buckwheat, rooibos tea and starfruit
methyl linoleate	Apple, buckwheat, gooseberry, elderberry, pineapple, plum and walnut
ethyl pyrazine	Asparagus, barley, beans, buckwheat, cocoa, coconut, coffee, oats, peanut, soybean, tamarind and wild rice
2,3-octanedione	Coffee, mushroom, pear, peanut, soybean, tea and milk products

**References:* www.vcf-online.nl; www.thegoodscentscompany.com/docs/doc1192811.html conditions. However, trace levels of these constituents were detected using the non-polar GC column. This is in agreement with two previous GC-MS studies.

Perez-Silva et al.[12] reported the absence of *p*-hydroxybenzoic acid in *V. planifolia* Jackson, and attributed this absence to the polarity of the column used. Also, Brunschwig et al.[20] noted the absence of these compounds in *V. tahitensis* and explained this was due either to low concentrations of the vanillyl compounds, or their co-elution with other compounds. On the other hand, Ehlers and Pfister[4] characterized *V. pompona* using HPLC and reported the presence of vanillic acid and *p*-hydroxybenzoic acid–and HPLC is known to be the most suitable analytical technique to identify and quantify the presence of these semi-volatiles.

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