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GC–MS and GC–olfactometry analysis of aroma compounds in a representative organic aroma extract from cured vanilla (*Vanilla planifolia* G. Jackson) beans

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Abstract

Volatile compounds from cured vanilla beans were extracted using organic solvents. Sensory analysis showed that the aromatic extract obtained with a pentane/ether (1/1 v/v) solvent mixture provided the extract most representative of vanilla bean flavour. Sixty-five volatiles were identified in a pentane/ether extract by GC–MS analysis. Aromatic acids, aliphatic acids and phenolic compounds were the major volatiles. By GC–O analysis of the pentane/ether extract, 26 odour-active compounds were detected. The compounds guaiacol, 4-methylguaiacol, acetovanillone and vanillyl alcohol, found at much lower concentrations in vanilla beans than vanillin, proved to be as intense as vanillin.

Keywords: Vanilla planifolia; Cured vanilla beans; Representative aroma extract; Aroma analysis; Gas chromatography–olfactometry

1. Introduction

Since the time of the Aztecs, vanilla has been highly prized for its aroma, and it is now considered the most important flavouring in the food and perfume industries. The development of vanilla aroma takes place during processing of green vanilla beans to produce cured vanilla beans, during which the green, odourless fruits become black, highly aromatic pods. The aroma compounds in cured vanilla beans from different countries: Madagascar, Tonga, Costa Rica, Java, Indonesia and Mexico, have been studied (Adedeji, Hartman, & Ho, 1993; Klimes & Lamparsky, 1976). Over one hundred volatile compounds have been detected, including aromatic carbonyls, aromatic alcohols, aromatic acids, aromatic esters, phenols and phenols ethers, aliphatic alcohols, carbonyls, acids, esters and lactones, of which the aldehyde vanillin is the most abundant (Adedeji et al., 1993; Klimes & Lamparsky, 1976; Ranadive, 1994). The level of the aldehydes, vanillin and *p*-hydroxybenzaldehyde and their respective acids (vanillic acid and *p*-hydroxybenzoic acid), in cured vanilla beans is used as an indicator of cured vanilla bean quality for commercial purposes. Although they are important, these compounds alone do not account for the flavour strength and extremely complex structure of this flavouring (Adedeji et al., 1993; Ranadive, 1994).

Aroma compounds from vanilla beans were extracted using several extraction procedures, prior to gas chromatography-mass spectrometry (GC–MS) analysis: extraction using alcohols and organic solvents (Dignum, Kerler, & Verpoorte, 2002; Galletto & Hoffman, 1978), direct thermal

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desorption (Adedeji et al., 1993; Hartman et al., 1992) and solid-phase microextraction (SPME) (Sostaric, Boyce, & Spickett, 2000). To determine the impact flavour compounds from a product, it is essential that the sensory characteristics of the aromatic extracts are as similar as possible to those of the original product before performing GC–MS and GC–olfactometry (GC–O) analysis (Priser-Monsigny & Saint Denis, 2001, chap. 16). Surprisingly, there are no studies in the literature with regard to the representativeness of vanilla aroma extracts subjected to GC–MS and GC–O analysis. Moreover, little has been published on the less abundant volatile flavour constituents of vanilla beans.

The objective of this study was to develop an extraction method for flavour compounds in vanilla beans, to obtain an aromatic extract as representative of vanilla beans as possible, so as to screen for aroma-active compounds by GC–O analysis and to monitor their formation during vanilla bean curing in a forthcoming study. Organic solvents were used to extract flavour compounds, since they enable the extraction of a wide range of organic compounds. Sensory evaluation of the extracts enabled us to identify the most representative aroma extract, which was subjected to GC–FID, GC–MS and GC–O analysis.

2. Materials and methods

2.1. Chemicals

The chemicals were of analytical grade. The reference compounds for GC–FID and GC–MS analysis: guaiacol, phenol, *p*-cresol, vanillin, acetovanillone, vanillyl alcohol, *p*-hydroxybenzaldehyde, *p*-hydroxybenzyl alcohol, vanillic acid, *p*-hydroxybenzoic acid, valeric acid, benzoic acid, myristic acid, anisic acid, 1-octanol, 2-phenylethanol, (*E*)-2,4-decadienal and ethyl vanillin were purchased from Fluka (Saint Quentin Fallavier, France), butyric acid, valeric acid, hexanoic acid, benzyl alcohol, anisyl alcohol, methyl cinnamate and γ-butyrolactone were from Interchim (Montluçon, France), 1,2-propanediol from Prolabo (Lyon, France), 4-vinylphenol from Lancaster (Mühlheim, Germany), and 1-hexanol and *n*-alkane (C5–C27) from Sigma–Aldrich Chimie (Saint Quentin Fallavier, France).

2.2. Extraction of volatile compounds

Cured vanilla beans (*Vanilla planifolia* G. Jackson) from the Tuxtepec region of Mexico were stored at −18 °C. A 300 g sample was chopped into approximately 2.5-cm pieces, frozen in liquid nitrogen, and ground by means of a blender to a fine powder (Dangoumill 300 ball mill. Prolabo, Paris, France). Five hundred milligrammes of powder were suspended in 10 ml of water, to which 80 ml of one of the following organic solvents were added: diethylether (E), a mixture of pentane/diethylether (P/E) (1:1 v/v) and pentane/dichloromethane (P/D) (2:1, v/v). The suspension was spiked with internal standard (100 μg *n*-hexanol) and homogenized with a Potter Elvejhem homogenizer for 2 min at ambient temperature. The upper organic phase was recovered; it was dried over anhydrous sodium sulfate and concentrated at 42 °C to a volume of 1 ml in a Vigreux column. The organic extracts were stored at −18 °C pending GC–FID and GC–MS analysis. The extractions were performed in triplicate.

2.3. GC–FID, GC–MS and GC–O analysis

A Varian 3380 gas chromatograph equipped with an FID detector was used for quantification. The levels reported are in ppm (mg/kg of cured vanilla) as internal standard equivalent. Volatiles were separated on a DB-Wax (J&W Scientific, Folsom, CA, USA) fused silica capillary column (30 m, 0.32 mm i.d., 0.25 μm film thickness), preceded by a 2 m × 0.32 mm uncoated precolumn. Hydrogen was the carrier gas (2 ml/min). The oven temperature was set at 40 °C for 3 min, then raised to 245 °C at 3 °C/min, and held at this temperature for 20 min. The on-column injector (injection volume 2 μl) was heated from 20 to 245 °C at 180 °C/min and held at this temperature for 90 min. The detector and injector temperatures were 250 °C.

The GC–O analyses were conducted using a Hewlett–Packard 5890 equipped with an FID detector and sniffing port. The column and analysis conditions were as above. The gas chromatography effluents of 4 μl of organic extract were split between the sniffing port and FID (1/1). The temperature of the FID and the sniffing port was 245 °C. The sniffing test on the vanilla bean P/E extract was performed in two chromatographic runs by two trained persons (in an alternative order at 20 min intervals) with reference compounds. If the testers did not use the same attribute for an aroma eluted by GC, only the descriptors belonging to the same aroma attribute were retained.

GC–MS analysis was carried out using a Hewlett–Packard 6890 gas chromatograph coupled to a Hewlett–Packard 5973 quadrupole mass spectrometer. The capillary column used and gas chromatography analysis conditions were as above. Helium was the carrier gas (1.1 ml/min). The injected volumes were 2 μl. The electron impact energy was 70 eV and the ion source and quadrupole temperatures were set at 230 °C and 150 °C, respectively. Electron impact (EI) mass spectra were recorded in the 40–600 amu range at 1 s intervals. Compounds were identified on the basis of linear retention index, and EI mass spectra from the literature or from reference compounds. The linear retention index (RI) was calculated using *n*-alkanes (C5–C27) as a reference.

2.4. HPLC analysis

The major volatile compounds in vanilla beans (vanillin, vanillic acid, *p*-hydroxybenzaldehyde and *p*-hydroxybenzoic acid) were quantified by HPLC analysis. One millilitre of organic extract obtained by extracting 500 mg of vanilla

powder with ether/pentane (see above) was evaporated to dryness under a nitrogen flux. The residue was added to 5 ml of the mobile phase (35% methanol– 65% 10^{-2} M H_3PO_4) and passed through a 0.45 μm filter prior to HPLC analysis.

The HPLC system was a Thermo Separation Products P100 fitted with a manual injector and a Licrospher 100 (Merck) RP18 (5 μm) column (250 mm long and 4 mm i.d). The injection volume was 20 μl and detection was conducted at 254 nm. The temperature of the column was maintained at 30 °C. An isocratic elution was applied with a flow rate of 0.7 ml/min. The compounds were quantified using the external standard technique. Solutions of vanillin, vanillic acid, *p*-hydroxybenzaldehyde and *p*-hydroxybenzoic acid, at concentrations from 1 to 100 mg/l, were separately prepared in the mobile phase and injected into the HPLC system described above to build the calibration curve.

2.5. Sensory evaluation

The representativeness of the vanilla bean aroma extracts obtained with the above three organic solvents was assessed by triangle tests to determine whether a perceptible difference exists between these extracts and vanilla bean powder (Stone & Sidel, 1985, chap. 5).

The panel consisted of 17 assessors. Fifteen-milligrammes of vanilla powder and organic aroma extracts were placed in a brown flask. The solvent from the organic aroma extracts was eliminated under a nitrogen flux. The flasks were then hermetically sealed. The assessors received, in each run, three coded samples, consisting of one of the organic aroma extracts and vanilla bean powder. They smelled the samples and indicated the different sample. The significance of the test was evaluated by binomial distribution from the tables (Stone & Sidel, 1985, chap. 5).

3. Results and discussion

3.1. Extraction of volatiles from vanilla beans

Cured vanilla beans used in this work were stored at −18 °C prior to analysis. The formation of the volatiles during the extraction process, up to adding of the organic solvent, can also be excluded, due to the use of liquid nitrogen to prepare the vanilla powder. Since the volatiles from vanilla beans represent a wide range of functional groups, organic solvents that differed in polarity were tested. Furthermore, solvents with a low boiling point and capable of giving an aroma extract representative of the original product (Abbott, Etiévant, Langlois, Lesschaeve, & Issanchou, 1993; Aznar, López, Cacho, & Ferreira, 2001; Moio et al., 1995; Sarrazin, Le Quéré, Gretsh, & Liardon, 2000) were chosen. The solvents used were ether, pentane/ether (1/1 v/v) and pentane/dichloromethane (2/1 v/v). Preliminary studies (results not shown) showed that water favours the extraction of volatiles, but that the quantity of water added must be controlled, since some water-soluble compounds (e.g., vanillin and acetic acid) may remain in the aqueous phase, thereby reducing their concentration in the organic phase. Acids and phenolic compounds were predominant in the vanilla extracts. The volatile compounds identified (65) included 25 acids, 15 phenolic compounds, 10 alcohols, 4 aldehydes, 4 heterocyclic compounds, 4 esters, 2 hydrocarbons and 1 ketone (Table 1). 2-Heptenal, (*E*)-2-decenal and 2-heptenoic acid were tentatively identified. These compounds were not reported hitherto in vanilla beans.

Clear differences were found between the numbers of aroma compounds identified in each organic aroma extract, starting from the same amount of vanilla powder. Extraction with ether gave 54 volatiles, that with P/E yielded the highest number of compounds (65 volatiles), whereas that with the P/D solvent yielded only 41 volatiles. Only 10 volatile compounds were reported in an ether extract from *V. planifolia* (*Vanilla fragrans*) beans from Madagascar (Nakazawa et al., 1981). They were mainly phenolic compounds and furfural. In a study of the changes of key compounds during the curing process, ten volatile compounds were reported in a pentane/dichloromethane (60:40) extract of vanilla beans (Dignum et al., 2002). Shiota and Itoga (1975) identified 22 volatiles in alcoholic extracts from vanilla beans. Re-extracting the alcoholic extract with P/D (1/1 v/v) allowed the identification of 1-octen-3-ol and methyl cinnamate, which in this study were not identified in the P/D (2/1, v/v) extract, but only in the E and P/E extracts. This difference could be due to the polarity of the solvents. A further difference with this study is that anisaldehyde was not detected in the present study. This is because anisaldehyde occurs in trace amounts in *V. planifolia*, whereas it is abundant in *Vanilla tahitensis* (Adedeji et al., 1993; Ranadive, 1994).

Benzyl ethers, such as vanillyl methyl ether, vanillyl ethyl ether, *p*-hydroxybenzyl methyl ether and *p*-hydroxybenzyl ethyl ether have been reported in cured vanilla beans (Galletto & Hoffman, 1978; Klimes & Lamparsky, 1976). In this study, only vanillyl methyl ether was detected. The absence of benzyl ethyl ethers may be explained by the fact that we did not use ethyl alcohol for vanilla bean extraction (Galletto & Hoffman, 1978).

p-Hydroxybenzoic acid, one of the abundant volatile compounds in vanilla beans, could not be detected under our GC–MS analysis conditions because of the polarity of the column used. Its presence in the organic extracts was demonstrated by HPLC analysis.

In terms of hydrocarbons, tricosane and pentacosane were identified in the P/E and P/D extracts, but not in the ether extract, which may be due to the polarity of this solvent. These hydrocarbons have previously been identified in vanilla beans (*V. planifolia*, *V. tahitensis* and *V. madagascariensis*) from Madagascar, La Réunion and Tahiti (Nakazawa et al., 1981).

Using a direct thermal desorption technique without prior solvent extraction, Adedeji et al. (1993) identified 61

Table 1
Volatile compounds detected in aroma extracts from cured vanilla beans obtained using various organic solvents

Compounds	RI_{DB-WAX}	$RI_{literature}$	E	P/E	P/D	Reliability of identification[a]
Phenols						
Guaiacol	1802	1840	*	*	*	1
4-Methylguaiacol	1897	–		*	*	1
Phenol	1946	1932	*	*		1
p-Cresol	2020	2041	*	*	*	1
4-Vinylguaiacol	2129	2200		*		2
Vanillyl methyl ether	2226	–		*	*	2
4-Vinylphenol	2298	–	*	*		2
Vanillin	2414	2449	*	*	*	1,3
Acetovanillone	2449	–	*	*	*	1
Vanillyl alcohol	2567	–	*	*	*	1
Vanilloylmethyl cetone	2572	–	*	*		2
p-Hydroxybenzaldehyde	2650	–	*	*	*	1,3
p-Hydroxybenzyl alcohol	2663	–	*	*		1
Vanillic acid	3060	–	*	*	*	1,3
p-Hydroxybenzoic acid[a]	–	–	*	*	*	3
Aliphatic acids						
Acetic acid	1396	1434	*	*	*	1
Propanoic acid	1492	1523	*	*	*	1
Isobutyric acid	1525	1557	*	*		2
Butyric acid	1584	1610	*	*		1
Isovaleric acid	1627	1674	*	*	*	1
Valeric acid	1694	1700	*	*	*	1
Hexanoic acid	1799	1829	*	*	*	1
Heptanoic acid	1909	1904	*	*	*	2
Octanoic acid	2014	2058	*	*	*	1
2-Heptenoic acid	2029	–	*	*	*	2
Nonanoic acid	2120	2110	*	*	*	2
Dodecanoic acid	2370	–	*	*		2
Myristic acid	2521	–	*	*		1
Pentadecanoic acid	2593	–	*	*		2
Hexadecanoic acid	2667	–	*	*	*	2
9-Hexadecanoic acid	2690	–		*		2
Heptadecanoic acid	2740	–	*	*		2
Stearic acid	2808	–	*	*		2
Oleic acid	2933	–	*	*		2
Linoleic acid	2954	–	*	*		2
Aromatic acids						
Benzoic acid	2320	2444	*	*		1
Benzene propanoic acid	2451	–	*	*	*	2
Cinnamic acid *(isomer 1)*	2558	–	*	*	*	1
Cinnamic acid *(isomer 2)*	2614	–	*	*	*	2
Anisic acid	2617	–	*	*		1
Alcohols						
1-Octen-3ol	1420	1438	*	*		1
2,3-Butanediol *(isomer 1)*	1500	1494	*	*	*	2
1-Octanol	1531	1553	*	*		1
2,3-Butanediol *(isomer 2)*	1537	–	*	*	*	2
1,2-Propanediol	1549	–	*	*		1
Benzyl alcohol	1822	1822	*	*	*	2
2-Phenylethanol	1857	1859	*	*	*	1
Benzene propanol	1990	1993	*	*		2
Anisyl alcohol	2207		*	*	*	1
Cinnamyl alcohol	2211	2207	*	*	*	2
Aldehydes						
2-Heptenal	1292	1243	*	*	*	2
(*E*)-2-decenal	1616	1611		*	*	2
(*E*,*Z*)-2,4-decadienal	1729	1710	*	*	*	1
(*E*,*E*)-2,4-decadienal	1774	1793	*	*	*	2

(*continued on next page*)

Table 1 (*continued*)

Compounds	$RI_{DB\text{-}WAX}$	$RI_{literature}$	E	P/E	P/D	Reliability of identification[a]
Esters						
Methyl salycilate	1716	1745		*		2
Methyl cinnamate	2011	2048		*		1
Anisyl formate	2467	–		*		2
Ethyl linolenate	3011	–	*	*	*	2
Hydrocarbons						
Tricosane	2300	2300		*	*	2
Pentacosane	2500	2500		*	*	2
Heterocyclics						
Furfural	1412	1449	*	*	*	1
γ-Butyrolactone	1566	1635	*	*	*	1
Pantolactone	1964	2033		*	*	2
1H-pyrrole-2,5-dione, ethyl-4-methyl	2198	–	*	*	*	2
Ketone						
3-Hydroxy-2-butanone	1237		*	*	*	1

(P/E) pentane/diethyl ether extract, (E) ether extract, (P/D) pentane/dichloromethane extract.
*, detected.
[a] Key for reliability of identification: 1, identified by linear retention index and mass spectrum of reference compounds available in the laboratory; 2, tentatively identified by linear retention index (RI) and mass spectrum similar to published data; 3, identified by retention times of reference compounds in HPLC analysis.

volatile compounds in Mexican vanilla beans, although only 9 of those were phenolic compounds, compared to the 15 identified in this study. Eight furanoid and pyranone compounds were detected by Adedeji et al. (1993), while they were not found in this study, except for furfural. They may have formed from sugars as a result of the desorption temperature (220 °C, 5 min) used during GC analysis of the vanilla bean samples.

Solid-phase microextraction (SPME), a solventless extraction technique, was used to analyse the volatile components in ethanolic extracts from vanilla beans from different origins and in synthetic vanilla flavourings (Sostaric et al., 2000). The aroma profile by this technique may be influenced by the fibre chosen, by the matrix and competition between compounds in terms of adsorption by fibres (Roberts, Pollien, & Milo, 2001; Sostaric et al., 2000).

3.2. Choice of an organic solvent to produce representative vanilla aroma extracts

The sensory evaluation of vanilla aroma extracts obtained with three organic solvents was performed to select an extract representative of the odour of the vanilla beans, since aroma may be modified by the extraction procedure (Etiévant & Langlois, 1999). It was essential to remove the organic solvents from the extract to prepare the sample for evaluation by the panellists. The aroma extracts were mixed with water and the organic solvents evaporated under vacuum, as described (Priser-Monsigny & Saint Denis, 2001, chap. 16). However, the aroma of the recovered aqueous extract was substantially modified, possibly because of volatile compound loss or modification during evaporation. Alternatively, solvent removal under a nitrogen flux has been used in various studies (Abbott et al., 1993; Ferreira, Hernández-Orte, Escudero, Lopez, & Cacho, 1999) and, in the present study, this was found to be the best way of removing the solvent without modifying the aroma of the extracts. The aroma extracts were presented in a brown flask, as were the powdered vanilla beans. We rejected the use of smelling strips for sensory analysis, as they modified the aroma of organic extracts, but they could be used for the sensory evaluation of coffee aroma extracts (Sarrazin et al., 2000).

In the sensory evaluation of the extracts (17 assessors) by triangle tests, only the aroma of the P/E extract was found not to differ from the original sample ($p < 0.05$). Accordingly, the P/E extract was chosen for further work to quantify aroma volatiles in vanilla beans and perform GC–O analysis.

3.3. Quantification of the aroma compounds in the P/E extract

Vanillin, vanillic acid, *p*-hydroxybenzaldehyde and *p*-hydroxybenzoic acid were the major compounds in the P/E aroma extract from vanilla beans (see Table 2), as in previous studies (Ranadive, 1994; Saltron, Langella, & Guerere, 2002). These compounds could not be quantified by GC/FID because of the saturation of the detector. They were therefore quantified by HPLC analysis. To verify whether P/E was capable of extracting these compounds efficiently, vanilla powder was also subjected to ethanol extraction by the reference Soxhlet method, which takes 16 h (NF ISO 5565-1, 2000). The concentrations of the above four compounds in the P/E extract were similar to those obtained with the Soxhlet method. Nakazawa et al.

Table 2
Concentrations of volatile compounds in pentane/ether extract from cured vanilla beans

Compounds	ppm[a]	SD
Phenols		
Guaiacol	9.3	±0.49
4-Methylguaiacol	3.8	±0.35
Phenol	1.8	±0.14
p-Cresol	2.6	±0.35
4-Vinylguaiacol	1.2	±0.06
Vanillyl methyl ether	<1	–
4-Vinyl phenol	1.8	±0.15
Vanillin[b]	19118	±1224.71
Acetovanillone	13.7	±0.92
Vanillyl alcohol	83.8	±2.97
Vanilloyl-methyl cetone	2.2	±0.28
p-Hydroxybenzaldehyde[b]	873.3	±55.65
p-Hydroxybenzyl alcohol	65.1	±4.88
Vanillic acid[b]	1315	±77.78
p-Hydroxybenzoic acid[b]	255	±13.44
Aliphatic acids		
Acetic acid	124.3	±11.10
Propanoic acid	1.7	±0.35
Isobutyric acid	1.7	±0.14
Butyric acid	<1	
Isovaleric acid	3.8	±0.21
Valeric acid	1.5	±0.01
Hexanoic acid	<1	
Heptanoic acid	1.9	±0.14
Octanoic acid	5.5	±0.49
2-Heptenoic acid	1.7	±0.21
Nonanoic acid	15.7	±1.73
Dodecanoic acid	2.2	±0.14
Myristic acid	12.4	±1.27
Pentadecanoic acid	13.4	±0.42
Hexadecanoic acid	126.6	±5.94
9-Hexadecanoic acid	5.7	±1.34
Heptadecanoic acid	5.7	±0.28
Stearic acid	13.9	±0.07
Oleic acid	16.3	±1.56
Linoleic acid	225.6	±17.25
Aromatic acids		
Benzoic acid	2.6	±0.35
Benzene propanoic acid	3.9	±0.28
Cinnamic acid *(isomer 1)*	3.4	±0.57
Cinnamic acid *(isomer 2)*	9.5	±1.13
Anisic acid		
Alcohols		
1-Octen-3ol	<1	–
2,3-Butanediol *(isomer 1)*	16.5	±1.77
1-Octanol	1.1	±0.08
2,3-Butanediol *(isomer 2)*	8.0	±0.14
1,2-Propanediol	<1	–
Benzyl alcohol	2.7	±0.21
2-Phenylethanol	1.0	±0.02
Benzene propanol	<1	–
Anisyl alcohol	2.4	±0.14
Cinnamyl alcohol	<1	–
Aldehydes		
2-Heptenal	2.1	±0.28
(*E*)-2-decenal	1.8	±0.16
(*E*,*Z*)-2,4-decadienal	1.4	±0.11
(*E*,*E*)-2,4-decadienal	1.2	±0.07
Esters		
Methyl salycilate	<1	–
Methyl cinnamate	1.1	±0.07
Anisyl formate	2.3	±0.35
Ethyl linolenate	13.5	±0.35
Hydrocarbons		
Tricosane	15.9	±2.19
Pentacosane	19.9	±1.48
Heterocyclics		
Furfural	<1	–
γ-butyrolactone	<1	–
Pantolactone	1.4	±0.14
1H-pyrrole-2,5-dione, ethyl-4-methyl	1.8	±0.35
Ketone		
3-Hydroxy-2-butanone	14.6	±0.85

[a] In mg/kg of cured vanilla.
[b] Quantified by HPLC (SD) standard deviation.

(1981) compared the effect of the solvent (ethanol, benzene, acetone, *n*-hexane, ether, ethylacetate and water) on extraction of the main compounds from *V. planifolia* beans. Vanillic acid, cinnamic acid and vanillin were among the major compounds, but in very small amounts compared to the present study. The levels of these compounds in vanilla beans from different countries reported by Adedeji et al. (1993) were lower than those found in this study. Vanillin represented 50% of the total quantified volatiles in Bourbon vanilla and 30% in Mexican vanilla whereas, in the present study, the vanillin concentration (19118 ppm) represented 85% of the volatile compounds. Adedeji et al. (1993) used a direct thermal desorption technique (220 °C) to analyse the volatiles from beans that may cause the thermal degradation and transformation of sugar into common volatile compounds such as 3,5-dimethyl-2,4(3H,5H)-furandione and 3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one. This last compound was detected at a high concentration (3880 ppm) in Mexican vanilla, being the third most abundant compound after vanillin and 2-furfural, and far more abundant than vanillic acid, *p*-hydroxybenzaldehyde and *p*-hydroxybenzoic acid (Adedeji et al., 1993). Of these compounds, only furfural was detected in this study, but at low level (less than 1 ppm).

The fatty acid fraction represented 0.45% and 1.5% of volatiles in vanilla beans, primarily comprising oleic and palmitic acids (Ramachandra Rao & Ravishankar, 2000). Relatively higher concentrations of linoleic and hexadecanoic acids were found in this study (225.6 and 126.6 ppm, respectively). Four benzyl ethers were identified in Bourbon vanilla, one of which was vanillylmethyl ether at a concentration of 13 ppm (Galletto & Hoffman, 1978). It was also identified in this study in both the P/E and P/D extracts, but at a concentration of less than 1 ppm. The high levels may be due to artifact formation as a result of extraction, as previously mentioned. Furthermore, fifteen

Table 3
Aroma-active compounds detected by GC–O analysis of a representative aroma extract from cured vanilla beans

Compounds	ppm	Odor quality	Intensity[a]
Phenols			
Guaiacol	9.3	Chemical, sweet spicy	+++
4-Methylguaiacol	3.8	Sweet, woody	+++
p-Cresol	2.6	Balsamic, woody, spicy	++
4-Vinylguaiacol	1.2	Chemical, phenolic	+
4-Vinylphenol	1.8	Sweet, woody	++
Vanillin	19118	Vanilla, sweet	+++
Acetovanillone	13.7	Vanilla, sweet, honey	+++
Vanillyl alcohol	83.8	Vanilla-like	+++
p-Hydroxybenzaldehyde	873	Vanilla-like, biscuit	++
p-Hydroxybenzyl alcohol	65.1	Vanilla-like, sweet	++
Aliphatic acids			
Acetic acid	124	Sour, vinegar	++
Isobutyric acid	1.7	Buttery	++
Butyric acid	<1	Buttery, oily	+
Isovaleric acid	3.8	Buttery, oily	++
Valeric acid	1.5	Cheese	+++
Alcohols			
2,3-Butanediol (isomer 2)	8.0	Floral, oily	+
Anisyl alcohol	2.4	Herbal	++
Aldehydes			
2-Heptenal	2.1	Green, oily	+
(*E*)-2-decenal	1.8	Herb-like, floral	++
(*E*,*Z*)-2,4-decadienal	1.4	Herb-like, fresh	++
(*E*,*E*)-2,4-decadienal	1.2	Fatty, wood	++
Esters			
Methyl salicylate	<1	Chalk	+++
Methyl cinnamate	1.1	Sweet	++
Ethyl linolenate	13.5	Sweet	++
Ketone			
3-Hydroxy-2-butanone	14.6	Buttery	+
Unknown[b]	6.2	vanilla-like, chemical	+++

[a] (+) Weak, (++) Medium, (+++) Strong.
[b] Mass fragmentation (91(90), 74(37), 69(34), 89(25), 57(24)) and RI (2528).

compounds occurring at levels of less than 1 ppm were found in vanilla beans in this study.

3.4. GC–O analysis of vanilla aroma extracts

An aroma extract obtained by P/E extraction of vanilla beans was subjected to GC–O analysis. Twenty-six aroma-active compounds were perceived by panellists (Table 3). Two of the twenty-six compounds detected were found at concentrations of less than 1 ppm, 13 at concentrations of less than 4 ppm, 6 at less than 20 ppm, 3 at less than 150 ppm and 2 at more than 150 ppm.

Ten phenolic compounds were detected in vanilla extracts as being aroma-active. Guaiacol, 4-methylguaiacol and acetovanillin, occurring at concentrations of 3.8–13.7 ppm, were similar in intensity to vanillin, which was detected at a concentration of more than 1000 times that of these compounds. Methyl salicylate, detected at a level of less than 1 ppm, was perceived as being as intense as vanillin. *p*-Cresol, methyl cinnamate and anisyl alcohol, occurring at concentrations of 1.1–2.4 ppm, were of medium intensity. Sweet, woody, balsamic, spicy, vanilla-like and toasted notes were attributed to phenolic compounds. Vanillic acid was not perceived by panellists, because its elution required a high temperature, which caused a burnt odour in the sniffing port. The aldehydes 2-heptenal and (*E*)-2-decenal, identified here for the first time in vanilla beans, were perceived as being of medium intensity, with green, oily and herb-like floral notes. Aliphatic, acetic, isobutyric, isovaleric and valeric acids were perceived by the panellists as having sour, buttery and oily notes.

In conclusion, this study is in agreement with previous observations of the contribution of minor constituents to the overall aroma of cured vanilla beans, through GC–O analysis of a representative aroma extract from cured vanilla beans.

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