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Chemical Investigation and Authenticity of Indian Vanilla Beans

T. V. JOHN* AND E. JAMIN

Corporate Research and Development, International Flavors & Fragrances Inc., Union Beach, New Jersey 07735, and Eurofins, Rue Pierre Adolphe Bobierre, B.P. 42301, F-44323 Nantes, France

Representative and validated samples taken from a 500 acre vanilla (*Vanilla planifolia* Andrews) plantation in India have shown significant deviations in aromatic profile, especially the relative amounts of vanillin (high) and *p*-hydroxybenzaldehyde (low) and the deuterium isotopic (SNIF-NMR) values. However, the carbon isotopic values (carbon 13 profiles) were generally in accordance with the previous findings on vanilla from other geographic origins.

KEYWORDS: Vanilla beans; authenticity; deuterium isotopic values; carbon isotopic values

INTRODUCTION

Vanilla being the world's most widely used flavoring, International Flavors & Fragrances Inc. (IFF) pursued a vertical integration program to produce high-quality vanilla beans. As a result, IFF has access to a 500 acre vanilla (*Vanilla planifolia* Andrews) plantation in India.

One of the objectives of this vanilla vertical integration program was to achieve full compliance with U.S. and European guidelines of vanilla beans (*1*, *2*). A vanilla germplasm program and genetic screening studies (DNA fingerprinting) ensured that the plants at the plantation are true *V. planifoli*a Andrews. The curing process also conforms to the guidelines of U.S. Standard of Identity for vanilla (*1*). The location of the plantation (southern India) is within 25° of the equator as are all other major vanilla-producing sites in the world (*3*).

The appearance, aroma, and flavor profile of properly cured Indian vanilla beans were similar or very close to those of beans from Madagascar (*4*; IFF internal data). However, chemical analysis has shown that the ratios of the major vanilla aromatic compounds—vanillin, *p*-hydroxybenzaldehyde (pHB), *p*-hydroxybenzoic acid (pHB acid), and vanillic acid—of the cured beans have been different from the very first crop compared to the International Organization of the Flavor Industry (IOFI) guideline values (*5*). This was the case despite the type of curing process (traditional bourbon or IFF's own accelerated process) used for curing the beans.

In view of the above, it was felt necessary to develop a validated and authentic database on the chemical nature of the Indian beans to establish the authenticity. Eurofins Laboratory was therefore approached to develop such a database. This paper covers the authenticated data on the aromatic profile, carbon isotopic values, and deuterium isotopic (SNIF-NMR) values of the Indian beans.

This study had two components: (1) main study—authenticity screening of mature vanilla beans; (2) supplementary study—authenticity screening of immature vanilla beans.

* Author to whom correspondence should be addressed (E-mail: t.v.john@iff.com).

Table 1. Vanilla Aromatic Ratios as per DGCCRF N.S. 5387

ratio between flavor components	ratio range
R1 = vanillin/pHB	10–20
R2 = vanillic acid/pHB	0.53–1.00
R3 = PHB acid/pHB	0.15–0.35
R4 = vanillin/vanillic acid	15–29
R5 = vanillin/pHB acid	53–110

The supplementary study was primarily intended to determine whether the age of the beans has any effect on the isotopic values.

According to the IOFI, three major analytical approaches are currently applied to check the authenticity of natural vanilla products from *V. planifolia* Andrews (*2*). They are (1) evaluation of the ratios between specific components (referred to in this paper as the aromatic profile), (2) isotope—mass spectrometry (referred to in this paper as carbon isotopic values), and (3) site-specific quantitative deuterium NMR (referred to as deuterium isotopic values). The aromatic profile and carbon isotopic values are based on guidelines developed by the DGCCRF (*5*).

Aromatic Profile. The first approach refers to the ratios of concentrations of some typical vanilla components that are determined by HPLC. This measures the amount of vanillin, pHB, vanillic acid, and pHB acid, as well as the ratios between those components. These specifications (ratios of vanilla components) were initially established by the French government in 1988 in a document titled DGCCRF N.S. 5387, which was eventually adapted by the IOFI as a guideline regarding vanilla authenticity (*6*). The ranges of these ratios for vanilla beans and standard vanilla extracts are given in **Table 1**.

Recent surveys (*7*) have shown that the products found on the European market, especially those imported from Madagascar, were showing slightly different ratios of vanilla components, and on the basis of these figures the Syndicat National des Industries de l'Aromatique Alimentaire (SNIAA), representing the flavor industry in France, has requested a revision of the ranges for the ratios. Very recently DGCCRF has published

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Table 2. Revised Vanilla Aromatic Ratios as per DGCCRF N.S. 2003-61

ratio between flavor components	revised range
R1 = vanillin/pHB	10–20
R2 = vanillic acid/pHB	0.53–1.5
R3 = PHB acid/pHB	0.15–0.35
R4 = vanillin/vanillic acid	12–29
R5 = vanillin/pHB acid	40–110

a notification (*5*) revising the ranges for the vanilla aromatic ratios, and the revised ranges are given in **Table 2**.

Carbon Isotopic Values. This analysis consists of the determination of the carbon 13 deviation of each flavor component (pHB, vanillin, pHB acid, and vanillic acid), defined as follows:

$$\delta^{13}C_X\ (‰) = [(^{13}C/^{12}C)_X - (^{13}C/^{12}C)_{VPDB})]/(^{13}C/^{12}C)_{VPDB} \times 1000$$

where X represents the compound of interest and VPDB is an international reference for $\delta^{13}C$ values.

Gas chromatography−combustion−isotope ratio mass spectrometry (GC-C-IRMS) is a hyphenated technique that enables the simultaneous on-line measurement of volatile compounds separated by GC and offers the advantage of a wide range of applications, from the vanilla beans to the finished vanilla-flavored food product (*8*). All of the literature available from both IRMS and GC-C−IRMS studies indicates that because vanilla is a Crassulacean acid metabolism (CAM) plant, the C13 values of the above components are enriched in carbon 13 versus their synthetic counterparts, thus allowing detection of frauds (*2*, *4*−*6*).

The DGCCRF N.S. 5387 (*1*) defined minimum values for the $\delta^{13}C$ of vanillin (−21 ± 0.5‰) and pHB (−22 ± 0.5‰). However, these values (especially the pHB one) have been criticized because they were not reflecting experimental findings on authentic beans (*7*). Moreover, it is more suitable to define minimum limits directly, including both the analytical uncertainty and the natural variability. It has been acknowledged by DGCCRF that these specifications needed to be changed, and they have proposed the following minima as new limits in the "note d'information no. 2003-61": −21.2‰ for vanillin and −19.2‰ for pHB (*5*). Moreover, additional minimum limits have been proposed for the corresponding acids: −24.0‰ for vanillic acid and −23.0‰ for pHB acid (*5*).

The results of the corresponding analyses performed on the Indian beans are reported under Results and Discussion.

Deuterium Isotopic values. With the aid of site-specific natural isotope fractionation−nuclear magnetic resonance (SNIF-NMR), it is possible to examine the components of various biomolecules to a greater degree of precision than was formerly possible (*9*). Whereas conventional methods are applicable only for characterization at a molecular level, SNIF-NMR is sensitive at the atomic level and produces a unique and distinctive isotopic "fingerprint" for a variety of substances.

This fingerprint is created on the basis of the atomic composition of biomolecules. The atoms of biological molecules occur in differing atomic masses of which the different versions are known as isotopes (*10*). Depending on its origin and the processing and production techniques applied to a substance, its molecule will differ with respect to the proportion of isotopes at each molecular position. By determining these parameters using NMR techniques, a typical isotopic profile can be created, which can be looked at as the molecule's "isotopic fingerprint".

SNIF-NMR analysis of the vanillin and of the pHB of the Indian beans was undertaken.

This analysis consists of the determination of site-specific isotopic ratios $(D/H)_i$ of each site within the molecules by quantitative deuterium NMR. It is the most sophisticated and the most powerful method to date to control the authenticity of vanilla components (*9*).

MATERIALS AND METHODS

Sampling. A total of 18 samples were selected for analysis using the full range of analytical methods described below.

Collection and Curing of Immature Beans for the Supplementary Study. The immature beans were available in late November 2001. A representative of the Institute of Marketecology (IMO) collected samples of freshly picked vanilla beans directly from the vanilla plantation. The IMO is an accredited Organic Certification Company with Headquarters in Switzerland. Eurofins provided a detailed protocol of bean sampling to the IMO representative. The beans have been collected from as varied a source as possible considering all aspects of the 500 acre plantation. The samples were uniquely marked so that their identity was not destroyed in the curing process. The beans thus collected were stored in marked and sealed boxes in a deep freezer for curing later, along with the mature beans (see below).

A total of 11 samples were collected. Five samples were selected to cover as much as possible the space of the plantation and were analyzed. The rest of the samples were stored for possible use, in case the results from the five samples revealed some significant differences between locations within the plantation.

Collection and Curing of Mature Beans for the Main Study. The mature beans were available in December 2001. A representative of Eurofins Scientific collected samples of freshly picked vanilla beans directly from the vanilla plantation. The beans were collected from as varied a source as possible considering all aspects of the 500 acre plantation. The samples were uniquely marked so that their identity was not destroyed in the curing process. Sampling points were coded and reported on a map of the plantation.

A total of 20 samples were collected. Eleven samples were selected to cover as much as possible the space of the plantation and were analyzed. The rest of the samples were stored for possible use, in case the results from the 11 samples revealed some significant differences between locations within the plantation.

Two additional samples of "green beans" were also collected from the same plantation and shipped separately (without curing at the Indian site) and cured within the Nantes (France) laboratory of Eurofins. These samples are referred to as "lab-cured" samples.

The mature beans as well as the stored immature beans were then cured at IFF's proprietary curing facility during Eurofins representative's visit in December 2001. After the initial phase of the curing, which was completed during this visit, the beans were kept in sealed and marked containers until the curing was completed.

Shipment. IMO Controls Pvt. Ltd. inspected the sealed containers on the day they were handed over to the shipping agent (a certificate of this control is available). At reception in Eurofins (Nantes, France), the seals were still intact.

Sample Reception. The samples were given a unique code in the Eurofins database system.

Table 3 exhibits examples on how the sample descriptions were maintained.

Analytical Methods: Determination of Vanillin, pHB, pHB Acid, and Vanillic Acid by HPLC (*11*). The beans were ground and extracted with 99% pure ethanol (Sigma Aldrich, Saint-Quentin Fallavier, France) using a Soxhlet extractor of 125 mL capacity. These extracts were analyzed on a 250 × 4.6 mm column filled with 5 μm Licrospher C18 stationary phase (Merck, Nogent-sur-Marne, France). The eluent consisted of a 80:20 (v/v) mixture of 10^{-3} M phosphoric acid buffer and methanol (Sigma Aldrich). UV detection was at 254 nm. HPLC equipment used included a Waters 2695 Alliance separations module and a Waters 996 photodiode array detector (Waters, St. Quentin en Yvelines, France).

Table 3. Sample Maintenance Method

ref no.	sample description	analyzed or stored
0136/0930	green vanilla pods seal no. 00040 and 00047 sampler, Sébastien Guiet date, July 12, 2001–Aug 12, 2001 curing, January 2002 at Eurofins Laboratories, Nantes, France	analyzed
0136/0931	green vanilla pods seal no. 00051 and 00056 sampler, Sébastien Guiet date, July 12, 2001–Aug 12, 2001 curing, January 2002 at Eurofins Laboratories, Nantes, France	analyzed
0137/2198	mature vanilla beans stake, EF 10 seal no. (collecting) 000088 seal no. (curing) 000232 seal no. (finish product) 000185 sampler, Sébastien Guiet date, July 12, 2001–Aug 12, 2001 curing, Oct 12, 2001–Nov 12, 2001 at Chennai	stored

The moisture content of the beans was determined according to ISO 5565-2 (*11*). The mass spectrometric determinations of the carbon isotope ratio were carried out as described in ref *8*. A Finnigan MAT Delta plus isotope ratio mass spectrometer coupled via a combustion interface to an HP 6890 gas chromatograph (Thermo-electron, Bremen, Germany) was used. The vanilla compounds were separated using a Chrompack CP-Sil 5CB 50 m × 0.32 mm × 1.2 μm capillary GC column. The temperature program was as follows: 15 min isothermal at 180 °C, raised to 250 °C at 5 °C/min. Injection was in split (1/20) mode, and the helium flow rate was 2 mL/min.

The determinations of the site specific isotope ratio $(D/H)_i$ were performed as described in ref *9*.

RESULTS AND DISCUSSION

Aromatic Profile (HPLC Determinations of Flavor Compounds). The raw results displayed in **Table 4** were not corrected for moisture, which might result in inappropriate conclusions, especially if one wants to compare the results from the various categories (lab-cured/immature/mature). Therefore, the moisture content of all the beans was measured, and then the results were expressed calculated on a 25% moisture content and given in **Table 5**.

Table 6 represents the ratios among the four main components computed from **Table 4** on the samples analyzed.

As shown in **Table 5**, the flavor compositions of the mature and lab-cured samples are quite similar, thus validating the use of lab-cured samples as relevant witnesses for the examination of isotopic data.

With regard to the ratios, the following observations can be made:

(1) The most striking observation concerns R1 (vanillin/pHB): all samples (including the lab-cured and the immature beans) have an R1 value above the highest limit of 20 defined in the DGCCRF note d'information (*5*). Because the same trend is observed for all of the categories, it seems that this is a specificity of this plantation as a whole, which could be due to a specific clone selection or specific growing/processing conditions.

(2) The R2 ratio (vanillic acid/pHB) is within the expected range for all of the samples, in accordance with the modified limit of 1.5.

Table 4. HPLC Results (Expressed in Percent w/w of the Raw Product)

ref no.[a]	type of sample	vanillin	pHB	vanillic acid	pHB acid
0136/0930	lab-cured	2.344	0.096	0.053	0.034
0136/0931	lab-cured	2.190	0.082	0.031	0.020
0137/2202	mature	2.623	0.091	0.085	0.029
0137/2203	mature	2.255	0.081	0.065	0.026
0137/2204	mature	2.136	0.087	0.053	0.027
0137/2205	mature	1.850	0.073	0.071	0.032
0137/2206	mature	2.356	0.074	0.084	0.028
0137/2209	mature	1.559	0.060	0.059	0.030
0137/2210	mature	1.847	0.075	0.084	0.030
0137/2212	mature	1.675	0.070	0.059	0.026
0137/2214	mature	2.327	0.087	0.074	0.030
0137/2215	mature	2.117	0.082	0.077	0.026
0137/2216	mature	2.618	0.092	0.094	0.032
mean		2.124	0.079	0.073	0.029
0137/2218	immature	1.304	0.063	0.056	0.028
0137/2221	immature	1.752	0.074	0.066	0.030
0137/2222	immature	1.500	0.061	0.053	0.022
0137/2225	immature	1.877	0.075	0.065	0.031
0137/2227	immature	1.773	0.073	0.061	0.035
mean		1.641	0.069	0.060	0.029

[a] Reference no. 0137/2202–0137/2216 are the 11 mature vanilla bean samples and ref no. 0137/2218–0137/2227 are the immature vanilla bean samples collected from the plantation.

(3) For R3 (pHB acid/pHB) all results are above the minimum limit, and approximately half of them are slightly higher than the upper limit of 0.35, with a maximum at 0.50. This observation, together with the high R1 values and average vanillin content, indicates that vanilla from this plantation tends to have lower pHB content than vanilla from the Indian Ocean origins on which the ranges are based.

(4) R4 (vanillin/vanillic acid) is also sometimes exceeded, especially for the most mature samples with highest vanillin content.

(5) On the other hand, R5 (vanillin/pHB acid) is always within the amended range.

Therefore, everything appears as if there was a relatively high rate of degradation of pHB to pHB acid (explaining high R1 and R3 ratios) and a relatively low rate of degradation of vanillin to vanillic acid (explaining high R1 and R4 ratios).

Carbon Isotopic Values. C13 deviations of the four main components of the vanilla beans are given in **Table 7**. The above $\delta^{13}C$ results are generally in accordance with the values published so far for vanilla components from other geographical origins, as thoroughly reviewed from the literature by Scharrer et al. (*12*), who also presented their own results. Moreover, the values for each compound are very homogeneous and do not indicate any significant difference among immature, maturem and (mature) lab-cured samples. This confirms the well-known fact that carbon 13 deviations are mainly governed by metabolic factors.

Most of the observed values are compliant with the authenticity limits recently proposed by DGCCRF (*8*), but these limits elicit the following comments:

(a) All of the measured $\delta^{13}C$ values of vanillin measured in this study are higher than the new limit of −21.2‰ defined by DGCCRF (*5*). However, on the basis of previous findings on authentic samples from various origins analyzed in several different laboratories, as reviewed in ref *12*, defining the minimum limit for vanillin at −21.5‰ would, in our opinion, be more suitable in order to avoid "false positive cases", as

Table 5. HPLC Results (expressed in Percent w/w of 25% Moisture Beans)

ref no.	type of sample	measured moisture (%)	vanillin (/25% moisture)	pHB (/25% moisture)	vanillic acid (/25% moisture)	pHB acid (/25% moisture)
0136/0930	lab-cured	29.7	2.499	0.102	0.057	0.036
0136/0931	lab-cured	30.7	2.371	0.088	0.063	0.022
0137/2202	mature	4.9	2.069	0.072	0.067	0.022
0137/2203	mature	31.3	2.462	0.088	0.071	0.029
0137/2204	mature	36.9	2.537	0.104	0.063	0.032
0137/2205	mature	21.9	1.777	0.070	0.068	0.030
0137/2206	mature	14.3	2.061	0.065	0.073	0.024
0137/2209	mature	44.9	2.121	0.082	0.081	0.041
0137/2210	mature	15.6	1.641	0.066	0.074	0.026
0137/2212	mature	35.9	1.960	0.082	0.069	0.030
0137/2214	mature	29.8	2.485	0.093	0.079	0.032
0137/2215	mature	33.3	2.380	0.092	0.086	0.029
0137/2216	mature	14.7	2.301	0.080	0.083	0.028
mean			2.163	0.081	0.074	0.029
0137/2218	immature	14.9	1.149	0.056	0.049	0.025
0137/2221	immature	30.9	1.902	0.080	0.071	0.033
0137/2222	immature	39.9	1.873	0.076	0.066	0.027
0137/2225	immature	21.9	1.801	0.072	0.062	0.029
0137/2227	immature	32.4	1.966	0.081	0.068	0.039
mean			1.738	0.073	0.063	0.031

Table 6. Ratios between Flavor Components

ref no.	type of sample	R1 = vanillin/ pHB	R2 = vanillic acid/pHB	R3 = pHB acid/ pHB	R4 = vanillin/ vanillic acid	R5 = vanillin/ pHB acid
0136/0930	lab-cured	24	0.55	0.35	44	70
0136/0931	lab-cured	27	0.38	0.25	70	109
0137/2202	mature	29	0.94	0.31	31	92
0137/2203	mature	28	0.80	0.32	35	86
0137/2204	mature	24	0.61	0.30	40	80
0137/2205	mature	25	0.97	0.43	26	59
0137/2206	mature	32	1.13	0.37	28	85
0137/2209	mature	26	0.99	0.50	26	52
0137/2210	mature	25	1.12	0.40	22	62
0137/2212	mature	24	0.84	0.37	29	65
0137/2214	mature	27	0.85	0.35	31	77
0137/2215	mature	26	0.93	0.31	28	82
0137/2216	mature	29	1.03	0.35	28	82
mean		27	0.92	0.36	29	74
0137/2218	immature	21	0.88	0.44	23	46
0137/2221	immature	24	0.89	0.41	27	58
0137/2222	immature	25	0.88	0.36	28	69
0137/2225	immature	25	0.87	0.41	29	62
0137/2227	immature	24	0.84	0.48	29	51
mean		24	0.87	0.42	27	56

Table 7. δ^{13}C Values (‰/VPDB) of Vanillin, pHB, Vanillic Acid, and pHB Acid

ref no.	type of sample	vanillin	pHB	vanillic acid	pHB acid
0136/0930	lab-cured	–20.5	–18.2	–21.5	–20.0
0136/0931	lab-cured	–20.6	–18.8	–22.3	–21.1
0137/2202	mature	–19.1	–18.0	–22.4	–21.7
0137/2203	mature	–20.2	–19.0	–22.7	–20.5
0137/2204	mature	–19.0	–17.7	–22.3	–20.5
0137/2205	mature	–20.6	–19.5	–24.0	–21.7
0137/2206	mature	–19.8	–18.7	–23.5	–21.3
0137/2209	mature	–20.4	–18.9	–24.3	–21.8
0137/2210	mature	–19.9	–18.7	–23.9	–21.7
0137/2212	mature	–20.5	–18.8	–24.4	–22.0
0137/2214	mature	–20.1	–18.7	–23.3	–21.4
0137/2215	mature	–20.7	–18.2	–23.3	–21.6
0137/2216	mature	–20.5	–18.4	–23.5	–22.7
mean		–20.1	–18.6	–23.4	–21.5
0137/2218	immature	–20.2	–18.2	–23.2	–20.4
0137/2221	immature	–20.3	–18.4	–22.9	–20.7
0137/2222	immature	–19.7	–17.4	–21.2	–19.6
0137/2225	immature	–20.4	–17.7	–22.9	–20.6
0137/2227	immature	–20.5	–18.6	–23.8	–21.5
mean		–20.3	–18.3	–23.3	–21.2

this value is commonly reported as the lowest observed δ^{13}C of vanillin from *V. planifolia* Andrews.

(b) With regard to pHB, Remaud et al. have recommended previously to set the authenticity limit at −19.5‰ (*9*), which tends to be confirmed by the distribution of δ^{13}C values observed in this study. Scharrer et al. have even reported values as low as −20.1‰ for the δ^{13}C of pHB from *V. planifolia* Andrews (*12*). Therefore, we also think that the action limit of −19.2‰ defined by DGCCRF (*5*) could lead to "false positive" results in some cases.

(c) A systematic impoverishment of each acid versus the corresponding aldehyde is observed, as already reported in previous studies (*8*). However, in the case of vanillic acid the impoverishment in carbon 13 versus vanillin is much stronger than what was observed in beans from Madagascar, Indonesia, and Reunion (*8*). Two samples of mature beans are even slightly lower than the proposed limit of −24‰. Because of the lower analytical precision in the case of acids measurement, and because there is less knowledge about their natural distributions, in our opinion such small deviations from the limit should not be regarded as significant by experts. Moreover, it is worth noting that all of the low values for vanillic acid tend to be correlated with low values of vanillin. Therefore, one should consider not only the individual values of each compound separately but also the overall C13 profile for the interpretation of results

Deuterium Isotopic Values: Site-Specific Isotope Ratios of Vanillin and pHB. $(D/H)_i$ ratios of all molecular sites numbered in **Figure 1** have been determined using SNIF-NMR analyses, and the results are given in **Tables 8** and **9**.

The biosynthesis of vanillin and pHB is very complex and still discussed among experts of the metabolic pathways. However, it is well-known that geographic parameters such as

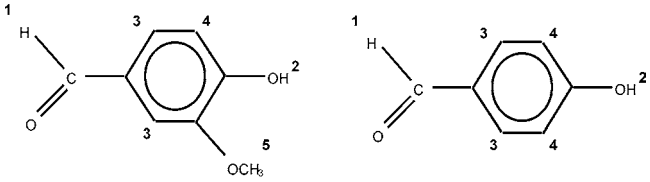

Figure 1. Numbering of molecular sites analyzed by SNIF-NMR (*9*).

Table 8. $(D/H)_i$ (Parts per Million) of Vanillin (18 Samples)

ref no.	type of sample	$(D/H)_1$	$(D/H)_3$	$(D/H)_4$	$(D/H)_5$
0136/0930	lab-cured	129.5	157.0	188.6	126.2
0136/0931	lab-cured	127.1	155.2	189.4	126.7
0137/2202	mature	124.1	157.4	183.3	126.5
0137/2203	mature	130.6	156.7	177.7	125.8
0137/2204	mature	130.4	156.7	183.9	127.0
0137/2205	mature	128.8	156.3	183.9	127.5
0137/2206	mature	124.6	155.2	178.3	125.6
0137/2209	mature	129.6	156.4	179.3	125.5
0137/2210	mature	128.7	156.0	182.7	127.0
0137/2212	mature	131.1	156.4	180.0	125.3
0137/2214	mature	130.1	157.9	180.0	126.5
0137/2215	mature	129.2	154.0	179.7	127.6
0137/2216	mature	124.2	156.3	176.0	125.8
mean		128.3	156.3	180.4	126.4
0137/2218	immature	124.5	155.6	184.0	123.5
0137/2221	immature	123.6	156.9	180.5	125.6
0137/2222	immature	126.9	156.7	178.3	124.9
0137/2225	immature	127.3	156.6	179.7	125.7
0137/2227	immature	126.3	157.3	182.4	125.8
mean		127.3	156.4	180.3	125.8

Table 9. $(D/H)_i$ (Parts per Million) of pHB (10 Samples)

ref no.	type of sample	$(D/H)_1$	$(D/H)_3$	$(D/H)_4$
0136/0930	lab-cured	269.4	152.9	183.0
0136/0931	lab-cured	276.0	154.7	188.0
0137/2202	mature	251.5	154.2	180.4
0137/2203	mature	256.3	152.3	181.3
0137/2204	mature	260.1	151.5	184.5
0137/2205	mature	251.6	154.7	184.1
0137/2206	mature	249.5	151.4	182.3
0137/2209	mature	253.8	153.5	181.6
0137/2210	mature	253.2	151.1	177.6
0137/2212	mature	256.5	153.0	186.5
mean		254.1	152.7	182.3

latitude, altitude, distance from the sea, and level of rainfalls influence the distribution of deuterium in local water and that this can have a variable influence on the site-specific ratios of a molecule depending on the way hydrogen atoms are incorporated at the various molecular sites (*13*).

In the case of vanillin, a variance analysis (at 95% confidence level) does not indicate any significant difference among the three types of samples, except for the $(D/H)_4$, which appears to be higher for the two samples cured in Nantes (no significant difference was found between mature and immature samples).

Although only two lab-cured samples are available for the comparison, this leads us to raise the hypothesis that the curing process contributes to this difference. However, the only ingredient/additive, other than the beans, used in the IFF curing process is water. The same was true in the case of the curing done at Nantes. However, the water used was not the same. It

Table 10. $(D/H)_i$ (Parts per Million) of Vanillin from Other Origins (49 Samples)[a]

vanillin	$(D/H)_1$	$(D/H)_3$	$(D/H)_4$	$(D/H)_5$
ex-beans	130.8	157.3	196.4	126.6
SD	3.1	3	2.5	1.7

[a] Adapted from ref *9*.

Table 11. $(D/H)_i$ (Parts per Million) of pHB from Other Origins (10 Samples)[a]

pHB	$(D/H)_1$	$(D/H)_3$	$(D/H)_4$
ex-beans	273.7	159	197.4
SD	8	5.6	3.1

[a] Adapted from ref *9*.

may be possible that the difference between the Nantes-cured and IFF-cured beans in the $(D/H)_4$ ratio may be due to the differences (deuterium level) in the water used. Literature data for meteoric water around the world are available (*14*). Unfortunately, the only information for India is the (D/H) of water from Delhi (149.5 ppm), which is similar to the value of tap water from Nantes. However, because the curing was done in southeastern India, where the climate is much more humid, one can expect a lower (D/H) value of water there, which is in line with the above hypothesis.

In the case of pHB, data are available for only mature samples, because these reflect more accurately the real production process. No significant difference was observed between the lab-cured and industrially cured samples, except for the $(D/H)_1$ of pHB, which appears to be higher for the two samples cured in Nantes. The aldehyde group is supposed to come from separate origins in vanillin and in pHB (*9*), which might explain the different findings for the two molecules. Conversely, the absence of effect of the curing on $(D/H)_4$ of pHB can be due to the difference of origin of the corresponding signal in the two molecules (see **Figure 1**).

The means and standard deviations resulting from the SNIF-NMR analysis of the above parameters in vanillin and pHB extracted from authentic vanilla beans grown mainly in Madagascar, Comores, Reunion, and Indonesia have been published by Eurofins in 1997 (*9*). These results are summarized in **Tables 10** and **11**.

When the results of **Tables 8** and **9** are compared to these values, no significant differences are observed for $(D/H)_3$ and $(D/H)_5$ of vanillin or for $(D/H)_3$ of pHB. On the other hand, the $(D/H)_4$ ratios of both molecules are systematically depleted in deuterium. A similar trend is also observed for $(D/H)_1$, especially for pHB (and also for some of the vanillin samples). These differential effects within the molecules can be due to the relative contribution of hydrogen atoms from water to the corresponding positions and/or to specific fractionation effects during biosynthesis.

On the basis of their order of magnitude, these deviations could be due to environmental factors. In particular, the altitude of the plantation (~900 m) must lead to an impoverishment of the water in deuterium versus that at sea level.

Interestingly, the discrimination power of the SNIF-NMR analysis (to detect adulteration of Indian vanilla with cheaper sources of vanillin and pHB) remains almost unchanged, as evidenced by the graphs based on principal component analyses (PCA) (see **Figures 2** and **3**): the projection of the samples analyzed in this study in the plane of the first two components

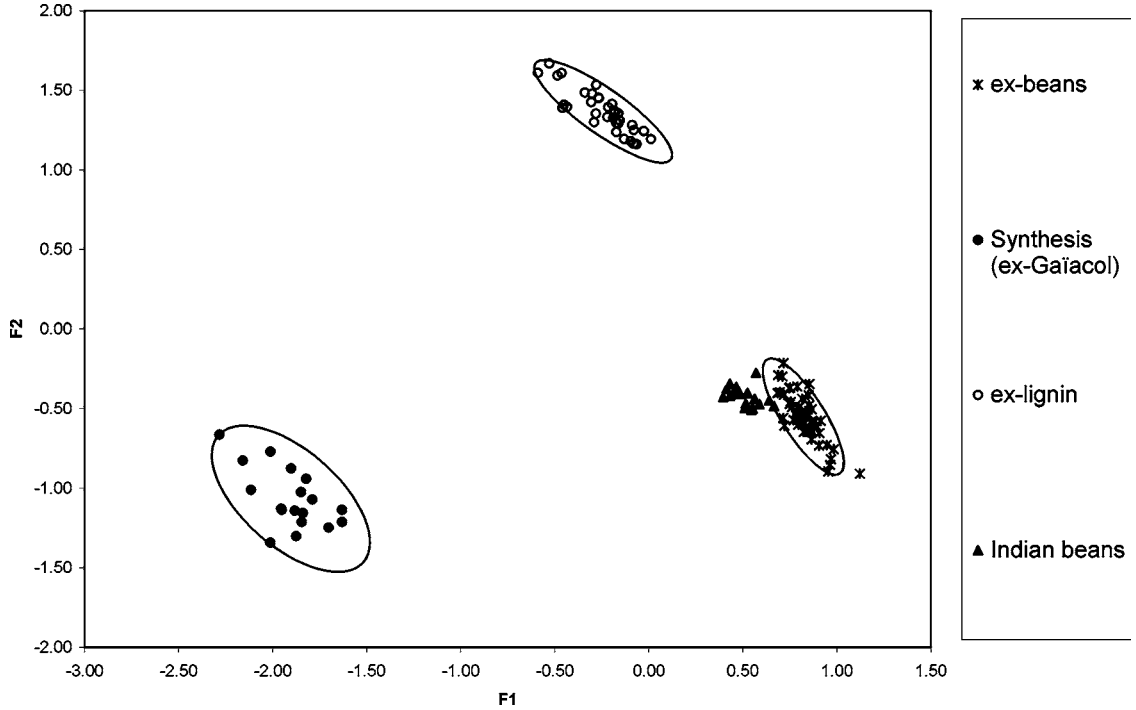

Figure 2. Differentiation of vanillin sources based on SNIF-NMR data: projection of the samples from this study ("Indian beans") in the plane of the first two canonical variables (F1 and F2) of the PCA based on previous results of "ex-beans", "synthetic (ex-guaiacol)" and "ex-lignin" samples analyzed in ref *9*. $(D/H)_1$, $(D/H)_3$, $(D/H)_4$, and $(D/H)_5$ were the initial parameters. The ellipses drawn correspond to the 95% confidence interval.

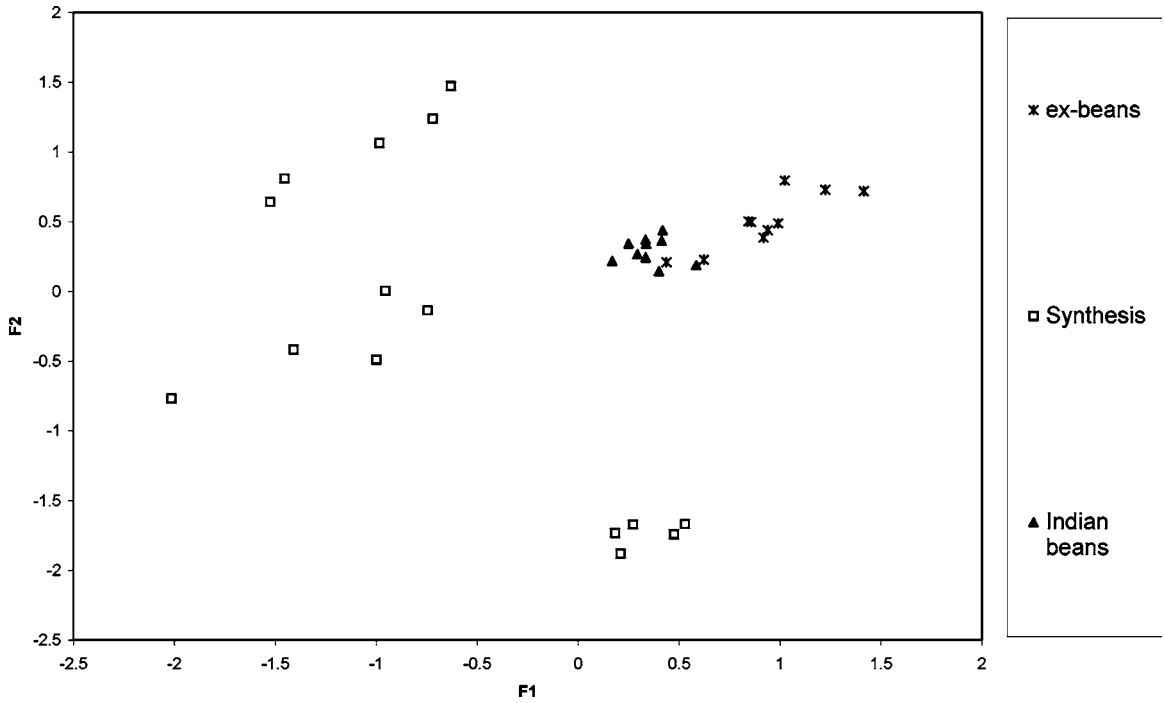

Figure 3. Differentiation of pHB sources based on SNIF-NMR data: projection of the samples from this study ("Indian beans") in the plane of the first two canonical variables (F1 and F2) of the PCA based on previous results of "ex-beans" and "synthetic" samples analyzed in ref *9*. $(D/H)_1$, $(D/H)_3$, and $(D/H)_4$ were the initial parameters.

(calculated previously) for vanillin and pHB, respectively, confirm the clear discrimination between Indian vanilla and synthetic or semisynthetic sources.

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LITERATURE CITED

(1) *U.S. Code of Federal Regulations* 21, 169.3, April 1, 2004.
(2) International Organization of the Flavour Industry. *Authenticity of Natural Vanilla Products*; Information Letter N 1271.

(3) Havkin-Frenkel, D.; Dorn, R. *Vanilla in Spices—Flavor Chemistry and Antioxidant Properties*; Risch, S. J., Ho, C.-T., Eds.; ACS Symposium Series 660; American Chemical Society: Washington, DC, 1996; pp 29−40.

(4) Purseglove, J. W.; Brown, E. G.; Green, C. L.; Robins, S. R. *Vanilla in Spices*; Longman Scientific and Technical: Harlow, U.K., 1988; Vol. 2, pp 644−735.

(5) Republic of France, Ministry of Economy, Finance, and Industry; Directorate General for Competition, Consumption, and Fraud Repression; Information Note 2003-61, June 16, 2003.

(6) Republic of France, Ministry of Economy, Finance, and Industry; Directorate General for Competition, Consumption, and Fraud Repression; Information Note 5387, Oct 17, 1988.

(7) Derbesy, M. Evolution des critères analytiques des vanilles de l'océan Indien. *Aromes, Addit. Ingredients* **2000**, *26*, 21−25.

(8) Fayet, B.; Fraysse, C.; Tisse, C.; Pouliquen, I.; Guerere, M.; Lesgards, G. Analyse isotopique par GC-SMRI du carbone 13 de la vanilline dans les crèmes glacées. *Analusis* **1995**, *23*, 451−453.

(9) Remaud, G.; Martin, Y.-L.; Martin, G. G.; Martin, G. J. Detection of sophisticated adulterations of natural vanilla flavors and extracts: application of the SNIF-NMR method to vanillin and *p*-hydroxybenzaldehyde. *J. Agric. Food Chem.* **1997**, *45*, 859−866.

(10) Galimov, E. M. *The Biological Fractionation of Isotopes*; Academic Press: London, U.K., 1985.

(11) ISO 5565-2 Vanilla [Vanilla fragrans (Salisbury) Ames], Part 2: test methods.

(12) Scharrer, A.; Mosandl, A. Progress in the authenticity assesment of vanilla. 2. $\delta^{13}C_{V-PDB}$ correlations and methodical optimisation. *Dtsch. Lebensm.-Rundsch.* **2002**, *98*, 117−121.

(13) Jamin, E.; Naulet, N.; Martin, G. J. Multi-element and multi-site isotopic analysis of nicotine from tobacco leaves. *Plant, Cell, Environ.* **1997**, *20*, 589−599.

(14) Yurtsever, Y.; Gat, J. R. Atmospheric waters. In *Stable Isotope Hydrology*; Technical Report Series; International Atomic Energy Agency: Vienna, Austria, 1981; No. 210, pp 103−142.

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