Authentication of Bitter Almond Oil and Cinnamon Oil: Application of the SNIF-NMR Method to Benzaldehyde

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The detection of adulterated bitter almond and cinnamon oils can be achieved by means of the SNIF-NMR method using benzaldehyde as a molecular probe. It is demonstrated that the site specific deuterium contents of benzaldehyde allow the determination of the origin of the molecule: synthetic (ex-toluene and ex-benzal chloride), natural (ex-kernels from apricots, peaches, and cherries and ex-bitter almond) and semisynthetic (ex-cinnamaldehyde extracted from cinnamon). A strategy resorting to the transformation of cinnamaldehyde into benzaldehyde has been exploited to study the origin of cinnamaldehyde. An analytical method for routine characterization of the genuineness of bitter almond and cinnamon oils is proposed. The repeatability of the ^{2}H-NMR measurements on benzaldehyde and the capability for both proving and quantifying adulterations are estimated.

Keywords: *Benzaldehyde; cinnamaldehyde; bitter almond oil; cinnamon oil; SNIF-NMR; authentication; adulteration; retroaldolization*

INTRODUCTION

The authentication of the origin of flavor compounds is a concern of the food and beverage industry. The use of natural flavors is often seen as a strong marketing advantage, but natural flavors, being more expensive than artificial/synthetic, are prone to adulteration (Culp and Noakes, 1992). Controls of the origin of flavor compounds are therefore currently routinely performed by the food and flavor industries. In this context, several approaches were developed in order to characterize the origin of bitter almond oils and cinnamon oils. These two essential oils form an important part of flavoring ingredients offered to the food industry (Clark, 1995). Bitter almond oil, which consists mainly of benzaldehyde, is derived in principle from bitter almonds (*Prunus amygdalus*). However, this denomination will be extended to other kernels containing amygdalin (the glycoside form of benzaldehyde) such as those of apricots, peaches, plums, and cherries (reaction 1, Figure 1). Cinnamon oil (or cassia oil), which is the volatile oil derived by steam distillation of the cinnamon tree (bark and pipes), contains mainly the *trans*-cinnamaldehyde molecule (Arctander, 1960).

Three general procedures are currently used to produce nature-identical benzaldehyde (Figure 1) (Shaath and Benveniste, 1991). The first one corresponds to a direct oxidation of toluene (reaction 2). The second one involves the hydrolysis of benzal chloride, itself produced by chlorination of toluene (reaction 3). The third one is the retroaldol reaction on cinnamaldehyde (reaction 4, path A). The cinnamaldehyde itself may be extracted from natural material (cinnamon oil), leading to the so-called benzaldehyde ex-cassia. In this case the resulting benzaldehyde is classified as semisynthetic and not natural, as advocated by the French decree no. 91-366, 1991) and European directives (directive no. 88/388/CEE 1988). The United States Bureau of Alcohol, Tobacco and Firearms clearly states that bitter almond can be the only source of benzaldehyde in alcoholic beverages which are labeled as "natural cherry". There appears, however, to be some debate on the natural status of benzaldehyde ex-cassia. For example, the United States Food and Drug Administration drew the attention of the manufacturers of benzaldehyde ex-cassia to the fact that the naturality of this type of benzaldehyde is strictly dependent on the reaction conditions (temperature, catalyst, etc.) (*Food Chem. News*, 1990). Interestingly, the main industrial procedure to manufacture cinnamaldehyde is path B of reaction 4 in Figure 1. Benzaldehyde, which is in this case the starting material, is obtained *via* either reaction 2 or reaction 3 (Figure 1).

The distinction between synthetic and natural benzaldehydes or cinnamaldehydes is more often based on isotopic methods. Radiocarbon (^{14}C) analysis was the first approach used for cinnamaldehyde (Hoffman and Salb, 1980) and benzaldehyde (Krueger, 1987). Synthetic benzaldehyde and cinnamaldehyde do not exhibit ^{14}C activity. Unfortunately, this technique was overcome by addition of ^{14}C-enriched benzaldehyde and cinnamaldehyde, as shown by Culp and Noakes (1990). Stable isotopes such as ^{2}H and ^{13}C were analyzed by stable isotope ratio mass spectrometry (IRMS) on benzaldehyde alone (Butzenlechner et al., 1989) and on both benzaldehyde and cinnamaldehyde (Culp and Noakes, 1990, 1992). This work identified specific ranges of δ^{13}C and δ^2H values for several origins of benzaldehyde and/or cinnamaldehyde. The total deuterium content was shown to be the most discriminating parameter, especially for an ex-toluene origin. However, these two IRMS approaches neither distinguish "ex-cassia benzaldehyde" from bitter almond oil nor quantify the adulterations with accuracy. The SNIF-NMR method is more efficient, since the four origins of benzaldehyde are differentiated (Guillou et al., 1991; Hagedorn, 1992;

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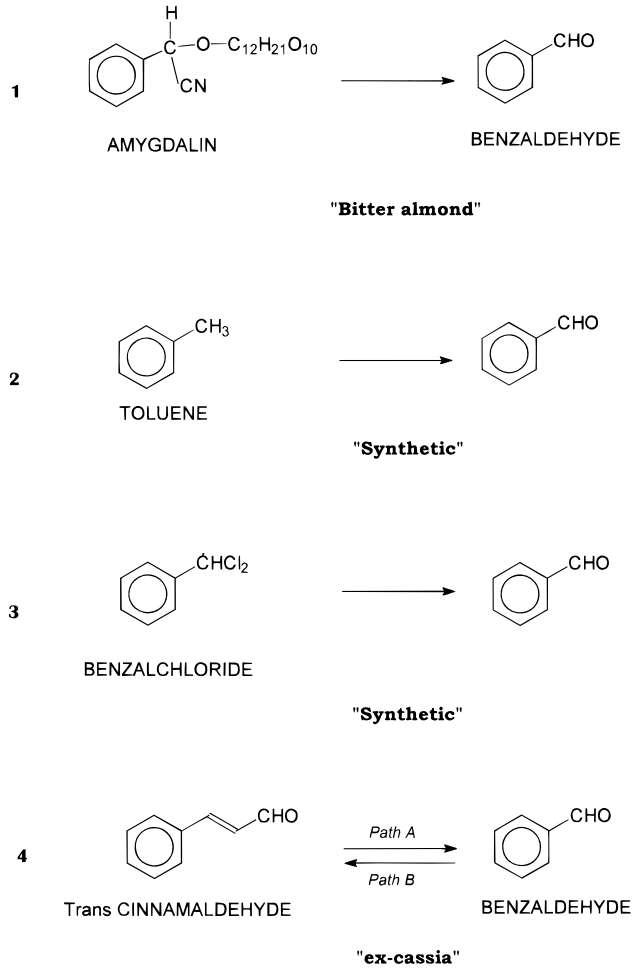

Figure 1. Main pathways for the production of benzaldehyde. Reaction 1 refers to the natural origin of benzaldehyde from hydrolysis of amygdalin extracted from almonds. Reactions 2 and 3 are the main industrial processes for manufacturing benzaldehyde from toluene and benzal chloride, respectively. Benzaldehyde ex-cassia is obtained by reaction 4 (path A). It should be noted that the double arrow does not indicate a chemical equilibrium but only the possibility to produce benzaldehyde from cinnamaldehyde and *vice versa* (paths A and B).

Remaud et al., 1992). This method was first introduced in the early 1980s by Martin and Martin (1981). Since then several applications have been developed (Martin et al., 1993). It is the official method of the European Community for detecting the addition of sugar in wines (OIV, 1990) and has become AOAC official method 995.17 for detecting added sugar in fruit juices (Martin et al., 1996). Since these preliminary works, improvements in NMR spectrometers, in the software used, and in the reference data have been achieved. We thought it necessary to update the results by presenting the latest progress of the technique concerning the analysis of benzaldehyde. We therefore include in the present paper the improvement of ^{2}H-NMR spectroscopy, the increase in reference data for benzaldehyde, and a statistical approach to quantifying adulterations.

Until now, the SNIF-NMR method has not been applied on cinnamaldehyde because its ^{2}H-NMR spectrum exhibits unfavorable signal overlaps, as shown in Figure 2. A more appropriate chemical derivative of cinnamaldehyde should be found. The transformation of a molecule of interest into another one which shows a suitable ^{2}H-NMR spectrum has been already applied (Remaud et al., 1995). The relevant deuterium NMR

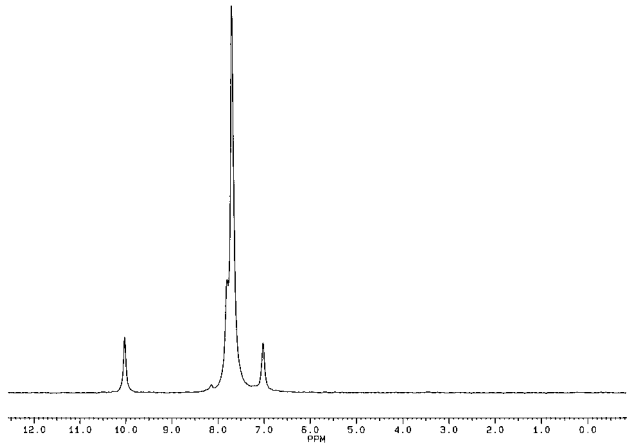

Figure 2. ^{2}H-NMR spectrum of pure cinnamaldehyde obtained on a 500 MHz spectrometer. One can note that only the signals of the formyl group and one ethylenic site are resolved.

results obtained on benzaldehyde, on one hand, and the possibility to transform cinnamaldehyde to benzaldehyde (path B of reaction 4 of Figure 1), on the other hand, made benzaldehyde the molecular probe of choice to study cinnamaldehyde. The retroaldolization of cinnamaldehyde to benzaldehyde ensures an efficient site connectivity, free from isotopic fractionation in the benzene ring and aldehyde function, under controlled transformation procedures. The study of the derivatization of cinnamaldehyde into benzaldehyde as a NMR probe and the creation of a database of benzaldehydes ex-cinnamaldehyde from several origins, allowing the authentication of cinnamon oil, was the other main objective of the present work.

MATERIALS AND METHODS

(a) Origin of the Products. The synthetic benzaldehyde and cinnamaldehyde samples were commercially available with a purity higher than 98%. They were used without further purification. Most of the samples of benzaldehyde used as references for natural origins were obtained, after hydrolysis and steam distillation, from deshelled pits of apricots, peaches, and cherries. The extraction of specific almond origins was performed by NATUREX (Avignon, France) on a pilot scale. Some extractions on a laboratory scale were also carried out according to reported procedures (Krueger, 1987; Culp and Noakes, 1990) [**CAUTION:** HCN Is Generated!]. Some authentic bitter almond oil (*P. amygdalus*) and other almond oil samples were also studied and were used as secondary references. In all cases the purity of benzaldehyde was higher than 97%.

The cinnamaldehyde samples from natural sources were extracted from cinnamon trees (pipes or bark or leaves) grown in Indonesia, China, Madagascar, Martinique (West Indies), Seychelles, and Sri Lanka and harvested over the period 1985−1995. The cinnamon oil was obtained either by direct hydrodistillation of the cinnamon material, followed by extraction with dichloromethane, or by soaking the cinnamon material in dichloromethane for several days and replacing the solution at least twice with fresh dichloromethane. The final step was, in both cases, the evaporation of the organic solvent under a light vacuum. The crude cinnamaldehyde was then submitted to a retroaldolization reaction.

(b) Retroaldolization Reaction. A prepurification of crude cinnamaldehyde was performed, in order to remove the other main constituents such as eugenol. This step was achieved on a chromatographic column using silica gel 60 (Merck, Darmstadt, Germany, 230−400 mesh). The elution of cinnamaldehyde was achieved with a mixture of pentane−ether (100−85% v/v of pentane). Each collected fraction was controlled by TLC (thin layer chromatography). The fractions

containing cinnamaldehyde were pooled before evaporation under a light vacuum. The resulting concentrate was ready for the transformation of cinnamaldehyde to benzaldehyde.

The creation of the aldehyde function was achieved by hydration in a basic medium. The addition of water did not produce stable products and readily yielded benzaldehyde and acetaldehyde (reaction 4, path A, Figure 1). The reaction took place in a flask containing 100 mL of water and 3% K_2CO_3. The organic concentrate containing cinnamaldehyde was then added to the flask. An efficient condenser allowed a reflux for about 7 h. A flow of nitrogen was applied on the top of the device at the beginning and at the end of the reaction. After that time the organic phase was extracted twice with pentane, which was removed by a slow distillation (**CAUTION**: the distillation should be very gentle in order to avoid loss of benzaldehyde, which would lead to isotopic fractionation). The base-catalyzed reaction was not complete: about 20−50% of cinnamaldehyde remained (routinely 30%).

The separation of benzaldehyde from cinnamaldehyde was performed on a short silica gel chromatographic column using the same silica gel as above. A flow of nitrogen was applied to each collected fraction, in order to prevent oxidation of benzaldehyde to benzoic acid. The elution was initiated with about 500 mL of pure pentane, and then benzaldehyde was collected with a pentane−ether mixture (95/5% v/v). Each collected fraction (about 80 mL) was controlled by TLC. It is important to stress, at this stage, that incomplete recovery of the benzaldehyde may produce isotopic fractionation, leading to erroneous interpretation of the isotopic results as demonstrated for other molecules (Remaud et al., 1997). The fractions containing benzaldehyde were gathered and were desiccated over sodium sulfate. A slow distillation (no overheating) of the organic solvents was then carried out. Finally, highly pure benzaldehyde was produced by passing a flow of dry nitrogen over the solution to remove the organic solvents completely. In order to prevent oxidation, benzaldehyde was kept under nitrogen.

(c) SNIF-NMR Method. Site specific $(D/H)_i$ ratios were obtained for all isotopomers of benzaldehyde from ^{2}H-NMR experiments, using an internal referencing procedure (Martin and Martin, 1995). The isotopic ratios $(D/H)_i$, measured in parts per million (ppm), are mean values resulting from at least three spectra.

A Bruker AM500 spectrometer was used for the ^{2}H-NMR experiments. The spectra (76.77 MHz) were recorded at 308 K using a 10 mm o.d. deuterium probe, broad band decoupling and,^{19} F lock (provided by C_6F_6). The quantitative measuring conditions were the following: 90° pulse (13 μs), repetition delay of $5T_1$ (6.8 s), sweep width, 4800 Hz; TD (time domain size), 32K; acquisition time, 3.4 s. The chemical shifts are expressed in ppm with respect to TMS. The reference used for $(D/H)_i$ calculations is certified tetramethylurea (TMU) purchased from the European Union Institute for Reference Materials and Measurements (Geel, Belgium). With about 1 mL of pure benzaldehyde, the spectrometer time was less than 7 h for recording three spectra with a signal to noise ratio above 150 for the reference signal.

The best possible results in terms of accuracy and precision are obtained when an advanced global least squares curve fitting procedure for spectra processing (Martin, 1994) is used. We used this new algorithm for the base line and phase corrections, together with the calculations of the signal areas (commercial information concerning the software used may be provided on request to the authors). It is worthwhile to note that any maladjustment of phase and base line can lead to erroneous and nonreproducible $(D/H)_i$ values, especially for sites 3 and 4.

Three types of parameters can be retrieved from a quantitative ^{2}H-NMR spectrum of benzaldehyde: (i) site specific deuterium ratios $(D/H)_i$ (in ppm), which are calculated from the area of the signal referred to that of the internal reference (TMU); (ii) molar fractions of the monodeuterated isotopomers f_i, which describe the natural distribution of ^{2}H in the different molecular sites; and (iii) the relative $R_{i/j}$ ratios ($R_{i/j} = F_j\, S_i/S_j$, where S is the signal height), which represent the actual number of deuterium atoms in site i with respect to site j, which is arbitrarily given its stoichiometric number of hydrogens, F_j. Parameters $R_{2/1}$, $R_{3/1}$, $R_{4/2}$, and $R_{4/3}$ are therefore the deuterium probability factors of sites 2, 3, and 4 in a situation characterized by the stoichiometric number $F_j = 1$ for the chosen reference site $j = 1$ or 3 and $F_j = 2$ when the reference site is $j = 2$. For a random distribution of deuterium, the four relative ratios $R_{i/j}$ would be $R_{3/1} = 1$ and $R_{2/1} = R_{4/2} = R_{4/3} = 2$. For benzaldehyde, the ^{2}H-NMR spectrum shows four peaks, and therefore four $(D/H)_i$ and four f_i are available (Figure 3). Among the selected $R_{i/j}$ parameters, $R_{2/1}$ and $R_{3/1}$ reflect the relationship between the deuterium content of aromatic and formyl sites. $R_{4/2}$ provides information on the relative deuterium distribution of the aromatic ortho and meta positions. $R_{4/3}$ is useful to understand the deuterium distribution between isotopomers in the meta and para positions. The above formalism is further explained in cited references (Martin and Martin, 1981, 1995).

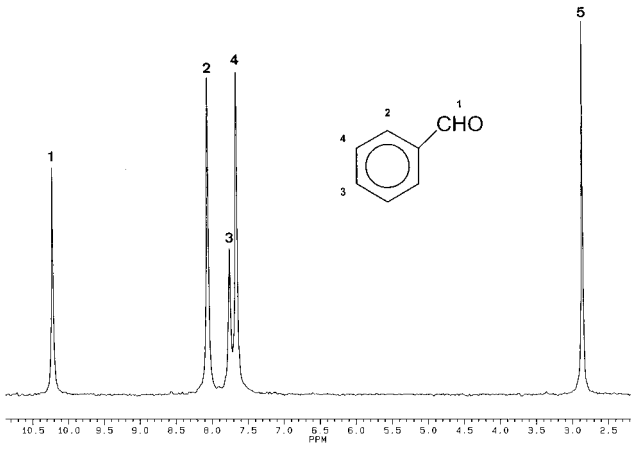

Figure 3. Typical ^{2}H-NMR spectrum of benzaldehyde obtained on a 500 MHz spectrometer from hydrolysis of benzal chloride (ex-benzal chloride). Officially calibrated TMU (tetramethylurea) is the internal reference (peak 5).

RESULTS AND DISCUSSION

(a) Reliability of the Method. Twelve variables being available from the ^{2}H-NMR spectra of benzaldehyde, it is worthwhile to discuss beforehand their individual efficiency for the differentiation of the four origins of benzaldehyde studied here. As a first approach, a one-factor analysis of variance, ANOVA, was carried out on the whole set of data (65 products). It appears that most of the variables considered are very efficient for distinguishing the groups. The differences between the means are ascertained with a probability higher than 99.99% but for $(D/H)_2$, $(D/H)_3$, and $(D/H)_4$ which are significant at the 99.0% level only. This first result leads to consideration of two series of variables: the isotope ratios $(D/H)_i$, which require the use of an internal reference (*N*,*N*-tetramethylurea, TMU) and the molar fractions f_i and relative $R_{i/j}$ ratios which depend only on the benzaldehyde studied. The variables in the first group ($(D/H)_i$) are indeed very useful in basic studies which tend to explain the relationships between isotopic fractionation and chemical or biochemical mechanisms. The variables in the second group are also discriminant, and the aim of the present work is to demonstrate the ability of SNIF-NMR to assess the origin of benzaldehydes. In this respect, a factor analysis which does not involve any *a priori* classification of the individuals in specified groups was computed successively with the two groups of variables, and the results are straightforward. On one hand, considering the isotope ratios only, two factors explain 79% of the overall variance (Figure 4a). On the other hand, the

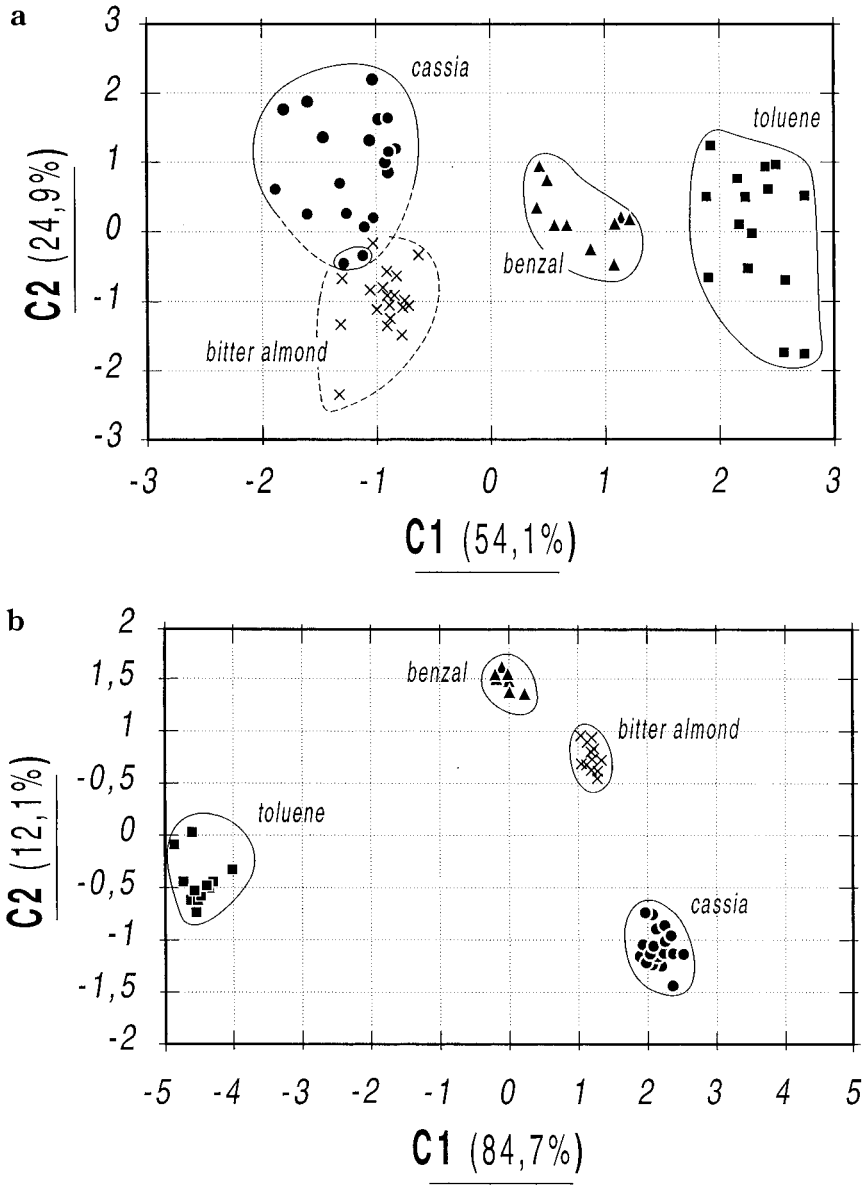

Figure 4. Results of the principal component analysis carried out on the 65 samples of benzaldehydes without any classification assumption: (a) analysis performed with the isotope ratios $(D/H)_i$ only; (b) analysis performed with molar fractions f_i and relative $R_{i/j}$ ratios.

Table 1. Repeatability *r*, Reproducibility *R*, and Mean *m* of f_i and $R_{i/j}$ Obtained in a Study of 15 Experiments (1 Outlier Was Removed)[a]

variable	*r*	*r* (%)	*R*	*R* (%)	*m*
f_1	0.0016	0.3	0.0041	0.7	0.5467
f_2	0.0017	0.9	0.0032	1.8	0.1775
f_3	0.0017	1.8	0.0084	9.0	0.0931
f_4	0.0015	0.8	0.0042	2.3	0.1828
$R_{2/1}$	0.0035	1.2	0.0035	1.2	0.2900
$R_{3/1}$	0.0031	2.4	0.0077	5.9	0.1307
$R_{4/2}$	0.0128	0.6	0.0202	1.0	2.0133
$R_{4/3}$	0.0277	1.2	0.0829	3.7	2.2353

[a] *r* and *R* are expressed in the unit of each variable and in %.

Table 2. Coefficients of Each Parameter Used for the Principal Component Analysis Carried Out on the 65 Benzaldehyde Samples without Any Classification Assumption

(a) Analysis Performed with the $(D/H)_i$ Ratio Only

	$(D/H)_1$	$(D/H)_2$	$(D/H)_3$	$(D/H)_4$
factor 1	0.60	0.15	−0.53	0.58
factor 2	0.05	0.97	0.21	−0.11

(b) Analysis Performed with the f_i and $R_{i/j}$ Parameters

	f_1	f_2	f_3	f_4	$R_{3/1}$	$R_{4/2}$	$R_{2/1}$	$R_{4/3}$
factor 1	−0.37	0.38	0.38	0.33	0.38	−0.25	0.38	−0.33
factor 2	−0.22	0.05	0.08	0.51	0.03	0.75	0.00	0.39

first two factors computed with the second group of variables (f_i and $R_{i/j}$), which have a high communality, explain 96.8% of the variance (Figure 4b). The comparison of the loadings of the first two factors computed with the $(D/H)_i$ data to those obtained from the molar fraction f_i and the intramolecular ratios $R_{i/j}$ elicits some comments. The coefficients for each variable are summarized in Table 2 (Table 2a should be associated with Figure 4a and Table 2b with Figure 4b). In the representation in Figure 4a, the first factor which expresses 54% of the variance depends mainly on $(D/H)_1$, $(D/H)_3$, and $(D/H)_4$, and the second factor (25% of the variance) is governed by $(D/H)_2$ (Table 2a). An interesting behavior of isotope ratios should be mentioned. Factor 1 is positively correlated to sites 1 and 4 and negatively to site 3: it contributes to differentiate cassia and bitter almond benzaldehydes (left side in

Table 3. Mean Values and Standard Deviations (SD) of f_i and $R_{i/j}$ of Benzaldehydes from the Main Origins: Toluene, Benzal Chloride, Bitter Almond, and Cinnamaldehyde Ex-Cassia

Origin	f_1	f_2	f_3	f_4	$R_{3/1}$	$R_{4/2}$	$R_{2/1}$	$R_{4/3}$
toluene ($n = 15$)[a]	0.5553	0.1704	0.0919	0.1832	0.125	2.039	0.277	2.237
SD	0.0164	0.0080	0.0044	0.0080	0.009	0.029	0.019	0.043
benzal chloride ($n = 10$)	0.1898	0.3130	0.1700	0.3274	0.673	2.010	1.477	2.212
SD	0.0048	0.0047	0.0062	0.0046	0.018	0.008	0.031	0.043
bitter almond ($n = 19$)	0.1666	0.3099	0.2040	0.3195	0.930	1.984	1.652	1.762
SD	0.0029	0.0038	0.0036	0.0029	0.017	0.015	0.031	0.028
cassia ($n = 21$)	0.1578	0.3460	0.2056	0.2906	0.983	1.622	1.972	1.627
SD	0.0051	0.0071	0.0065	0.0048	0.047	0.032	0.081	0.036

[a] The number n corresponds to the number of samples used.

Figure 4a) which have a natural benzene ring with respect to the benzaldehydes from fossil origin (right side of Figure 4a). This demonstrates the interest of being able to measure the deuterium content of site 3 by using a high magnetic field. The case of Figure 4b is slightly different since the first factor (85% of the variance) depends in a very similar way on the eight variables (f_i and $R_{i/j}$) whereas only the parameters related to site 4 (f_4, $R_{4/2}$, and $R_{4/3}$) have a significant influence on the vertical axis corresponding to the second factor (12% of variance) (Table 2b).

An important step in the determination of the reliability of an analytical method is the determination of its reproducibility. Before considering an interlaboratory comparison, an internal reproducibility was estimated using the same concepts as those defined in the ISO 5725 norm (ISO Standard 5725, 1986). Sixteen experiments were carried out on the same benzaldehyde ex-toluene, and each experiment was replicated three times over a period of 1 year. The results are given in Table 1 for the eight discriminant variables retained (f_i and $R_{i/j}$). One experiment has been considered as an outlier from the Dixon test carried out on the means, and the variances appear to be homogeneous.

The repeatability of the NMR experiment, which is on the order of 1% except for f_3 and $R_{3/1}$, may be considered as quite satisfactory (Table 1). The reproducibility of the determinations is usually twice the repeatability, and this observation expresses the great importance of the preparation of the product for the NMR analysis. For this reason, it was necessary to examine carefully the repeatability of the retroaldolization reaction, which is a good chemical way to transform cinnamaldehyde into benzaldehyde and is commonly used in the industry. Eight replications of the chemical experiment were carried out on a sample of synthetic cinnamaldehyde, and the benzaldehyde obtained was purified and examined by ^{2}H-NMR, according to the same protocol described in the experimental part. As mentioned before, the repeatabilities of the isotope ratio determinations, which are on the order of 3−4%, are higher (thus worse) than those of the dimensionless variables f_i and $R_{i/j}$ (1−3%) and, similarly to the previous discussion, the variables involving the benzenic site 3 are more poorly estimated. Thus, to conclude, as explained above, $(D/H)_i$ mostly finds its advantage for study of chemical and biochemical mechanisms and for checking the total deuterium content, while f_i and $R_{i/j}$ may be sufficient for analytical purposes. In addition, we have verified that the SNIF-NMR method produces repeatable data for the benzaldehyde molecule and can be safely applied to its origin identification.

(b) Authentication of Bitter Almond Oil. Table 3 summarizes the isotopic data (f_i and $R_{i/j}$) of benzaldehydes from the four main origins. The synthetic benzaldehydes are obtained from either toluene or benzal chloride, and the semisynthetic products are prepared from cassia. The natural benzaldehyde corresponds to ex-bitter almond origin. These different groups are clearly distinguished, and no overlapping occurs. This is illustrated by the discriminant analysis shown in Figure 5. There is a nearly random isotopic distribution on benzene moiety for the synthetic origins; i.e., the ratios $R_{4/2}$ and $R_{4/3}$ are close to 2. The deuterium repartition between the aldehyde function and the benzene ring is very different when comparing the synthetic products on the one hand and the natural or semisynthetic products on the other hand. Interestingly, random distribution of deuterium between the formyl site and the para position of the benzene ring is almost achieved for the ex-bitter almond and ex-cassia products: $R_{3/1}$ is close to 1. Generally speaking, the aldehyde function shows a higher deuterium content in the synthetic origins than in the cassia and bitter almond benzaldehydes. Indeed, the oxidation reactions of toluene or benzyl derivatives are characterized by important direct kinetic isotope effects on the order of 5−6 (Heck, 1996). The elimination of the light hydrogen atom from the methyl or methylene group is then privileged, and the remaining hydrogen in the aldehydic group is strongly enriched. The main difference between the benzaldehyde ex-cassia and the benzaldehyde ex-bitter almond is found in the lower deuterium content of site 4 and the higher deuterium content of site 3 for the former. The existence domains of the four groups

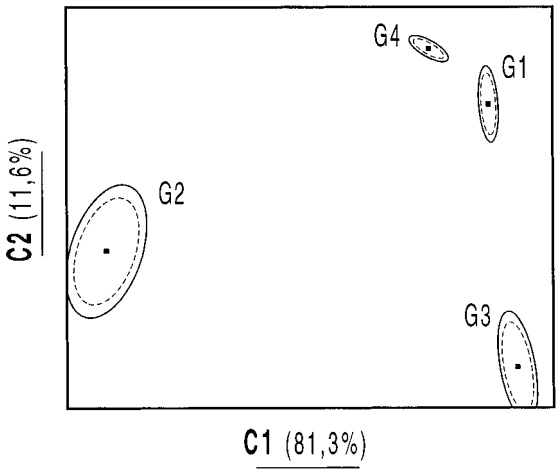

Figure 5. Representation of the reference groups of benzaldehyde (G1, ex-bitter almond; G2,: ex-toluene; G3, ex-cinnamaldehyde from cinnamon oil; and G4, ex-benzal chloride) projected in the plane of canonical variables obtained by discriminant analysis (Krzanowski, 1988) based on the training set of 65 samples reported in this work. The molar fractions f_1, f_2, f_3, and f_4 and the internal ratios $R_{3/1}$, $R_{4/2}$, $R_{2/1}$, and $R_{4/3}$ were the initial parameters. 81.3% of the discrimination is achieved according to the x axis and 11.6% according to the y axis. The ellipses drawn correspond to the 95% (broken line) and 99% (solid line) confidence interval.

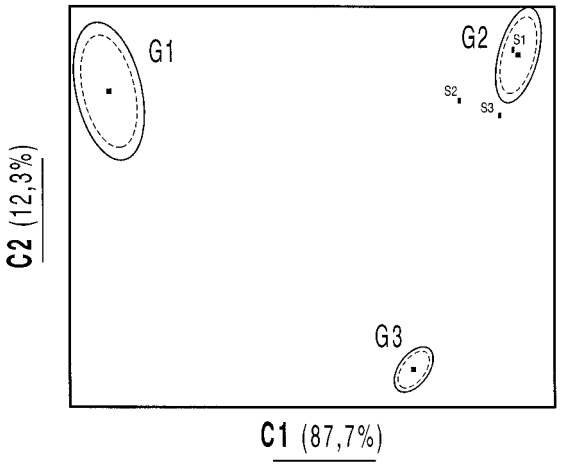

Figure 6. Quantification of adulterations of cinnamon oil. Synthetic cinnamaldehyde was added to the natural cinnamon oil (sample 1: S1), and then the retroaldolization was performed on the mixtures to analyze the corresponding benzaldehydes. Graphical representation of the results is in the same projected plane as in Figure 5, but using three groups (G1, ex-toluene; G2, ex-cassia; and G3, ex-benzal chloride). 87.7% of the discrimination is achieved according to the *x* axis and 12.3% according to the *y* axis. Samples 2 (S2) and 3 (S3) correspond to benzaldehyde obtained from natural cinnamon oil where about 20% of cinnamladehyde ex-toluene and about 15% of cinnamaldehyde ex-benzal chloride were added, respectively.

of benzaldehydes considered were computed for two confidence levels (99% and 95%) and are represented in Figure 5.

The quantification of adulteration of bitter almond oils using the deuterium content of the four sites of benzaldehyde has been described previously (Remaud et al., 1992), and this will not be developed here.

(c) Authentication of Cinnamon Oil. The samples used as references for the study of the origins of cinnamaldehydes, which appear in Table 3, correspond to benzaldehydes issued from cinnamaldehydes. No difference was noticed between industrial and laboratory processes (as described in the experimental section). The only point of interest is that the deuterium content of the aldehyde function is slightly higher (about 10%) in benzaldehyde ex-cinnamaldehyde purified in the laboratory than in commercial benzaldehyde of the same origin. The isotopic effects associated with the addition of water on the double bond of cinnamaldehyde according to our experimental procedure are, therefore, very similar to those of industrial processes: no effect on the benzene ring and a secondary isotopic effect on the C−H bond corresponding to the aldehyde function. Experimentally, we have observed that there is no significant effect on the isotopic data when the conversion yield of the retroaldol reaction varies even from 50% to 80%. The results obtained on the repeatability of the retroaldolization (see above) are within the repeatability of the ^{2}H-NMR measurement on benzaldehyde; no other effects are detectable. Thus a common database can be used for the authentication of both benzaldehyde ex-bitter almond and cinnamaldehyde. Figure 5 could be used for the determination of benzaldehyde whatever its origins or its manufacturing process.

The detection threshold of the adulteration of cinnamon oil with synthetic cinnamaldehyde is illustrated in Figure 6. A cinnamon oil (sample S1) was adulterated with about 20% cinnamaldehyde ex-toluene in one case (sample S2) and with about 15% cinnamaldehyde ex-benzal chloride in another (sample S3). Figure 6 reveals that, even with a relatively low amount of synthetic cinnamaldehyde, the resulting benzaldehyde cannot be classified as 100% issued from cinnamaldehyde ex-cinnamon. Adulteration of about 10% with either cinnamaldehyde ex-toluene or ex-benzal chloride would be detected as illustrated by the position of the samples of interest in Figure 6. The quantification can be performed as previously demonstrated (Remaud et al., 1992). On the basis of the global deuterium content determined by IRMS techniques, such a detection level is not possible when adulterations are performed with cinnamaldehyde ex-benzal chloride (Culp and Noakes, 1990).

CONCLUDING REMARKS

The original proposal of the present paper is the use of benzaldehyde as a unique molecular probe for the SNIF-NMR method to assess the authenticity of both bitter almond oils and cinnamon oils. In the first case, benzaldehyde is the main constituent and is used directly. In the second case, cinnamaldehyde is transformed into benzaldehyde *via* a controlled retroaldolization reaction. The deuterium repartition in benzaldehyde represents a fingerprint which can be used to distinguish the four main origins of benzaldehyde: ex-toluene (synthetic), ex-benzal chloride (synthetic), ex-cassia (semisynthetic), and ex-bitter almond (natural). The possibility of using ^{2}H-NMR to measure the deuterium content of the four sites of benzaldehyde leads to a powerful tool in term of authentication and quantification of adulteration. The interest of ^{2}H-NMR at a very high magnetic field over the IRMS technique (^{2}H and ^{13}C) is demonstrated in the detection of as little as 10−15% of synthetic cinnamaldehyde in cinnamon oil.

ABBREVIATIONS USED

AOAC, Association of Official Analytical Chemists; IRMS, isotope ratio by mass spectroscopy; NMR, nuclear magnetic resonance; OIV, Office International de la Vigne et du Vin; SNIF-NMR, a registered trademark from EUROFINS; TMS, tetramethylsilane; TMU, tetramethylurea.

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LITERATURE CITED

Arctander, S. *Perfume and flavor materials of natural origin*; Arctander: Elizabeth, NJ, 1960.

Butzenlechner, M.; Rossmann, A.; Schmidt, H. L. Assignment of bitter almond oil to natural and synthetic sources by stable isotope ratio analysis. *J. Agric. Food Chem.* **1989**, *37*, 410−412.

Clark, G. S. An aroma chemical profile: benzaldehyde. *Perfum. Flavor.* **1995**, *20*, 53−60.

Culp, R. A.; Noakes, J. E. Identification of isotopically manipulated cinnamic aldehyde and benzaldehyde. *J. Agric. Food Chem.* **1990**, *38*, 1249−1255.

Culp, R. A.; Noakes, J. E. Determination of synthetic components in flavors by deuterium/hydrogen isotopic ratios. *J. Agric. Food Chem.* **1992**, *40*, 1892−1897.

European Directive: article 1, paragraph 2, point b of directive no. 88/388/CEE of June 22, 1988.

Food Chem. News **1990**, March 5, 19−20.

French Directive: article 4 of decree no. 91-366 of April 11, 1991.

Guillou, C.; Remaud, G.; Martin, G. J. Application of deuterium NMR and isotopic analysis to the characterization of foods and beverages. *Trends Food Sci. Technol.* **1991**, *2*, 85−89.

Hagedorn, M. L. Differentiation of Natural and Synthetic Benzaldehydes by ^{2}H Nuclear Magnetic Resonance. *J. Agric. Food Chem.* **1992**, 40, 634−637.

Heck, G. Caractérisation de procédés de transformation chimique par analyse isotopique spécifique multi-élement. [Characterization of the chemical transformation processes by using multielement specific isotopic analyses.] Ph.D. Dissertation. The University of Nantes, France, 1996.

Hoffman, P. G.; Salb, M. Radiocarbon (^{14}C) method for authenticating Natural Cinnamic Aldehyde. *J. Assoc. Off. Anal. Chem.* **1980**, *63*, 1181−1183.

Krzanowski, W. J. *Principles of Multivariate Analysis, a User's Perspective*; Clarendon Press: Oxford, 1988.

Krueger, D. A. Determination of adulterated natural bitter almond oil by carbon isotopes. *J. Assoc. Off. Anal. Chem.* **1987**, *70* (1), 175−176.

Martin, G. G.; Remaud, G.; Martin, G. J. Isotopic methods for control of natural flavours authenticity. *Flavour Fragrance J.* **1993**, *8*, 97−107.

Martin, G. G.; Hanote, V.; Lees, M.; Martin, Y. L. Interpretation of combined ^{2}H SNIF-NMR® and ^{13}C SIRA-MS analyses of fruit juices to detect added sugar. *J. Assoc. Off. Anal. Chem.* **1996**, *79* (1), 62−72.

Martin, G. J.; Martin, M. L. Deuterium labelling at the natural abundance level as studied by high field quantitative ^{2}H NMR. *Tetrahedron Lett.* **1981**, *22* (36), 3525−3528.

Martin, G. J.; Martin, M. L. Stable isotope analysis of food and beverages by NMR spectroscopy. *Annu. Rep. NMR Spectrosc.* **1995**, *31*, 91−104.

Martin, Y. L. A global approach to accurate and automatic quantitative analysis of NMR spectra by complex least-square curve-fitting. *J. Magn. Reson., Ser. A* **1994**, *111*, 1−10.

Norme ISO 5725: 1986, Fidélité des méthodes d'essai—Détermination de la répétabilité et de la reproducibilité d'une méthode d'essai normalisée par essais interlaboratoires. In *Méthodes Statistiques—Recueil de Normes ISO 3*; ISO: Geneva, 1989; pp 413−463.

Office International de la Vigne et du Vin. Détection de l'enrichissement des moûts, des moûts concentrés, du sucre de raisin et des vins par application de la résonance magnétique nucléaire du deutérium (RMN-FINS, SNIF-NMR). In *Recueil des Méthodes Internationales d'Analyse des Vins et des Moûts*; OIV: Paris, France, 1990, pp 107−118.

Remaud, G.; Martin, G. J.; Martin, G. G. Identification de l'origine des cinnamaldéhydes et des benzaldéhydes par RMN du deutérium (SNIF-NMR). In *Xièmes Journées Internationales Huiles Essentielles, Digne Les Bains*, September 3−5, 1991, Digne Les Bains, France; Rivista Italiana EPPOS; Istituto Tetrahedron: Milan, Italy, 1992.

Remaud, G ; Jamin, E.; Martin, Y. L.; Martin, G. G.; Martin, G. J. Latest applications of the SNIF-NMR® method and other isotopic analyses to the authentification of fruit juices and derivatives. EUROFINS 3rd European Symposium on food authenticity, Oct 11−13, 1995, Nantes, France.

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.

Shaath, N. A.; Benveniste, B. Natural oil of bitter almond. *Perfum. Flavor.* **1991**, *16*, 17−24.

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