A New Approach to the Characterization of Volatile Signatures of Cork Wine Stoppers

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This work describes a new method for the rapid characterization of volatile compounds of cork wine stoppers by a dynamic headspace method coupled to mass spectrometry (DHS-MS). This technique does not require any pretreatment of the sample. The global volatile signature of the cork is evaluated without real chromatographic separation of its constituents. For data analysis, the mass fragments of each spectrum (50 < m/z <180 amu) were considered as potential descriptors of the composition of the cork sample headspace. Chemometrics methods (principal component analysis and partial least-squares regression) were applied to extract useful information by selecting the most significant mass fragments (m/z) that allowed a good classification of the cork samples studied. In the present work, geographical origin of cork samples could be successfully characterized and predicted.

KEYWORDS: Dynamic headspace; mass spectrometry; volatile signature; chemometrics; PCA; PLS

INTRODUCTION

Food product characterization based on an analysis of their aroma compounds is a widely used technique. Generally, analytical solutions imply the use of gas chromatography−mass spectrometry (GC-MS). Among food or agricultural products, wine corks have been extensively studied because of the many difficulties encountered by wine makers in avoiding cork taint, the most frequent wine off-flavor. Using various solvent extraction procedures, about 100 volatile compounds have been identified in cork by GC or GC-MS *(1−4).* Nevertheless, GC-MS is very time-consuming, not only because of the pretreatment step (extractions) but also because of long data interpretations. Recent research has shown that the rapid analysis of the global volatile fraction of foods by mass spectrometry without chromatographic separation produces signals (signatures) containing significant information. Thus, direct coupling of mass spectrometry with extraction methods such as dynamic headspace (DHS-MS), static headspace (SHS-MS), or solid-phase microextraction (SPME-MS) affords a "fingerprint" of analyzed products *(5−13),* with or without preconcentration of their volatile fraction. Owing to their rapidity, these nonseparative methods can be used for the classification and prediction of the volatile signatures of the products.

In this study, a new analytical approach is proposed for the rapid characterization of low- and medium-molecular-weight compounds from cork wine stoppers using a dynamic headspace method coupled online with a mass spectrometer (DHS-MS). The volatile signature of the sample is evaluated without complete separation of its constituents, and the potential of the instrument to discriminate volatile signatures of corks, with

regard to their geographical origin, is tested. In practice, the discriminating power of the DHS-MS was evaluated by using three different geographical cork origins. A tentative identification of the corks' volatile signature constituents was conducted by a series of separative analyses on the same extraction device with the dynamic headspace system, coupled to gas chromatography/mass spectrometry (DHS-GC/MS). The spectra of the molecules separated by DHS-GC/MS and mass profiles obtained by the headspace analysis of the samples with the DHS-MS method were both compared with a library of mass spectra and with cork constituents identification results published in other studies.

EXPERIMENTAL SECTION

Origin and Preparation of Products Analyzed. The cork samples were supplied by a Champagne producer (SIBEL, France). The samples were of three geographical origins: Spain (NAV and CAS), Portugal (LIM and HEF), and Morocco (TEM). They were stored in the dark at room temperature in polyethylene bags, and just before use, they were ground to powdered form and kept in aluminum foil.

Sample Preparation. Each sample of powdered cork was placed in a stainless steel tube. The tube was filled before the analyses and was put in a dynamic desorber CHROMPACK TCT system (Thermal Desorption Cold Trap injector, CHROMPACK France, Les Ulis, France). The quantity of powdered cork used was 350 and 150 mg for the DHS-GC/MS and DHS-MS methods, respectively.

Isolation and Desorption of Volatiles. The volatile components were isolated and concentrated using the dynamic headspace desorber Chrompack, with adsorption on a poly(dimethylsiloxane) capillary trap (Supelco SPB-1, 0.53 mm i.d., film thickness 5.0 μm) which was maintained at room temperature to prevent water condensation, which causes analytical artifacts *(14).* Each sample was heated to 150 °C under helium flow (N55 99.995% pure) at a constant pressure of 1 bar above atmospheric pressure. The tube trap adsorbed the volatile substances

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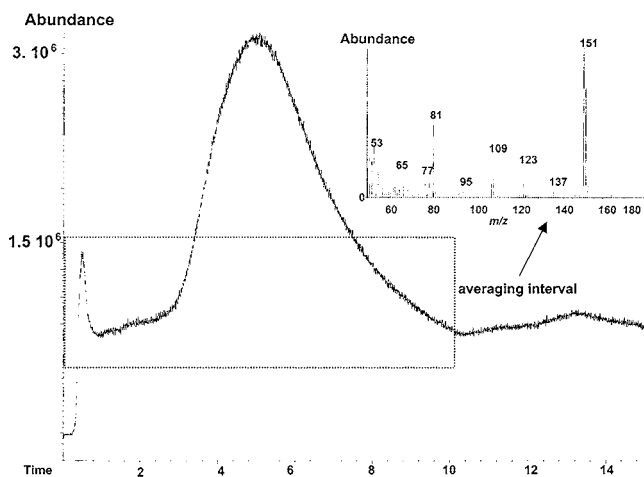
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Figure 1. Total ion current of Portuguese (LIM) cork sample obtained by DHS-MS.

Table 1. Volatile Compounds Proposed as Tentative Identification in the Dynamic Headspace of Corks Samples

peak no.	retention time (min)	molecules
1	2.247	acetic acid
2	6.714	2-furancarboxaldehyde
		2*H*-pyran-2-one
3	11.569	5-methyl-2-furancarboxaldehyde
4	21.531	decanal
5	21.914	(2*E*,4*E*)-2,4-nonadienal
6	25.494	2-(1,1-dimethylethyl)phenol
7	25.800	(2*E*,4*E*)-2,4-decadienal
8	27.046	2-methoxy-4-(2-propenyl)phenol
9	27.306	4-butoxybutanoic acid
10	28.606	4-hydroxy-3-methoxybenzaldehyde
11	28.848	tetradecane
12	30.454	6,10-dimethyl-5,9-undecadien-2-one
13	31.816	terpene
14	32.088	terpene
15	35.165	1,2-benzenedicarboxylic acid, diethyl ester
16	35.790	hexadecane
17	39.223	alcane

flushed from the sample by the helium. During the isolation step, the helium flow at the exit of the trap was released into the atmosphere through the flow controller. Extraction lasted 12 min for DHS-MS analysis and, as larger amounts are required for GC, 30 min in the case of DHS-GC/MS. At the end of the extraction time, helium was driven to the analytical apparatus while the trap was concomitantly heated at 230 °C for 6 min in order to desorb the volatile components.

DHS-GC/MS Measurements. For the DHS-GC/MS analysis, the volatiles were separated by gas chromatography under a flow rate of 0.8 mL/min. An HP 5890 Series II gas chromatograph (Agilent Technologies, Avondale, PA) and an HP 5970 mass-selective detector (MSD) (Agilent Technologies, Palo Alto, CA), directly interfaced to the chromatograph, were used for this study. The MSD parameters were set to the values determined by the automatic tuning procedure, and the mass range was 37−300 atomic mass units (amu). Mass spectra were searched against the *nbs49k* mass spectral library (Office of Standard and Reference Data, NIST). The separation was performed with a DB-5MS (95% methyl and 5% phenyl) bonded phase column (J & W Scientific, 25 m × 0.2 mm i.d., film thickness 0.33 μm). Operating conditions were as follows: initial temperature 50 °C for 3 min, then 50−200 °C at 4 °C/min. Three replicates were done for each type of sample.

DHS-MS Measurements. The trap was under a constant pressure of 1 bar above atmospheric pressure with a helium flow rate of 100 mL/min. After the extraction and the desorption steps, the molecules passed through a transfer line (SGE DB-1, 1 m × 0.25 mm i.d.), held at 200 °C in the chromatograph oven, which ensured sufficient flow resistance for the turbomolecular pump to maintain the vacuum in the spectrometer. The volatile components were detected by mass spectrometry with electron impact ionization at 70 eV. The *m/z* range considered in these conditions was 50−180 amu. This range is smaller than in the case of DHS-GC/MS because, on one hand, fragments less than 50 amu have various origins and low specificities (*15*) and, on the other hand, preliminary experiments showed no significant fragments above 180 amu. The signal (total ion current) took the form of an asymmetrical peak ~10 min wide (**Figure 1**). Background noise was evaluated by averaging the mass spectra recorded after the peak, at between 12 and 14 min. Finally, the mean abundance values of all the mass fragments recorded between 0 and 10 min were used in the calculations, after removal of the overage background spectrum. About 10 replicates were done for each of the five types of samples, generating a matrix of 51 individuals and 131 variables X (51 × 131).

Data Analysis. PCA was used as the first step for data exploration, as it allows a visualization in two- or three-dimensional space of the variability of a data set, without the constraint of any initial hypothesis concerning relationships among samples and variables. PCA was performed using MATLAB software (MATLAB for Windows, version 4.0, The Mathworks Inc., Natick, Ma).

Partial Least Squares-2 (PLS-2). PLS regression has become the most commonly used method for multivariate regression because the

predictive ability is often better than that for principal component regression (PCR) and because fewer principal components are required, simplifying interpretation (*16−18*). PLS-2 is a PLS regression method used for multiple *Y* variables. In the present case, matrices used to build the PLS-2 discriminant analysis model are the matrix of the raw data X (51 × 131) and the matrix of variables to predict Y (51 × 3). *Y* is the matrix "origins" coded 1 and 2:

$$1 = \text{does not belong to the group}$$

$$2 = \text{belongs to the group}$$

PLS-2 was performed using Win ISI software (Infrasoft International, LLC, version 1.50.)

When dealing with predictive regression methods, it is necessary to check the consistency of the model by determining the optimal number of PCs to use for the prediction of new unknown samples. The goodness of the calibration model is measured by the standard error of cross-validation (SECV). It is calculated by using the square of the differences between expected and predicted values, divided by the number of individual values,

$$\text{SECV} = \sqrt{\frac{(Y - \bar{Y})^2}{n}}$$

where *Y* is the expected value, $\bar{Y}$ is the predictive value, and *n* is the number of individuals. On the other hand, the external validation principle is totally independent of the determination of numbers of PCs to use in the model. The model predictive ability is evaluated by the calculation of the standard error of prediction (SEP), which is similar to SECV but divides by *n′*, the number of individual values of the validation matrix. The validation matrix is recorded for an independent sample set.

RESULTS AND DISCUSSION

DHS-GC/MS Results. From the 12 chromatographic analyses, tentative identifications were done for 17 peaks on the basis of their retention times and mass spectra (**Table 1**). The molecules proposed cover a wide range of chemical families, most of them already published in other studies: organic acids [4-butoxybutanoic acid, acetic acid (*1, 2*)]; furans [2-furancarboxaldehyde, 2*H*-pyran-2-one (*1, 2*)]; aldehydes [decanal, (2*E*,4*E*)-2,4-nonadienal, (2*E*,4*E*)-2,4-decadienal (*2*)]; phenols [2-methoxy-4-(2-propenyl)phenol, 2-(1,1-dimethylethyl)phenol, 4-hydroxy-3-methoxybenzaldehyde (vanillin) (*1−3*)]; and hydrocarbons [tetradecane, hexadecane, alcane].
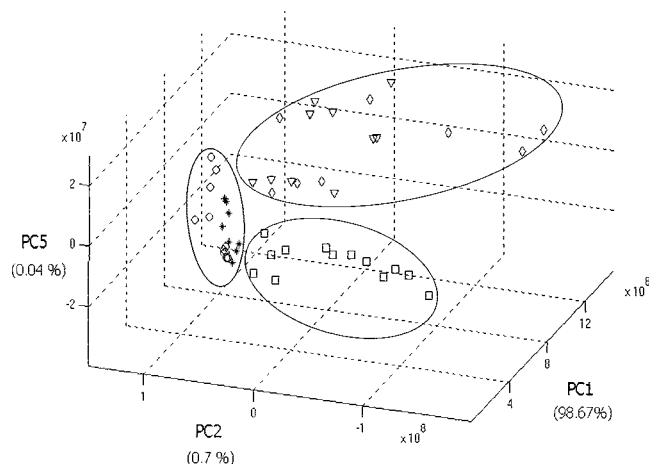
Figure 2. Three-dimensional PCA plot of data matrix *X* (51 × 131). (◇) CAS and (▽) NAV (Spanish samples), (∗) HEF and (○) LIM (Portuguese samples), and (□) TEM (Moroccan samples).

The identity of vanillin was confirmed by comparing its retention time and mass spectrum with those of a commercial reference (Sigma, Saint-Quentin Fallavier, France).

DHS-MS Results. *PCA Results.* Principal component analysis was applied to the desorption profiles of Moroccan, Spanish, and Portuguese cork samples, without any pretreatment of the data. **Figure 2** shows the PCA projection of the samples on the first, second, and fifth principal components, which are the most relevant to visualize the groups of origin. The three PCs collectively account for 99.41% of the total variability of the samples. The first PC seems to be the most important for distinguishing the groups and accounts for 98.67% of variation. The PCA shows that the samples are organized into three well-separated groups. PC2 separates the Portuguese group (G3) from the other two groups, while PC5 separates the Spanish group from the two others. No subdivision due to the time of analysis is observed. The loadings associated with each PC are presented in **Figure 3**, which shows which *m/z* variable most contributes to the separation between the groups. Variables which contribute to PC1 are the *m/z* = 50, 57, 60, 61, 81, 109, 123, 151, and 152 amu; to PC2, *m/z* = 57, 60, 61, 134, and 178 amu; and finally to PC5, *m/z* = 57, 60, 68, 73, 97, 112, 126, 134, 160, and 178 amu. The most important of these ions were used to search through the mass spectral library. Results are listed in **Table 2**. PC3 and PC4 were rejected for classification purposes, as the corresponding score plot demonstrated strong overlap of the three groups, indicating low information content concerning geographical origin of the cork in PC3 and PC4.

PLS Results. PLS-2 was used to build a discrimination model and to predict group membership. The SECV minimum was observed with six principal components. The calibration gave a regression coefficient of 0.8106 and an SECV of 0.2066, to be compared to the *Y* range, namely from 1 to 2. The independent validation set of this model was carried out on a new data file (31 × 131) collected at later dates: 3 months and 8 months after the first acquisitions of the calibration set. This step allows the validation of the statistical model and the checking of the reproducibility of the instrument. **Figure 4** illustrates the model-predicted group membership values of both the calibration data *X* (51 × 131) and the validation data X_v (31 × 131). This model gave an SEP of 0.2659. The correct origin is well predicted for all samples, though for 6 corks among 31, the value predicted for one of the wrong origins is high enough to be within the confidence interval at the level *p* = 0.05.
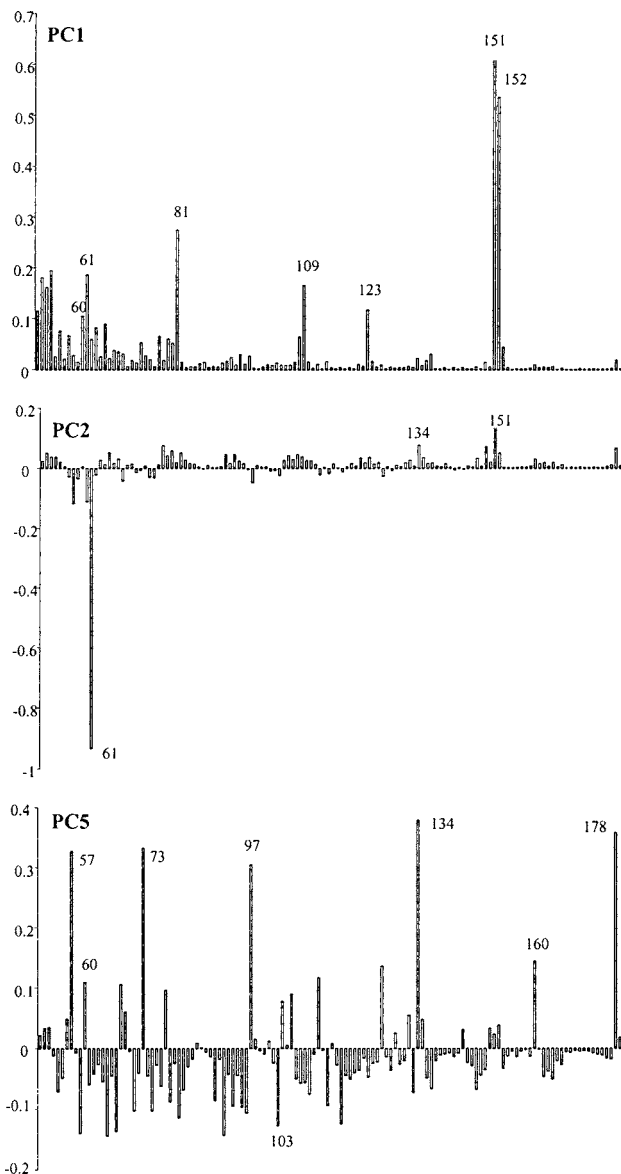

Figure 3. PCA loadings plot of the data matrix *X* (51 × 131). Variables that strongly contribute to PC1, PC2, and PC5 are highlighted.

Table 2. Fragments (*m/z*) Which Have the Highest Contribution in the Discrimination between the Samples and the Possible Chemical Origin for These Fragments

m/z (amu)	possible molecules	refs
50	benzaldehyde	*1, 2*
57	2-ethylhexanol	*1, 2*
60	acetic acid	*1–3*, DHS-GC/MS
61	2,3,4,6-tetrachlorophenol	*1–3*
97	4-hydroxy-3-methoxybenzoic acid	*1–3*
109	1,7,7-trimethylbicyclo[2.2.1]heptan-2-one (camphor)	*1, 2*
134	1-ethyl-2,3-dimethylbenezene	*1–3*
81, 151, 152	4-hydroxy-3-methoxybenzaldehyde	*1–3*, DHS-GC/MS
97, 134, 160	2,4,6-trichlorophenol	*1–3*

Data Filtering Using PLS Results. PLS is a supervised method where a model is built to predict a controlled variable. PLS can highlight which variables have a significant role in the model. In the present case, these selected variables may then be used to perform another PCA on the data. The selection of *m/z* variables is thus based on the contribution to the PLS model. Variables with a contribution less than 0.4 in the first principal
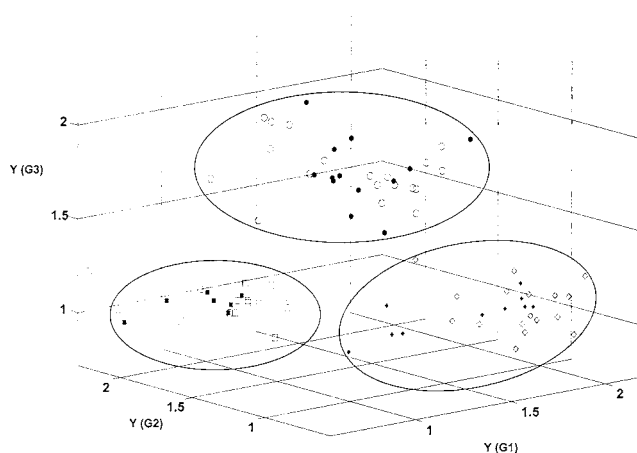
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Figure 4. PLS plot results representing projection of the samples onto the three axes of the predicted PLS values. *X* is the Spanish group's predicted value (G1), *Y* is the Moroccan group's predicted value (G2), and *Z* is the Portuguese group's predicted value (G3). (◇) Spanish calibration samples, (□) Moroccan calibration samples, and (○) Portuguese calibration samples. (◆) Spanish validation samples, (■) Moroccan validation samples, and (●) Portuguese validation samples.
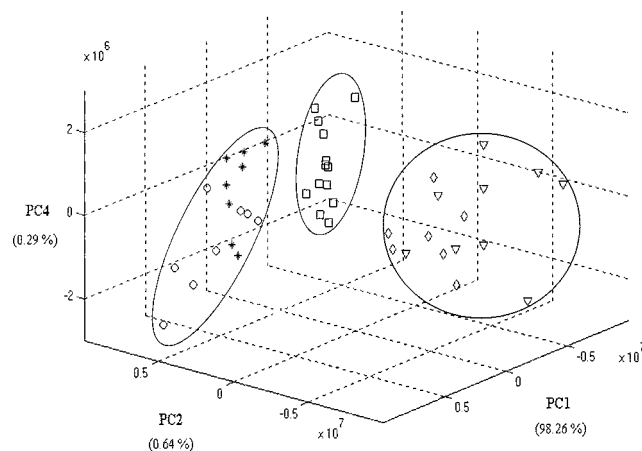

Figure 5. Three-dimensional PCA plot of data matrix X_R (51 × 32). (◇) CAS and (▽) NAV (Spanish samples), (∗) HEF and (○) LIM (Portuguese samples), and (□) TEM (Moroccan samples).

component of the PLS were rejected, resulting in a reduced data set, X_R. The size of X_R (51 × 32) is less than 25% of the original data matrix X (51 × 131). PCA was then applied to the reduced matrix X_R (51 × 32) in order to visualize the data compression effect. **Figure 5** represents the projection of the X_R individuals onto PC1 (98.26%), PC2 (0.64%), and PC4 (0.29%). The groups are better separated compared to the previous PCA.

The dynamic headspace—mass spectrometry method described in this paper offers promise for obtaining fast results for the characterization of volatile signatures. DHS-MS in conjunction with multivariate analysis represents a simple and effective solution to determine cork origin on the basis of the rapid analysis of its volatile fraction. However, chemical information about the origin may be masked by the presence of the other compounds. Data filtering may be required to extract the most discriminant variables when unsupervised data analysis methods such as PCA are used. This study demonstrates the

ability of DHS-MS to characterize the geographical origin of cork, and further developments are expected in the field of cork taint.

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