JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Mass Spectrometry Based Sensor Strategies for the Authentication of Oysters According to Geographical Origin

JEREMY RATEL,[†] PHILIPPE BERGE,[†] JEAN-LOUIS BERDAGUE,[†] MIREILLE CARDINAL,[§] AND ERWAN ENGEL*[,†]

UR370 QuaPA, Laboratoire Typicité Aromatique et Authentification, Institut National de la Recherche Agronomique (INRA), F-63122 Saint-Genès-Champanelle, France, and Laboratoire Génie Alimentaire, Institut français de recherche pour l'exploitation de la mer (IFREMER), F-44311 Nantes, France

This study was undertaken to investigate the relevance of using the pyrolysis−MS (Py-MS) technique to discriminate the production area of oysters harvested over two years and to assess from the data of the second year of harvest the potential of an alternative MS-based technique, the solid phase microextraction−MS (SPME-MS), to perform this discrimination. Oysters were harvested in various areas of France, and models of discrimination according to harvest season were built from Py-MS fingerprints and from virtual SPME-MS fingerprints obtained by summing the mass spectra generated by the SPME-GC-MS system. The treatment of the Py-MS data by a 21–12–3 artificial neural networks led to a correct classification of only 89.2% of the oyster samples according to shoreline. The misclassifications thus did not allow use of the Py-MS technique as a relevant tool for authentication of oyster origin. The assessment of the potential of the virtual SPME-MS fingerprints to discriminate the production area of oysters was undertaken on a part of the sample set. The virtual SPME-MS data were pretreated according to two methods, filtering of raw data (FRD) and comprehensive combinatory standard correction (CCSC), a recently developed chemometric method used for the correction of instrumental signal drifts in MS systems. The results obtained with the virtual SPME-MS fingerprints are promising because this technique, when the data were pretreated by the CCSC method, led to a successful discrimination of the oyster samples not only according to shoreline but also according to production region. This study confirms that an efficient correction method (CCSC) of instrumental drifts can considerably increase the discriminative information contained in the volatile fraction of food products.

KEYWORDS: Oysters; geographical origin; authentication; pyrolysis−MS; SPME-MS; artificial neural networks; instrumental drift correction

INTRODUCTION

The rapid worldwide intensification of the trade of raw or processed food materials for human consumption has increased health hazards and consequently consumer concern about the origin and the conditions of production of foods. Seafoods such as oysters can constitute a significant threat for the health of the consumer, given the possible exposure of these food chains to a wide spectrum of contaminants: pathogenic bacteria (*1*); heavy metals such as arsenic and mercury (*2, 3*); and chemical pollutants such as the polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and organochlorine pesticides (OCPs) (*4*). The certification of the geographical origin of oysters is thus very important considering the safety challenge, in particular when contaminated oysters from a particular area must be withdrawn from the market, but also considering the economic implications related to the influence of the area of production on the sensory qualities of the oysters (*5*) and thus to the protection of trademarks and the reputation of oyster producers. Dégremont et al. (*6*) suggested that the growth performances of oysters are mainly influenced by the environmental conditions in the area of production. Moreover, the climate and composition of plankton in the area of production were shown to influence the composition of oyster flesh (*5, 7*). The discrimination of oyster geographical origin based on analysis of oyster composition could thus be envisaged.

The analytical methods used for the authentication of animal products consist of either searching molecular constituents revealing the origin of the product (*8–11*) or generating a

* Corresponding author (telephone +33 4 7362 4589; fax +33 4 7362 4731; e-mail erwan.engel@clermont.inra.fr).
[†] Institut National de la Recherche Agronomique.
[§] Institut français de recherche pour l'exploitation de la mer.

10.1021/jf072207i CCC: $40.75
Published on Web 12/21/2007

fingerprint of the product and comparing this fingerprint with those of reference products available in databases (*12, 13*). The latter strategy is rapid, easy to carry out, and allows products to be discriminated without a priori knowledge of the compositional differences between the products provided that the databases used are time consistent and relevant to the authentication question at issue.

Cardinal et al. (*14*) found the analysis by Curie-point pyrolysis−mass spectrometry (Py-MS) of oyster flesh to be a promising technique to identify rapidly the geographical origin of oysters. In their work the processing of Py-MS fingerprints by artificial neural networks (ANNs) allowed correct classification of 89% of oyster samples collected over one year according to production area. Another approach consisting of analyzing the volatile fraction of oysters could be relevant to the discrimination of their geographical origin. This approach was already found to be promising for discriminating milk and cheese according to their conditions of production (*13, 15*). The solid phase microextraction−MS (SPME-MS) technique is particularly suitable for analyzing the volatile fraction of oysters, as it allows an efficient extraction of the volatile compounds at a moderate temperature (*16*).

The aim of this work was to determine the relevance of using the Py-MS technique to discriminate the geographical origin of oysters harvested over a longer period of time (two years instead of one year) and to assess the potential of another MS-based technique, that is, virtual SPME-MS, to discriminate the geographical origin of oysters using the data of the second year of harvest.

MATERIALS AND METHODS

Materials. Oysters (*Crassostrea gigas*) were sampled in France from sites of production distributed among seven production regions: Normandy (NO), northern Brittany (NB) and southern Brittany (SB), Bourgneuf Bay (BO), Marennes-Oléron Bay (MO), Arcachon Bay (AR), and Thau Lagoon (TH). These regions could be grouped into three major shorelines (*14*): the English Channel (NB, NO), the Atlantic coast (SB, BO, MO, AR), and the Mediterranean coast (TH). The harvest of oysters was carried out over four periods of the year, that is, November−December (P1), March−April (P2), June (P3), and September−October (P4), and during two campaign seasons, the first (C1) in 2000–2001 (P1, P2, P3, P4) and the second (C2) in 2003–2004 (P1, P2, P3).

Sample Preparation. *Sample Preparation for Py-MS Analysis.* Immediately after harvest, oysters were opened and their seawater contents were removed. A mass of 100 g of fresh oyster flesh from each site was ground using a model T25 Ultra-Turax homogenizer (Janke and Kunkel, IKA Labortechnik) to obtain a representative sampling of oyster flesh from each production site. The samples were put in a polyethylene low-density bag protected from light by aluminum foil and then stored at −80 °C. A mass of approximately 20 g of sample was transferred into a 50 mL glass flask (VWR International France, Fontenay-sous-bois, France), placed under nitrogen atmosphere, and closed with a butyl-Teflon septum (VWR International France). The sample was thawed at 4 °C overnight and then transferred into 29 × 104 mm, 50 mL polycarbonate centrifuge bottles under nitrogen atmosphere and closed with butyl-Teflon septum caps (Beckman Instruments, Fullerton, CA). After a 50000*g* centrifugation for 20 min at 4 °C, a volume of 1 mL of the aqueous phase was pipetted and diluted with 7 mL of deionized water. This dilution step was necessary to reduce the quantity of material analyzed and thus to minimize pollution in the transfer area of the pyrolysis mass spectrometer and to avoid a gradual loss of instrument sensitivity. The aqueous solutions obtained were transferred into glass flasks and stored under nitrogen atmosphere at −20 °C. One day before analysis, the aqueous solutions were left to thaw at 4 °C. A volume of 2 μL from each solution was pipetted, deposited on a clean iron−nickel foil (SS Scientific Ltd., Hellingly, U.K.), and dried at 150 °C for 7 min in an oven. Foils were introduced into silica pyrolysis tubes (Ets. Maillères, Aubière, France) as described by Berdagué et al. (*17*). A Viton O-ring was placed around each tube for airtightness during pyrolysis. Three replicates were performed for each sample.

Sample Preparation for Virtual SPME-MS Analysis. The oyster flesh was ground as described above. A mass of approximately 3 g of sample was transferred into a 10 mL vial (VWR International France), and 0.6 g of NaCl (Prolabo, Paris, France) was added to decrease the solubility of the volatile substances in the oyster flesh sample and therefore to increase their concentration in the headspace of the samples (*18*). The vials were set under nitrogen atmosphere and sealed with butyl-Teflon septum caps (VWR International France). The samples were protected from light by aluminum foil and thawed at 4 °C overnight prior to analysis.

Oyster Analysis. *Py-MS Analysis.* The pyrolysis mass spectrometer used in this study was a Cp-Py-MS RaPyD 400 (Horizon Instruments Ltd., Sussex, U.K.). The foil in the pyrolysis tube was heated at 530 °C for 3 s with a temperature rise time of 0.6 s chosen to provide a balanced fragmentation of the carbohydrate, lipid, and protein fractions (*14*). The pyrolysate then entered an expansion chamber heated at 160 °C and was diffused through a molecular beam tube to the ionization chamber of the mass spectrometer. To minimize secondary fragmentation of the pyrolysate, low-voltage electron impact ionization (33.9 eV) was used. Nonionized molecules were retained in a cold trap cooled by liquid nitrogen. The ionized fragments were focused by the electrostatic lens of a set of source electrodes, accelerated, and then directed into the quadrupole mass analyzer. The mass spectrometer scanned the ionized pyrolysate 65 times during pyrolysis. Data were collected as 191 mass fragments over the range of *m/z* 50−240.

SPME-GC-MS Analysis and Construction of Corresponding Virtual SPME-MS Fingerprints. (1) Addition of Standards to Oyster Samples. As described previously by Deport et al. (*19*), three standards were chosen to correct the instrumental signal drifts by applying the comprehensive combinatory standard correction (CCSC) method to the virtual SPME-MS data. The standards used were 1-bromobutane (S1; purity = 99.7%; retention index = 729), bromobenzene (S2; purity = 99.5%; retention index = 940), and 1-fluoronaphthalene (S3; purity = 99.0%; retention index = 1207) (Sigma Aldrich Chimie, St-Quentin-Fallavier, France). The retention indices of these standards were distributed evenly over the sample GC chromatograms. They were added in the salt−ground oyster mix to obtain a final concentration of approximately 0.1 ppm for each standard. Then the vials were sealed with butyl-Teflon septum caps, protected from light by aluminum foil, and kept at 4 °C overnight.

(2) Analysis Parameters. The samples kept at 4 °C were installed on a Peltier tray cooler (Gerstel, Mülheim an der Ruhr, Germany) set at 6 °C. The extraction of volatile compounds was carried out using a model MPS2 multipurpose sampler (GERSTEL, Baltimore, MD), which managed the following steps: preheating of the sample during 45 min at 40 °C in the stirrer (500 rpm), trapping of the volatile compounds of the headspace during 60 min at 40 °C with a 75 μm carboxen/polydimethylsiloxane SPME fiber for Merlin Microseal (Supelco, Bellefonte, PA), and thermic desorption of the trapped volatile compounds by introduction of the fiber in the GC injector. The compounds condensed at the head of the column were analyzed by a model 6890 GC (Hewlett-Packard, Avondale, PA) after the interface had been heated for 2 min at 280 °C and automatic splitless injection onto a 60 m × 0.32 mm i.d., 1 μm, SPB5 capillary column (Sigma Aldrich, St. Louis, MO). The oven temperature was successively held at 40 °C for 5 min, increased to 190 °C at a gradient of 3 °C min^{-1}, and further increased to 230 °C for 2 min according to a gradient of 10 °C min^{-1}. The GC column was connected to a model 5973A mass spectrometer (Hewlett-Packard). The temperature of the column in the transfer section between the GC oven and MS source was 280 °C. The temperature was fixed at 180 °C in the MS source and at 150 °C in the MS quadrupole. The electron impact energy was set at 70 eV, and data were collected in the range of *m/z* 33−230 at a scan range of 1.68 scan s^{-1}.

The principle of the construction of virtual SPME-MS fingerprints of the volatile fraction is shown in **Figure 1**: the mass spectra, which

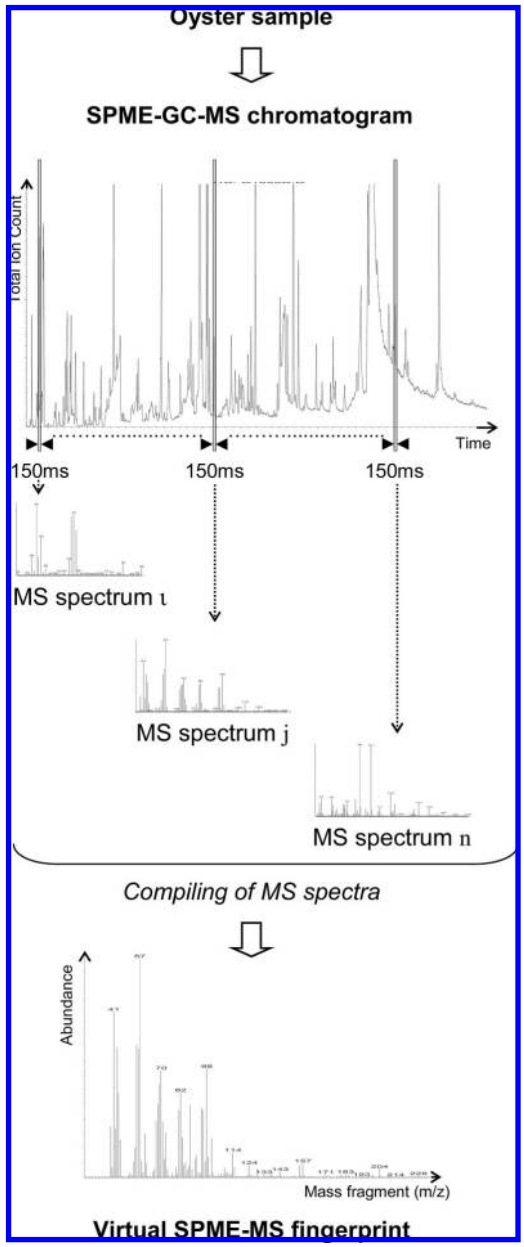

Figure 1. Scheme of the construction of a virtual MS fingerprint of an oyster sample from a GC-MS chromatogram of this tissue. The mass spectra, which were acquired every 150 ms of the GC-MS chromatogram, were summed and then converted in a MS fingerprint characterized by the abundance of 198 mass fragments ranging from *m*/*z* 33 to 230.

were acquired every 150 ms of the SPME-GC-MS chromatogram, were summed, resulting in a virtual SPME-MS fingerprint characterized by the abundance of 198 summed mass fragments ranging from *m*/*z* 33 to 230.

Data Treatment. Data were processed using the Statistica Neural Networks Software release 6.1 package (Statsoft, Maisons-Alfort, France).

Py-MS Data. *(1) Data Set.* Discrimination of the oyster production area was carried out on the two data sets of the Py-MS fingerprints of the oysters collected on the three shorelines during the C1 and C2 harvest campaigns: C1 = [471 mass spectra × 191 mass fragments], the 471 mass spectra originating from 157 oyster samples × 3 replicates; and C2 = [255 mass spectra × 191 mass fragments], the 255 mass spectra originating from 85 oyster samples × 3 replicates.

(2) Data Pretreatment. (a) Filtering of the Instrumental Noise. A mass fragment was considered to bring information and preserved for further treatment when the average abundance (calculated from C1 and C2 data matrices)/noise level ratio was greater than 2 (*20*).

(b) Median Filtering of Replicates. The median abundance of each mass fragment standing out from the background noise was selected from the three replicates to obtain one median mass spectrum for each oyster sample (*12*).

(c) Normalization of Data. The abundance of the selected fragments was then corrected by internal normalization consisting of the expression of each mass fragment abundance as a percentage of the sum of all mass fragment abundances.

(d) Filtering of Mass Spectra. Principal component analysis (PCA) was carried out on the filtered and normalized data sets for the C1 and C2 campaigns to visualize the structure of the data and to remove outlying samples.

(e) Merging of the Two Data Sets. To correct the drifts of analytical system occurring during and between the analyses of the C1 and C2 samples (*21, 22*), a factor was applied to each mass fragment F_i of the C2 data set:

$$F_{i,\mathrm{C2,corrected}} = F_{i,\mathrm{C2,noncorrected}} \times \frac{\bar{F}_{i,\mathrm{C1,noncorrected}}}{\bar{F}_{i,\mathrm{C2,noncorrected}}}$$

where $F_{i,\mathrm{C1}}$ and $F_{i,\mathrm{C2}}$ are the mass fragments from C1 and C2 data matrices, respectively, and $\bar{F}_{i,\mathrm{C1}}$ and $\bar{F}_{i,\mathrm{C2}}$ are the averages of the abundances of the mass fragments from C1 and C2 data matrices, respectively.

Then data from the two matrices were pooled into a single matrix containing all of the normalized mass fragments of the oyster samples. Principal component analyses were carried out on the pooled raw data and on the pooled corrected data to visualize the signal drift correction.

(3) Discrimination of Production Area. (a) Classification by Discriminant Analysis (DA). The selection of the relevant mass fragments from the compiled matrix for the discrimination of production area within each harvest period was performed by DA according to a stepwise algorithm (stepwise algorithm with *p* inclusion and *p* exclusion values of <0.05). The maximum number of discriminative mass fragments retained for computing each discriminant model within period was at maximum equal to the number of samples divided by 10.

(b) Classification by Artificial Neural Network (ANN). A three-layer network architecture was chosen to classify the oysters according to production area because of its ability to model most functions and to solve complex nonlinear problems (*23*). Network training was performed with the "standard back-propagation" algorithm (*24*). Learning, validation, and test were performed using 108, 53, and 53 samples, respectively. The oyster samples were distributed in each data set in a balanced way according to the two harvest campaigns, the four harvest periods, and the geographical origins. The computations of the network during the cross-validation steps were stopped when the validation error stopped decreasing (*24*).

Virtual SPME-MS Data. *Data Set.* The potential of the virtual SPME-MS method to discriminate oyster production area was evaluated with part of the oysters collected during the C2 harvest campaign. Three oyster samples from three production sites per production region were analyzed for each of the three harvest periods, and only two oyster samples from two sites in the Thau Lagoon were studied during period 2 of C2. Sixty-two samples were analyzed by virtual SPME-MS.

Filtering of Raw Data (FRD). The raw data were filtered by one-way analysis of variance (ANOVA) at the 5% level of significance (model: abundance of mass fragment = production area).

Pretreatment of Raw Data. Pretreatment of virtual fingerprints by comprehensive combinatory standard correction (CCSC) was compared to FRD to assess its ability to correct the raw data for the influence of the instrumental drifts: the mixture of three selected internal standards being analyzed together with the oyster sample, the abundance of each mass fragment normalized by the sum of the abundances of specific ion of standards, selected among the $\sum_{p=1}^{3} C_3^p$ possible sums, where *p* represents the number of standards involved in a given sum, that enabled the best product discrimination (*19*). The specific ion abundance of the standards used in the CCSC corrections of virtual SPME-MS fingerprints was quantified on the basis of the abundance of the standard specific ion quantified in SPME-GC-MS. Finally, a one-way ANOVA (model: CCSC pretreated abundance = production area, $p < 0.05$) was

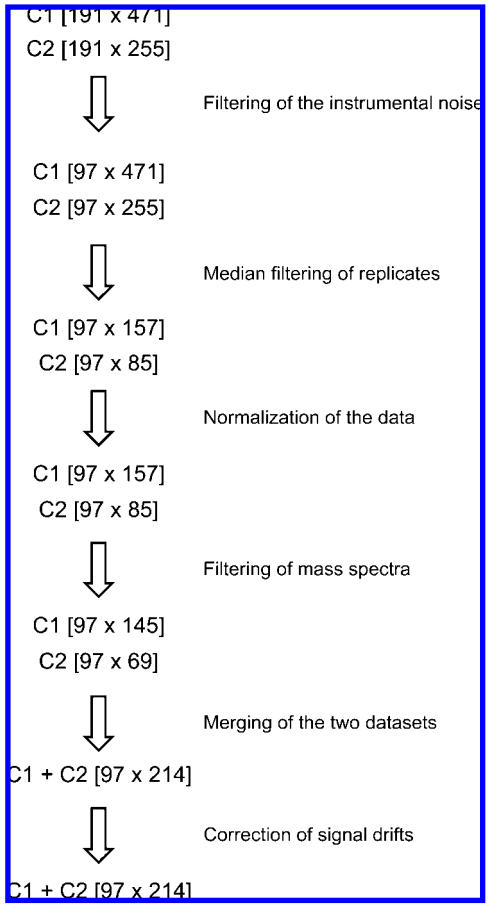

Figure 2. Data pretreatment steps of the Py-MS fingerprints of the oysters collected during two harvest campaigns, the first one (C1) in 2000–2001 and the second one (C2) in 2003–2004. Data matrices [$i \times j$] obtained after each pretreatment step included the number of mass fragments (i) and the number of mass spectra (j), which originated from the number of oyster samples $\times$ 3 replicates.

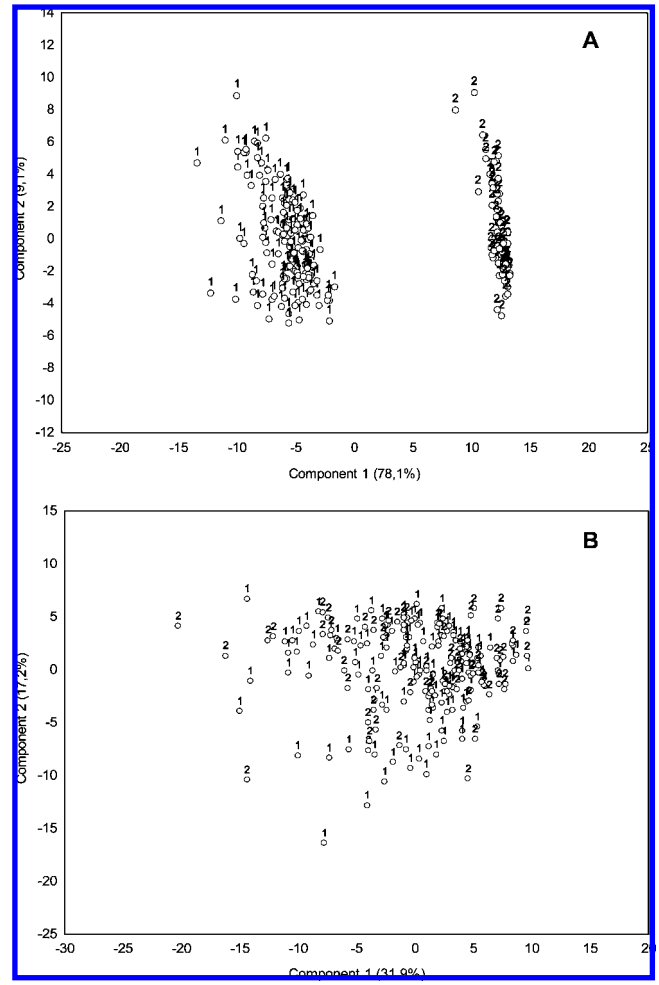

Figure 3. Normed PCAs carried out on the compiled matrix [97 $\times$ 214] resulting from the merging of the pretreated C1 and C2 data sets (**A**) without fingerprint correction of the between-campaign differences and (**B**) with correction of the between-campaign differences by the factor $F_{i,C2,corrected} = F_{iC2,noncorrected} \times (\bar{F}_{i,C1,noncorrected}/\bar{F}_{i,C2,noncorrected})$, where $F_{i,C1}$ and $F_{i,C2}$ are the mass fragments from C1 and C2 data matrices, respectively, and $\bar{F}_{i,C1}$ and $\bar{F}_{i,C2}$ are the averages of the abundances of the mass fragments from C1 and C2 data matrices, respectively. Samples with the number 1 belong to the C1 data set, and samples with the number 2 belong to the C2 data set.

processed for each mass fragment to select the discriminative corrected abundances. When several corrected abundances were significant for the same mass fragment, the combination of standards with the highest Fisher's F value was selected.

Discrimination of Production Area. DA was carried out on both FRD and CCSC pretreated data within each harvest period to discriminate oysters according to their production area (shoreline or region). The discriminant analyses were processed with a best subset algorithm. The classification models of oysters according to their production area were built for each data set. The number of discriminant variables in the models were set as the lowest number of discriminative fragments giving 100% of well-classified samples on the FRD or CCSC data set.

RESULTS AND DISCUSSION

Discrimination of Production Area by Py-MS. *Py-MS Data Pretreatment.* The different steps of the Py-MS data pretreatment are illustrated in **Figure 2**. After the noise-filtering step, 97 mass fragments were selected for further processing. The median filtering of the replicates used to stabilize the variance of the data produced two new data matrices with 157 and 85 samples for oysters collected during the C1 and C2 harvest campaigns, respectively. The abundance of the mass fragments selected by median filtering was processed by internal normalization to correct the intensity of the Py-MS signal for the variations in the oyster sample mass analyzed. This step led to a better distribution of sample plots along the PCA first map and allowed the aberrant samples in the data sets to be highlighted. As suggested by Sebastián et al. (*12*), the presence of aberrant samples can be explained by fluctuations in the quality of the vacuum in the MS source, by pollution in the transfer lines and/or source, and by the aging of the electron multiplier. Finally, 12 and 16 samples from the C1 and C2 harvest campaigns, respectively, were eliminated, leading to two new data sets with 145 and 69 samples, respectively. After the two data matrices had been merged into a single [97 $\times$ 214] matrix, the PCA first map clearly showed two groups of samples matching the two harvest seasons (**Figure 3A**). The differences observed between the two groups could be explained by variations in environmental conditions (streams, climate) between the two harvest seasons, which could have influenced the type of plankton consumed by the oysters and consequently their flesh composition (*6, 7*), and by an instrumental drift of the analytical system between the two analytical campaigns (*21, 22*). In **Figure 3A**, the between-campaign drift was directed mainly according to the first principal component, and it justified the linear correction applied to the data to merge both groups. **Figure 3B** shows

Table 1. Oyster Discrimination According to Their Shoreline of Production Obtained by Discriminant Analysis Processed on the Pretreated Py-MS Fingerprints

		discrimination of shoreline of production[a]		
harvesting period[b]	oyster samples	no. of discriminative mass fragment ($p < 0.05$)	mass fragments selected for modelization (m/z)	model performance (% of well-classified samples)
P1	81	7	53/66/67/72/78/80/93	88.3
P2	51	4	58/72/74/79	93.8
P3	52	5	59/65/72/84/94	94.1
P4	30	3	57/75/82	96.7

[a] Oyster samples were grouped in three shorelines of production: the English Channel, the Atlantic coast, and the Mediterranean coast. [b] Oyster samples analyzed by Py-MS were collected during two seasons (2000–2001 and 2003–2004) and four annual periods: November–December (P1), March–April (P2), June (P3), and September–October (P4).

that after the correction applied to each mass fragment of the C2 campaign data matrix, the two groups of plots corresponding to the two harvest campaigns fully overlapped in the PCA first map, suggesting that the between-campaign drift was mainly the result of instrumental drifts.

Discrimination of Production Area. DAs performed on data sets corresponding to the four harvest periods enabled between 88.3 and 96.7% of the oyster samples go be classified according to shoreline (**Table 1**). The lowest model performance was obtained on period P1, whereas seven discriminative mass fragments were entered in the model. The relatively poor performance of the linear model used for the discrimination is consistent with previous results suggesting the use of ANNs for discriminations based on Py-MS data (*12, 14, 25, 26*). With the ANNs, production area, harvest season, and their interactions were taken into account in a single model. The 17 different discriminative mass fragments retained by the stepwise algorithm in the DA models (**Table 1**) were used in the ANNs to limit the number of input variables and to prevent the data from overfitting during ANN training. The network consisted of three layers containing 21 inputs (the 17 mass fragments selected by DAs and the 4 harvest periods), one hidden layer containing 3 outputs (the 3 shorelines), and 12 nodes, corresponding to network architecture of the type 21–12–3. The discrimination model enabled correct classification of 89.2% of the oyster samples according to shoreline, showing that the cause of misclassification was not only the type of data treatment but could also be the use of the Py-MS technique. These misclassifications could result from an insufficient capacity of Py-MS to reveal differences between composition of oysters (*14*). Also, the preprocessing of Py-MS data was perhaps insufficient to correct the instrumental drifts, which are known to affect the reproducibility of the Py-MS systems (*21, 22*) and to mask part of the discriminative information (*19*).

Discrimination of Production Area by Virtual SPME-MS. Only the mass fragments from virtual SPME-MS fingerprints for which abundance was significantly influenced by the shoreline factor ($p < 0.05$) after FRD pretreatment were selected for each of the three harvest periods studied (**Table 2**). The classification models built from these discriminative mass fragments allowed correct classification of 90.5, 100, and 85.7% of the samples in periods 1, 2, and 3, respectively. The classification errors could be explained by insufficient sensitivity of the virtual SPME-MS system with respect to the differences in composition between oysters of different origins or instrumental drifts in the SPME-GC-MS system. To correct these instrumental drifts, which may be gradual or sudden, linear or nonlinear, and generally difficult to predict, particularly when they occur simultaneously (*27*), the virtual SPME-MS data were processed by the CCSC method developed by Deport et al. (*19*). It revealed 2.9, 1.4, and 12.1 times more discriminative mass fragments from the virtual SPME-MS data than did the FRD pretreatment for periods 1, 2, and 3, respectively (**Table 2**). Additionally, the corresponding discriminative models allowed 100% correct within-harvest period classifications of oyster samples according to shoreline using only three or four

Table 2. Oyster Discrimination According to Their Shoreline and Their Region of Production Obtained by Discriminant Analysis Processed on the Pretreated Virtual SPME-MS Fingerprints

					discrimination of production area		
			filtering of MS data		model performance		
	harvesting period[a]	oyster samples	data set pretreatment	no. of discriminative mass fragment ($p < 0.05$)	% of well-classified samples	no. of mass fragments	mass fragments selected for discrimination (m/z)
shoreline of production[b]	P1	21	FRD[c]	26	90.5	3	41/49/55
			CCSC[d]	75	100	3	114 S3[e]/247 S2S3/49 S3
	P2	21	FRD	16	100	4	35/73/232/180
			CCSC	22	100	4	35 S1/150 S3/134 S3/137 S3
	P3	20	FRD	10	85.7	3	144/157/158
			CCSC	121	100	3	98 S2/95 S2/162 S2S3
region of production[f]	P1	21	FRD[c]	11	85.3	5	169/102/64/61/35
			CCSC[d]	24	100	5	101 S3[e]/114 S3/169 S1/73 S3/195 S2S3
	P2	21	FRD	16	100	4	183/73/149/161
			CCSC	15	100	4	161 S3/182 S3/ 243 S2/47 S1S3
	P3	20	FRD	23	95.2	4	172/138/61/144
			CCSC	87	100	4	102 S2/182 S3/189 S3/237 S2S3

[a] Oyster samples analyzed by virtual SPME-MS were collected during three annual periods: November–December (P1), March–April (P2), and June (P3). [b] Oyster samples were grouped in three shorelines of production: the English Channel, the Atlantic coast, and the Mediterranean coast. [c] FRD, filtering of raw data. [d] CCSC, comprehensive combinatory standard correction. [e] Best standard or combination of standards used to correct each fragment abundance with the CCSC: S1, 1-bromobutane; S2, fluorobenzene; S3, 1-fluoronaphthalene. [f] Oyster samples originated from seven regions of production: Normandy (NO), northern Brittany (NB), southern Brittany (SB), Bourgneuf Bay (BO), Marennes-Oléron Bay (MO), Arcachon Bay (AR), and Thau Lagoon (TH).

discriminative mass fragments (**Table 2**). This result confirms that, by reducing the effect of instrumental drifts, the CCSC preprocessing method increases both the number and the discriminative power of markers of product differentiation and therefore the efficiency of the method to extract useful information from stabilized MS databases (*19, 28*).

The discrimination of shorelines having been successful after CCSC pretreatment of virtual SPME-MS data, the discrimination of oyster origin at a smaller geographical scale (production region) was undertaken. The CCSC revealed 2.2 and 3.8 times more mass fragments discriminating the production region than did FRD pretreatment in periods 1 and 3, respectively (**Table 2**). The CCSC did not improve the number of discriminative mass fragments in period 2, probably because the origin-related compositional differences between oysters were large enough to be revealed by FRD pretreatment. The corresponding discriminative models built after CCSC pretreatment allowed a 100% correct within-harvest-period classification of oyster samples according to production region using only four or five discriminative masses (**Table 2**). In contrast with previous results obtained by Cardinal et al. (*14*) with the Py-MS technique, 100% of the oysters collected over one year of harvest were correctly classified by virtual SPME-MS according to their production area, showing that this technique is a promising tool for authentication. Further investigation is needed to validate the discriminative potential of the virtual SPME-MS fingerprints coupled with CCSC data pretreatment for the discrimination of geographical origin on a broader range of oyster samples, origins, and harvest campaigns.

The Py-MS technique led to an unsuccessful discrimination of the oyster samples according to their production area even at the largest geographical scale studied. The results of the discrimination of the production area using virtual SPME-MS data, here generated by a SPME-GC-MS system, are promising because this technique could classify correctly the oyster samples according to shoreline and also production region within each harvesting period, when the virtual SPME-MS data were pretreated by the CCSC method. The virtual SPME-MS technique has several advantages compared to the Py-MS for the discrimination of the geographical origin of oysters. First, it gives access to the information related to the volatile fraction of animal products, which depends directly upon the modifications of the animal metabolism induced by the environmental conditions. Second, it did not require any prior preparation of the sample before analysis. Third, it allows using the CCSC method for the correction of analytical instrumental drifts and then revealing the discriminative information contained in the volatile fraction of food products. Indeed, the temperature applied to the sample during the Py-MS analysis makes impossible the use of heat labile standards required by the CCSC method. Fourth, the SPME-GC-MS analysis allows additionally the use of the information supplied by the GC separation and thus may explain the molecular origin of the compositional differences observed between oysters from different production areas.

A collection of data of plankton composition and environmental conditions has been undertaken to increase the information from virtual SPME-MS data and thus to improve the robustness of this technique for the discrimination of oyster origin. The identification of molecular markers of geographical origin from the SPME-GC-MS signal is also being investigated.

ACKNOWLEDGMENT

We thank the IFREMER coastal laboratories, which ensured the sampling of oysters, especially Port en Bessin, La Trinité sur Mer, Concarneau, Bouin, La Tremblade, Arcachon et Sète.

LITERATURE CITED

(1) Desenclos, J. C. A.; Klontz, K. C.; Wolfe, L. E.; Hoecheri, S. The risk of Vibrio illness in the FL raw oyster eating population, 1981–1988. *Am. J. Epidemiol.* **1991**, *134*, 290–297.
(2) Guo, H. R. Cancer risk assessment for arsenic exposure through oyster consumption. *Environ. Health Perspect.* **2002**, *110*, 123–124.
(3) Karouna-Renier, N. K.; Snyder, R. A.; Allison, J. G.; Wagner, M. G.; Rao, K. R. Accumulation of organic and inorganic contaminants in shellfish collected in estuarine waters near Pensacola, FL: contamination profiles and risks to human consumers. *Environ. Pollut.* **2007**, *145*, 474–488.
(4) Bayen, S.; Lee, H. K.; Obbard, J. P. Exposure and response of aquacultured oysters, Crassostrea gigas, to marine contaminants. *Environ. Res.* **2007**, *103*, 375–382.
(5) Pennarun, A. L.; Prost, C.; Haure, J.; Demaimay, M. Comparison of two microalgal diets. 1. Influence on the biochemical and fatty acid compositions of raw oysters (*Crassostrea gigas*). *J. Agric. Food Chem.* **2003**, *51*, 2006–2010.
(6) Dégremont, L.; Bédier, E.; Soletchnik, P.; Ropert, M.; Huvet, A.; Moal, J.; Samain, J. F.; Boudry, P. Relative importance of family, site, and field placement timing on survival, growth, and yield of hatchery-produced Pacific oyster spat (*Crassostrea gigas*). *Aquaculture* **2005**, *249*, 213–229.
(7) Piveteau, F.; Gandemer, G.; Baud, J. P.; Demaimay, M. Changes in lipid and fatty acid compositions of European oysters fattened with skeletonema costatum diatom for six weeks in ponds. *Aquacult. Int.* **1999**, *7*, 341–355.
(8) Vasta, V.; Ratel, J.; Engel, E. Mass spectrometry analysis of volatile compounds in raw meat for the authentication of the feeding background of farm animals. *J. Agric. Food Chem.* **2007**, *55*, 4630–4639.
(9) Sebastiàn, I.; Viallon-Fernandez, C.; Berge, P.; Berdagué, J. L. Analysis of the volatile fraction of lamb fat tissue: influence of the type of feeding. *Sci. Aliments* **2003**, *23*, 497–511.
(10) Schmidt, O.; Quilter, J. M.; Bahar, B.; Moloney, A. P.; Scrimgeour, C. M.; Begley, I. S.; Monahan, F. J. Inferring the origin and dietary history of beef from C, N and S stable isotope ratio analysis. *Food Chem.* **2005**, *91*, 545–549.
(11) Banks, M. A.; Hedgecock, D.; Waters, C. Discrimination between closely related Pacific oyster species (*Crassostrea*) via mitochondrial DNA sequences coding for large subunit rRNA. *Mol. Mar. Biol. Biotechnol.* **1993**, *2*, 129–136.
(12) Sebastiàn, I.; Viallon, C.; Tournayre, P.; Berge, P.; Berdagué, J. L. Pyrolysis−mass spectrometry characterisation of ovine fat tissues according to diet. *Analusis* **2000**, *28*, 141–147.
(13) Pérès, C.; Viallon, C.; Berdagué, J. L. Solid-phase microextraction–mass spectrometry: a new approach to the rapid characterization of cheeses. *Anal. Chem.* **2001**, *73*, 1030–1036.
(14) Cardinal, M.; Viallon, C.; Thonat, C.; Berdagué, J. L. Pyrolysis−mass spectrometry for rapid classification of oysters according to rearing area. *Analusis* **2000**, *28*, 825–829.
(15) Pérès, C.; Begnaud, F.; Eveleigh, L.; Berdagué, J. L. Fast characterization of foodstuff by headspace mass spectrometry (HS-MS). *Trends Anal. Chem.* **2003**, *22*, 858–866.
(16) Pawliszyn, J. In *Applications of Solid Phase Microextraction*; RSC: Cambridge, U.K., 1999.
(17) Berdagué, J. L.; Rabot, C.; Bonneau, M. Rapid classification of backfat samples selected according to their androstenone content by pyrolysis−mass-spectrometry. *Sci. Aliments* **1996**, *16*, 425–433.
(18) Canac-Arteaga, D.; Begnaud, F.; Viallon, C.; Berdagué, J. L. Effect of adding hygroscopic salts on the analysis of the volatile fraction of cheese. *Analusis* **2000**, *28*, 973–979.

(19) Deport, C.; Ratel, J.; Berdagué, J. L.; Engel, E. Comprehensive combinatory standard correction: a calibration method for handling instrumental drifts of gas chromatography–mass spectrometry systems. *J. Chromatogr., A* **2006**, *1116*, 248–258.
(20) Begnaud, F.; Berdagué, J. L. Influence of ionisation energy on the quality of static head space-mass spectrometry spectral fingerprints. *Sens. Actuators, B* **2002**, *81*, 2–3.
(21) Goodacre, R.; Kell, D. B. Correction of mass spectral drift using artificial neural networks. *Anal. Chem.* **1996**, *68*, 271–280.
(22) Goodacre, R.; Timmins, E. M.; Jones, A.; Kell, D. B.; Maddock, J.; Heginbothom, M. L.; Magee, J. T. On mass spectrometer instrument standardization and interlaboratory calibration transfer using neural networks. *Anal. Chim. Acta* **1997**, *348*, 511–532.
(23) Haykin, S. In *Neural Networks: A Comprehensive Foundation*; Macmillan Publishing: New York, 1994.
(24) McClelland, J. L.; Rumelhart, D. E. In *Explorations in Parallel Distributed Processing: A Handbook of Models, Programs and Exercises*; MIT Press: Cambridge, MA, 1988.
(25) Goodacre, R.; Kell, D. B. Rapid and quantitative analysis of bioprocesses using pyrolysis MS and neural networks: application to indole production. *Anal. Chim. Acta* **1993**, *279*, 17–26.
(26) Goodacre, R.; Kell, D. B.; Bianchi, G. Rapid assessment of the adulteration of virgin olive oils by other seed oils using pyrolysis mass spectrometry and artificial neural networks. *J. Sci. Food Agric.* **1993**, *63*, 297–307.
(27) Pérès, C.; Begnaud, F.; Berdagué, J. L. Standard gas addition: a calibration method for handling temporal drifts of mass spectrometry-based sensors. *Anal. Chem.* **2002**, *74*, 2279–2283.
(28) Engel, E.; Ratel, J. Correction of the data generated by mass spectrometry analyses of biological tissues: application to food authentication. *J. Chromatogr., A* **2007**, *1154*, 331–341.

Received for review July 24, 2007. Revised manuscript received October 1, 2007. Accepted October 3, 2007.

JF072207I