JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Assessment of Strawberry Aroma through Solid-Phase Microextraction−Gas Chromatography and Artificial Neuron Network Methods. Variety Classification versus Growing Years

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In a previous work, the SPME-GC-MS method (chemical analysis) coupled with KSOM-ANN treatment of the results (statistical algorithm) has proved to be efficient to classify 70 strawberry samples harvested in the same year, through the 17 varieties to which they belonged, in a two-dimensional map. As an extension, the present study confirms that these results were not dependent on the year of strawberry production and discusses what effects were observed between results obtained in different years. Samples of different strawberry varieties were harvested during the three campaigns of 2000, 2001, and 2002 and analyzed independently. The chemical data matrix obtained in each case allowed the verification of the proposal that the same discriminative effect could be obtained independently of the year of production by using maps of different sizes. Therefore, 30 measures obtained from samples of 9 varieties in 2000, 54 measures from 13 varieties in 2001, and 80 measures from 20 varieties in 2002 were correctly classified by using 20, 35, and 56 hexagon maps, respectively. In a second analysis based on the 2002 production, the chemical differences between variety aromatic features were noted through the increasing size of the map used. Finally, results relative to 7 varieties cultivated in 2001 and 2002 and stored under exactly the same conditions were computed together for elaborating a single map. An interesting effect of double classification according to the year and the varieties was observed.

KEYWORDS: Strawberry variety aroma constituents; SPME analysis; Kohonen SOM; computational method; discrimination; characterization; plant breeding

INTRODUCTION

To provide an efficient and running analytical tool to strawberry plant breeders who have to characterize and compare the aromatic properties of new cultivars to those already known, a chemical method using headspace solid-phase microextraction (HS-SPME), gas chromatography−mass spectrometry analysis (GC-MS) (*1*−*6*) coupled with a statistical treatment of the results by artificial neuron networks (ANN), has been recently developed by the authors and published in this journal (*7*). Following this approach, the method was based on the relative determination of 23 constituents and allowed to chemically characterize aromas of 17 strawberry varieties, to fully discriminate them and also to recognize unknown samples belonging to these varieties. For this purpose, the Kohonen self-organizing maps (KSOM) (*8*) algorithm was applied to the matrix of results and proved to be very efficient. Actually, a 24-hexagon map was sufficient for obtaining the full discrimination of the 70 analyzed samples, gathering all samples from the same variety in the same hexagon and having no hexagons containing several varieties. Moreover, unknown samples belonging to the panel of the studied varieties were correctly classified by the whole method and projected into the units of the map corresponding to the right variety, thereby providing a recognition function to this analytical approach.

More recently, Dutta et al. (*9*) used the same approach to discriminate different qualities of tea by using a similar process. To our knowledge and except for this very recent example, no other methods using natural noses through any sensorial analysis techniques or artificial noses through any physical or chemical analysis systems have been described as being able to realize such discriminative classification and recognition performances. In these conditions, the proposed method dealing with the aroma chemical constitution appears to be of real interest for plant breeders for whom the discriminative classification of existing varieties is the inescapable first step for positioning a new cultivar comparatively to parent varieties and all others previously existing. Then, it was necessary to verify that these results

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10.1021/jf035376l CCC: $27.50
Published on Web 04/06/2004

Table 1. Presentation of the 25 Selected Varieties through the Three Campaigns with Their Corresponding Measure Codes

code	variety	2000	2001	2002
A	Capron Royal	A1–A2	A1–A2–A3–A4	
B	CF129	B1–B2	B1–B2–B3–B4–B5–B6	B1–B2–B3–B4
C	Ciflorette	C1–C2–C3–C4	C1–C2–C3–C4	C1–C2–C3
D	Cigaline	D1–D2–D3–D4	D1–D2–D3–D4	D1–D2–D3–D4
E	Cilady	E1–E2–E3–E4	E1–E2–E3–E4	E1–E2–E3–E4
F	Ciloé	F1–F2–F3–F4	F1–F2–F3–F4	F1–F2–F3–F4
G	Cireine	G1–G2	G1–G2–G3–G4	G1–G2–G3
H	Cigoulette	H1–H2–H3–H4–H5–H6		H1–H2–H3–H4–H5–H6
I	Earliglow			I1–I2–I3–I4
J	Pajaro		J1–J2–J3–J4	J1–J2–J3–J4
K	CF1116		K1–K2–K3–K4	K1–K2–K3–K4
L	Capitola		L1–L2–L3–L4	L1–L2–L3–L4
M	Marabella		M1–M2–M3–M4	
N	Sengana		N1–N2–N3–N4	
O	CF2024	O1–O2		
P	Selene		P1–P2–P3–P4	
Q	Cifrance			Q1–Q2–Q3–Q4–Q5–Q6
R	Darselect			R1–R2–R3–R4–R5–R6
S	Madeleine			S1–S2–S3–S4
T	CF1110			T1–T2–T3
U	CF1693			U1–U2–U3–U4
V	CF2036			V1–V2–V3–V4
W	CF2337			W1–W2–W3
X	CF2559			X1–X2–X3–X4
Y	CF2838			Y1–Y2–Y3
total		**9**	**13**	**20**

were not dependent on the year of production of the strawberries and to compare the influence of map size on the discriminating effect through several years.

The objective of the following study was thus to validate the use of this method for characterizing aromas of different strawberry varieties harvested during the three campaigns of 2000, 2001, and 2002, respectively. According to the previously described procedure, 25 varieties have been analyzed over this period and some of them have been selected for two or three years. The data matrix thus obtained was used to elaborate annual maps for verifying that the same discriminating effect could be obtained independently of the strawberry year of production and by using this procedure. At the same time and based on the 2002 production, the chemical differences between variety aromas were noted through the increasing size of the corresponding map. Finally, results relative to seven varieties commonly cultivated in 2001 and 2002, and stored under exactly the same conditions, were computed together for elaborating a single map in which their relative distribution was studied.

MATERIALS AND METHODS

Varieties. All strawberry samples were provided by the producer partner in charge of the selection program (CIREF, Prigonrieux, France), and the 25 selected varieties are listed in **Table 1**. Those named with the reference code CF (followed by a number) are new cultivars presently developed in the CIREF breeding program. The 9, 13, and 20 varieties studied in 2000, 2001, and 2002, respectively, were harvested at full maturation and immediately frozen and kept at −18 °C until being analyzed. Nevertheless, the samples of 2000 and 2001 came from two or three pickings performed on two or three different dates of each campaign, whereas all of the samples of 2002 were harvested on the same day (**Table 2**). In addition, the samples of 2000 were analyzed after one year whereas those of the 2 other campaigns were analyzed within 3 months after freezing.

Chemicals. To identify the 23 selected volatile compounds, standards provided by Sigma-Aldrich (Saint Quentin-Fallavier, France) were used and are presented in **Table 3**. All purities were >95%. Stock solutions of each standard at 1000 mg/L and mix solutions at 25 mg/L were prepared in methanol, under N_2 atmosphere.

GC-MS Analysis. A Varian 3400 GC coupled with a Finnigan Mat ITS 40 ion trap mass spectrometer (Thermo Finnigan, Les Ulis, France) was used for the 2000 and 2001 campaigns. Analytes were separated on a PTE5 30 m × 0.32 mm × 0.25 μm column (Supelco, Saint Quentin Fallavier, France) by applying the following temperature program: 40 °C for 3 min, increased from 40 to 60 °C at 2 °C/min and from 60 to 130 °C at 10 °C/min, held at 130 °C for 8 min, increased from 130 to 280 °C at 20 °C/min, and then held at 280 °C for 1.5 min. The 2002 campaign was treated exactly the same with a Trace GC coupled with a Polaris ion trap mass spectrometer (ThermoQuest, Les Ulis, France) fitted with a CombiPal autosampler (AlphaMos, Toulouse, France). Similarly, both MS detectors were tuned under the same conditions. Thus, all identifications were based on the comparison of retention times and mass spectra of the compounds with those of standards (**Table 3**). For each compound, relative quantification was performed by replotting the full-scan data by using selective ions and measuring the corresponding peak area.

Sample Preparation. About 200 g of strawberries was defrosted for at least 2 h and blended at 13500 rpm (Ultraturrax) for 1 min. Aliquots of 50 g of blended fruits were weighted into four Teflon vials and then centrifuged at 3000*g* and 4 °C for 15 min. Supernatant were collected in a 50 mL volumetric flask and immediately submitted to the SPME procedure.

In 2000 and 2001, each sample of each studied variety was prepared as described above and analyzed in duplicates. Each so obtained chromatogram provided a "measure" constituted by a set of 23 relative concentrations corresponding to the 23 selected aromatic constituents. The couples of measures relative to the variety α were denoted α_1 and α_2, α_3 and α_4, and, eventually α_5 and α_6, according to the harvest date of the corresponding sample, as indicated in **Table 2**.

In 2002, all samples of the 20 studied varieties were harvested on the same day. Most of these samples were also analyzed in duplicate, affording couples of measures denoted β_1 and β_2, β_3 and β_4, and, eventually, β_5 and β_6, in relation with the analyzed sample. Besides these, five samples have been analyzed only once, providing isolated measures for C_3 (Ciflorette), G_3 (Cireine), T_3 (CF1110), W_3 (CF2337), and Y_3 (CF2838), respectively.

SPME Procedure. Because the 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB) fiber (Supelco) has already proved its efficiency with volatile flavor components (*10*, *11*), this fiber was chosen to perform SPME analyses. Except for 2002 samples for which

Table 2. Harvest Dates of 2000 and 2001 for Each Sample

code	variety	measure code	harvest date
		2000	
A	Capron Royal	A1–A2	May 31
B	CF129	B1–B2	May 2
C	Ciflorette	C1–C2	May 2
		C3–C4	May 9
D	Cigaline	D1–D2	May 2
		D3–D4	May 9
E	Cilady	E1–E2	May 2
		E3–E4	May 9
F	Ciloé	F1–F2	May 2
		F3–F4	May 31
G	Cireine	G1–G2	May 9
H	Cigoulette	H1–H2	May 2
		H3–H4	May 9
		H5–H6	May 31
O	CF2024	O1–O2	May 31
		2001	
A	Capron Royal	A1–A2	May 30
		A3–A4	June 7
B	CF129	B1–B2	May 17
		B3–B4	May 17
		B5–B6	May 23
C	Ciflorette	C1–C2	May 17
		C3–C4	May 23
D	Cigaline	D1–D2	May 17
		D3–D4	May 23
E	Cilady	E1–E2	May 17
		E3–E4	May 23
F	Ciloé	F1–F2	May 17
		F3–F4	May 23
G	Cireine	G1–G2	May 30
		G3–G4	June 6
J	Pajaro	J1–J2	May 17
		J3–J4	May 23
K	CF1116	K1–K2	May 17
		K3–K4	May 23
L	Capitola	L1–L2	May 17
		L3–L4	May 23
M	Marabella	M1–M2	May 17
		M3–M4	May 23
N	Sengana	N1–N2	May 17
		N3–N4	May 23
P	Selene	P1–P2	May 17
		P3–P4	May 23

SPME was performed with an autosampler, all other samples were extracted with a manual holder (Supelco) according to the previously described procedure. Thus, for each measure, a 5 mL aliquot of a defined strawberry preparation was transferred into a Teflon-lined septum cap vial, equipped with a glass-coated magnetic stirring bar. To favor the transfer of the analytes from the aqueous solution to the headspace, 10% of NaCl was added. Then, the solution was stirred at 1000 rpm at 35 °C. After a 5 min equilibration time between the solution and the headspace, the PDMS/DVB fiber was exposed for 30 min in the headspace. The PDMS/DVB fiber was then withdrawn and introduced into the injector port of the GC for desorption at 250 °C during 3 min in the splitless mode. Then the split valve was opened, but the fiber was kept for 10 min in the injector for cleaning. Concerning the samples of 2002, the extraction protocol was conducted in the same way, but equilibration, extraction, and desorption times were controlled by the automatic system.

Data Treatment. *Preprocessing.* As previously described (*7*), chemical data were largely different from each other, within samples analyzed in a given year, in terms of magnitude levels of the observed signals. To standardize these data, each signal, x, relative to each selected aromatic constituent in a given variety was transformed into a y value according to the relationship

$$y = \log(x + 1)$$

and then converted into a centered reduced variable z

$$z = (y - \bar{y})/\sigma_y$$

Table 3. Peak Number, Retention Time, and Mass Spectra Description of the 23 Selected Compounds (Underscored Ions Were Used for Quantification)

peak	compound	RT (min)	fragments (abundance)
1	pentanone-2	2.10	71 (35%); 86 (100%); 87 (89%)
2	methyl butanoate	2.27	74 (100%); 71 (56%); 103 (17%)
3	ethyl butanoate	4.34	71 (100%); 88 (23%); 116 (24%)
4	butyl acetate	4.80	56 (62%); 61 (100%); 116 (25%)
5	butanoic acid	5.59	71 (68%); 60 (100%); 89 (78%)
6	(*E*)-2-hexenal	5.24	69 (84%); 83 (67%); 98 (3%)
7	(*Z*)-3-hexen-1-ol	6.03	67 (100%); 83 (48%); 95 (5%)
8	2-methylbutanoic acid	6.09	73 (39%); 74 (100%); 87 (27%)
9	heptanone-2	7.07	58 (38%); 97 (4%); 115 (100%)
10	methyl hexanoate	8.40	87 (35%); 99 (20%); 131 (100%)
11	ethyl hexanoate	12.45	99 (25%); 115 (11%); 145 (100%)
12	hexanoic acid	13.33	60 (100%); 73 (47%); 117 (7%)
13	DMF[a]	15.22	69 (34%); 85 (7%); 142 (100%)
14	DHF[a]	15.53	69 (100%); 83 (45%); 128 (2%)
15	linalol	16.37	71 (24%); 81 (100%); 154 (0.5%)
16	octanoic acid	18.93	101 (35%); 60 (100%); 145 (31%)
17	methyl anthranilate	21.37	119 (24%); 120 (28%); 151 (100%)
18	eugenol	22.06	103 (41%); 149 (42%); 164 (100%)
19	γ-decalactone[a]	25.13	110 (12%); 153 (56%); 171 (100%)
20	δ-decalactone[a]	26.16	71 (85%); 153 (31%); 171 (100%)
21	(*Z*)-nerolidol	28.04	121 (46%); 69 (100%); 149 (15%)
22	(*E*)-nerolidol	29.07	121 (46%); 69 (100%); 149 (15%)
23	γ-dodecalactone[a]	31.11	85 (100%); 199 (10%); 181 (5%)

[a] DMF, 2,5-dimethyl-4-methoxy-3(2*H*)-furanone; DHF, 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone; γ-decalactone, 4-hydroxydecanoic acid lactone; δ-decalactone, 5-hydroxydecanoic acid lactone; γ-dodecalactone, 4-hydroxydodecanoic acid lactone.

where $\bar{y}$ and σ_y are the mean and the standard deviation of the calculated y value, respectively.

Processing by Using Kohonen Maps. To extract the structure of the high-dimensional data matrix formed by the 23 chemical components analyzed in each strawberry sample within each year, the unsupervised neural network method KSOM was used as indicated below. Unlike the well-known supervised neural networks (for instance, the back-propagation neural network), KSOM can be used with only a few samples for each variety (*12*).

Equipment similar to that described in the former publication (*7*) was used. Calculations have been performed with a personal computer, equipped with an Intel Pentium III-500 processor. For the SOM development, *R* software (*13*) has been used with a program file written by the authors (*14*).

For each year, the chemical data relative to all of the measures made from the selected varieties were computed, affording series of maps from which the discriminative efficiency of the method was studied. In a final step, a unique computed map was performed, by using results obtained in 2001 and 2002 from samples relative to seven varieties selected through both of these years.

RESULTS AND DISCUSSION

Chromatographic Analysis. As already demonstrated in the previous work, the analysis method coupling HS-SPME and GC-MS and using parameters indicated in **Table 3** proved to be efficient to provide for each variety and each year a type of "chemical signature" that appeared to be clearly different when compared to all of the others. This means that the variation of this signature from one measure to another one, and from one sample to another of the same variety, is much smaller than that between two samples of two different varieties, respectively. On the contrary, the comparison of signatures provided by samples of the same variety but produced in different years revealed an obvious difference as indicated in **Figure 1**, where chromatograms relative to three samples of the same variety

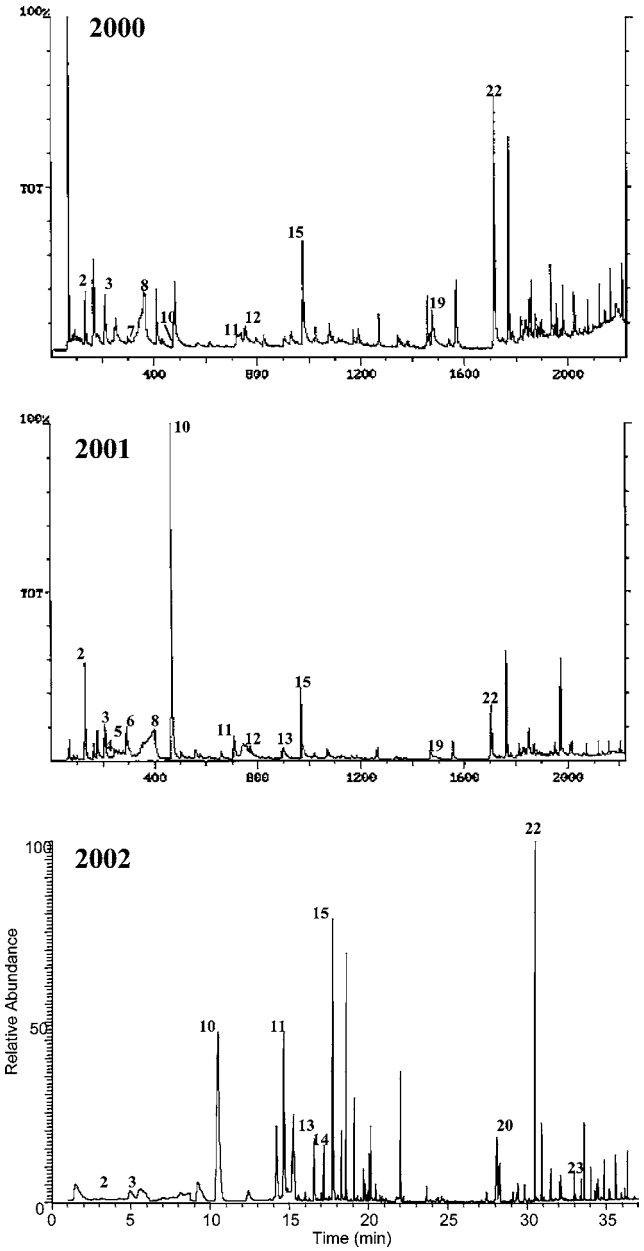

Figure 1. Chromatograms from Ciloé (F) variety of the 2000, 2001, and 2002 campaigns. For peak identification, see **Table 3**.

Ciloe (F) were exemplified through the three years of production. For example, relative intensities of peaks 10, 15, and 22 were obviously observed in a different order of importance when chromatograms obtained in 2000, 2001, and 2002 were compared. From this point, it was assumed that combining these two parameters, variety and year of production, would induce major difficulties in terms of ordination and classification of varieties. Then, the reading of all these chemical signatures by using the KSOM method appeared to be of major interest.

Discriminative Effect. The KSOM method was then applied to the 9 varieties (30 measures) of 2000, the 13 varieties (54 measures) of 2001, and the 20 varieties (80 measures) of 2002, independently. Each of the three maps shown in **Figure 2**, and corresponding to the 3 years of production, respectively, has been selected according to their size correlated to their discriminating efficiency.

For the year 2000, the total discrimination of the 9 varieties was obtained with a map of 20 hexagons, where no units contained samples of different varieties (**Figure 2a**). In 2001, the same result was observed with a map of 35 hexagons, but for discriminating 13 varieties through a larger data matrix elaborated from 54 different measures (**Figure 2b**). In the same way, 56 neurons were necessary for the same efficiency for the treatment of the 20 varieties and 80 measures operated on 2002 samples (**Figure 2c)**.

Beyond this first observation, it must be noted that throughout all of these maps, couples of measures realized from the same sample are never separated into different cells except in one case, the map of year 2002, where R_5 (Darselect) appeared in a contiguous hexagon of that containing R_6 and all of the other measures relative to this variety. This feature confirmed the observation made in the previous paragraph relative to the chromatogram description, that the variability of the method due to the SPME procedure (between measures) is at a low level compared to the difference existing between different varieties.

Nevertheless, it appeared clearly in each map that the grouping of all the samples relative to one variety in the same or neighboring hexagons was not as effective every year. In 2002, this grouping was verified for all of the varieties as long as the reading of the signatures of real samples came out in the same hexagon for 14 varieties, whereas they were located into contiguous units for the 6 others (E, F, G, I, R, and S). On the contrary, in 2001, 4 varieties (E, L, M, and N) among the 13 studied have got their respective signatures placed in nonadjacent hexagons, but always with the two measures relative to the same sample in the same one. In 2000, the same phenomenon was observed for the varieties F, C, and H, whereas D and E came out in the same hexagon or in two adjacent ones, respectively.

In fact, these surprising differences characterized by the method between samples of the same variety in 2000 and 2001 must be correlated with indications given in **Table 2** that deals with the dates at which these samples had been harvested. The samples of the four varieties in 2001 and the three others in 2000 for which a lack of grouping was observed had been taken on different dates and from plants at different levels of their biological cycle in the season. In these conditions, one can understand that the flavors produced by these samples could present some major differences. This was not the case in 2002, when all of the samples had been collected on the same date. Of course, the influence of the stage of the plant cycle on the aromatic composition of the fruit has no reason to be of the same importance whatever the variety is, and this could explain why some of the samples of some varieties stayed grouped in the corresponding maps, whereas they had also been taken at different dates.

Nevertheless, the variety Cigoulette (H), as analyzed in 2000, presents an interesting featuring. The three analyzed samples had been collected at three different dates, May 2, 9, and 31, respectively, affording three couples of measures H_1/H_2, H_3/H_4, and H_5/H_6, with a better grouping for the two first separated by only 7 days than between the last two, harvested with a 22 day difference. If the aromatic constitution of a variety moves according to time during one season, it can be considered as reasonable to observe a larger difference for 3 weeks than for a period of only 1.

Influence of the Map Size. As already indicated and commented in the frame of one year of production, the level of the discrimination effect is strongly dependent on the number of neurons characterizing the map. Consequently, batteries of maps were computed for each year to study the evolution of this effect. The series of maps trained in 2002 were revealed to

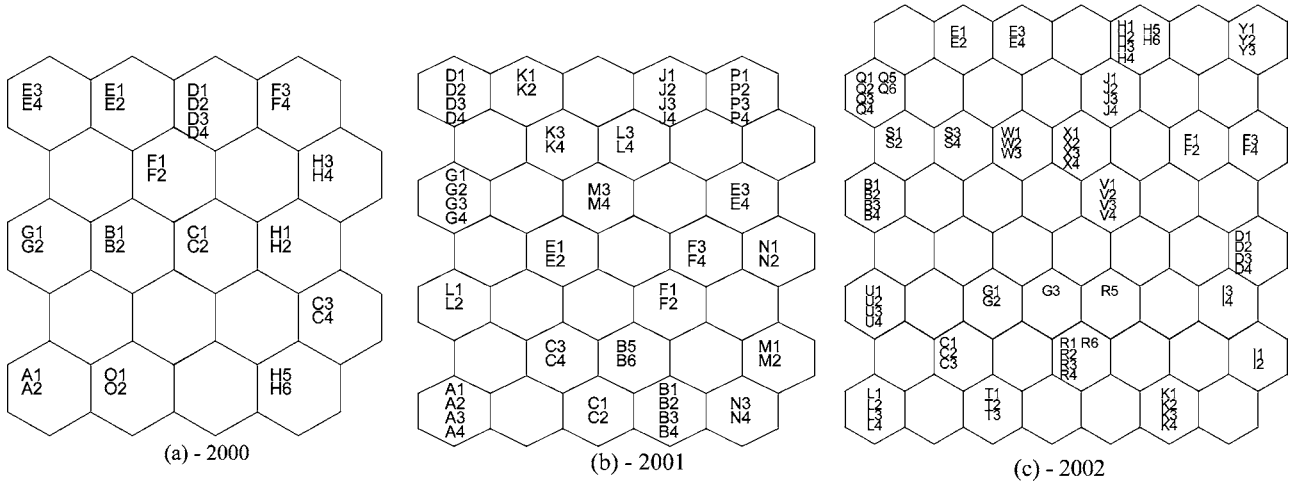

Figure 2. Distribution of the studied variety samples through three production years onto a 20-unit map for 2000 (**a**), onto a 35-unit map for 2001 (**b**), and onto a 56-unit map for 2002 (**c**).

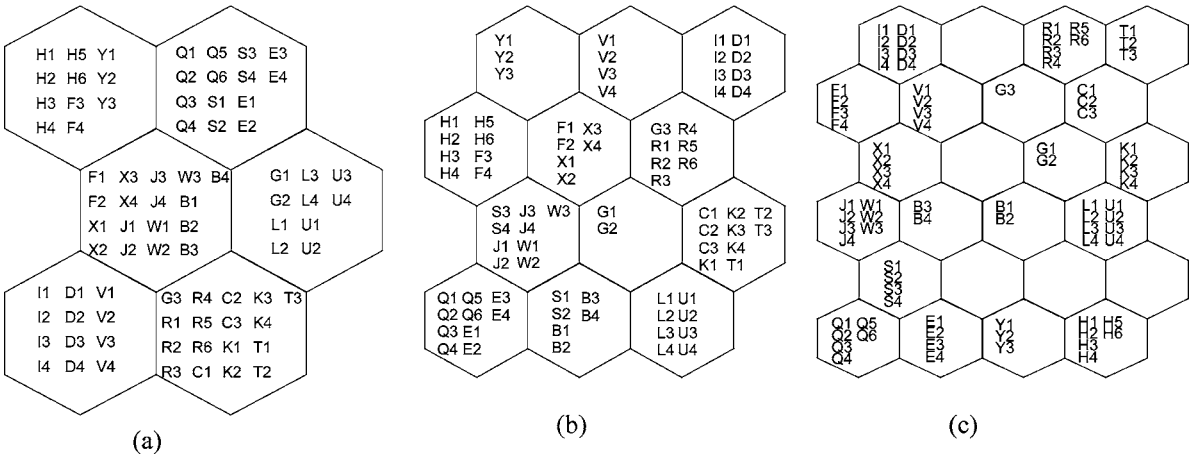

Figure 3. Distribution of the studied variety samples in 2002 onto a 6-unit map (**a**), a 12-unit map (**b**), and a 24-unit map (**c**).

be more adapted for supporting comments as long as all of the samples analyzed this year were more representative of the corresponding varieties because they have been harvested on a unique date.

Then, three intermediate maps containing 6, 12, and 18 hexagons have been computed and drawn in **Figure 3**. First of all, it was interesting to note that throughout all of this increasing of the map size, samples relative to one variety always appeared as grouped in the same unit or in two adjacent ones, indicating that even in small maps (**Figure 3a**), the distribution of samples was organized mainly according to variety chemical characteristics. In the 12 unit map, despite the small number of neurons, the variety CF2838 (Y) was already discriminated in one hexagon. This fact emphasized the chemical typicality of this variety. The same observation can be made about the variety CF2036 (V). At the same time, the variety Cireine (G) began to separate from all of the others, but kept one of the samples, G3, located in the same case as Darselect (R). This particular point should translate for these two varieties to a chemical proximity that appeared once more in the following map (**Figure 3c**) with G3 kept attached to the case where R samples were located. At this level of resolution, it is noteworthy that some varieties still appeared in the same hexagons, such as Capitola (L) and CF1693 (U), Earliglow (I), and Cigaline (D) or even Pajaro (J) and CF2337 (W), and this type of chemical proximity should be more intense for D and I, which stayed in adjacent neurons in the final map of 56 hexagons (**Figure 2c**) characterized by a total discrimination.

Discrimination According to the Year of Production. To compare samples harvested in different years, seven varieties cultivated in 2001 and 2002 have been selected with the criteria that no major differences between samples of the same variety had been shown in both years, in the preceding discussion. In the following, these samples are noted with an additional index relative to the year of production, but refer to the same data as those used in the other paragraphs of this study. So, samples of CF129, Ciflorette, Cigaline, Ciloe, Cireine, Pajaro, and CF1116 were double-indexed as B*x*.01, C*x*.01, D*x*.01, F*x*.01, G*x*.01, J*x*.01, and K*x*.01, respectively, in 2001, and as B*x*.02, C*x*.02, D*x*.02, F*x*.02, G*x*.02, J*x*.02, and K*x*.02, respectively, in 2002, with *x* corresponding to the measure used.

In a first approach, measures relative to samples collected in 2002 have been plotted into the map computed from data obtained with the same varieties in 2001, and the result is shown in **Figure 4a**. It appeared clearly that some samples were correctly recognized as belonging to the corresponding variety (as is the case of B and C), but this was not the case for the majority of them. In the same way, samples harvested in 2001 were plotted into maps computed from data relative to the same varieties in 2002 (**Figure 4b**), and the same kind of results can be observed. This clearly indicated that the variability of the aroma chemical constitution drastically moved between these

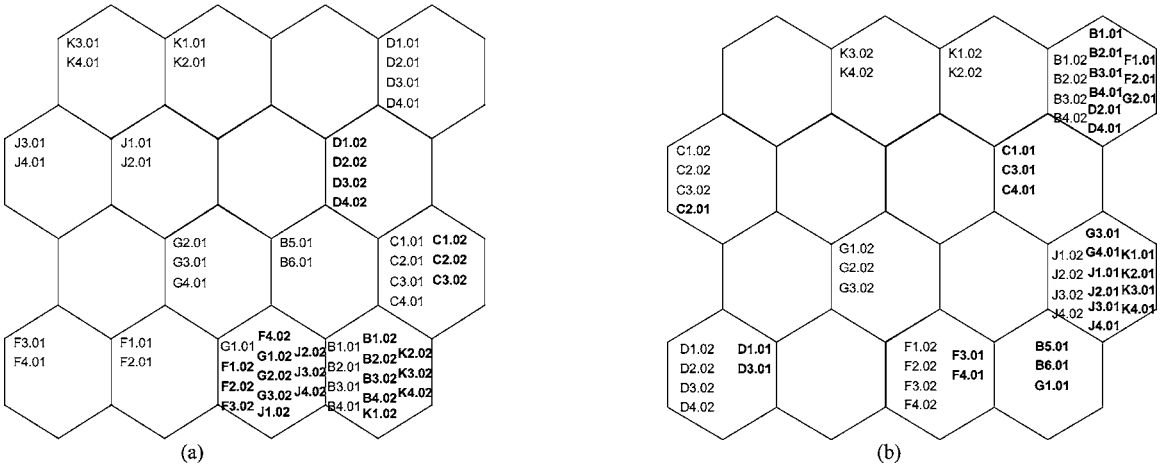

Figure 4. (**a**) Plotting of samples of 2002 in the map built with samples of 2001 (second index α.01). (**b**) Plotting of samples of 2001 in the map built with samples of 2002 (second index α.02).

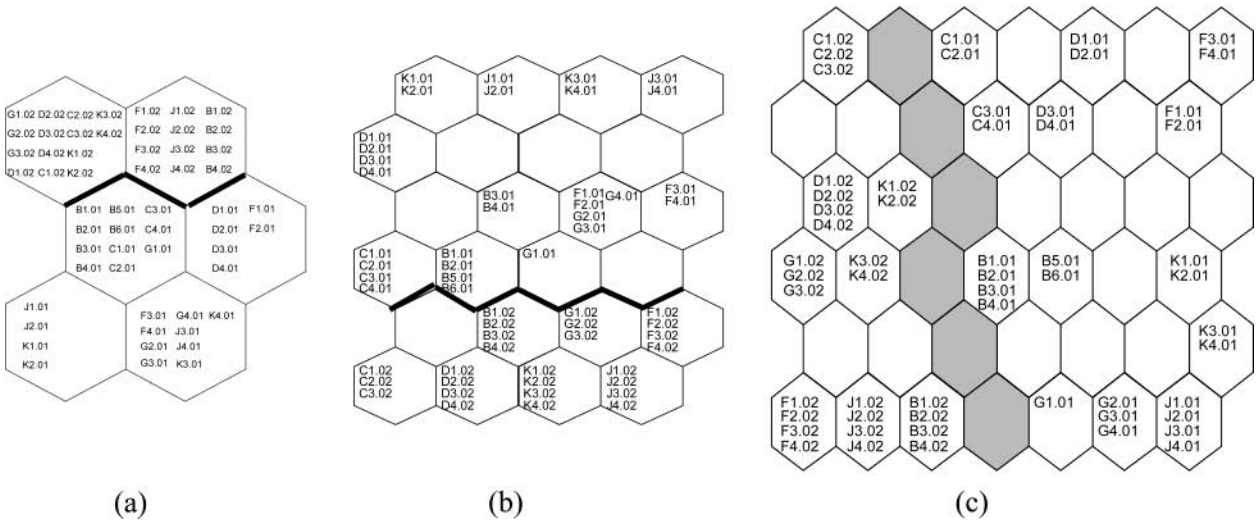

Figure 5. Distribution of 2001 (second index α.01) and 2002 (second index α.02) cumulated samples in 6-unit (**a**), 24-unit (**b**), and 42-unit maps (**c**).

two years and, more precisely, that a map computed from samples collected in one year is not usable for significantly classifying samples of the same varieties produced in a following year. Actually, this observation correlated with the other one made in the same study and indicated that the stage of evolution in the plant cycle had a notable influence on the aroma composition, for a defined variety and a defined year.

In a second analysis, a battery of maps was computed from data gathering samples measured in both years. Some of the corresponding maps are reproduced in **Figure 5**, where it can be seen that a first discrimination was operated very soon between all of the samples, according to the year of production. In the first indicated map carried out of six hexagons (**Figure 5a**), a border was clearly separating samples from each year, independently of the varieties. This separation was confirmed in all of the following maps realized with more hexagons as exemplified in **Figure 5b** (24 neurons) and **Figure 5c** (42 neurons).

Beyond this first observation, it came out that the 42 hexagon map provided a total discriminating classification of all the samples according to the varieties and independently of the year. In this way, two levels of discrimination were effectively observed between all of the samples measured along the two years, the first one according to the year and the second one according to the variety.

Conclusion. This work clearly confirmed that the HS-SPME/GC-MS technique is an efficient analytical tool to globally and rapidly characterize the chemical nature of strawberry variety aromas, according to a defined selection of constituents. The reliability of the described method is at such a level that a data treatment tool such as KSOM allows classification and recognition of measured values which are exactly considered like an aromatic signature characterizing a defined variety. The consequent variety discriminating capacity of the method was found to be not year-dependent. Moreover, depending on the initial grid size chosen by the operator, parameters such as the year of production or the period of harvesting were also differentiated and characterized in the same operation. In contrast, this work clearly indicated that the observed variability of the aromatic constitution of a particular variety upon several years prevented the use of a map established from samples harvested in one year throughout another year of production. Nevertheless, a kind of proximity between samples collected in different years has been observed in the case of some varieties less sensitive to this variability. For comparison, the map obtained by computing the samples corresponding to the seven varieties cultivated in both 2001 and 2002 showed an interesting example of a double-discrimination level, with the year of production in the first step and the variety in the second one. In the same way, other

parameters such as those able to induce differences or similarities between variety aroma composition are presently investigated in the laboratory.

LITERATURE CITED

(1) Pawliszyn, J. *Solid-Phase Microextraction: Theory and Practice*; Wiley-VCH: New York, 1997.

(2) Zhang, Z.; Pawliszyn, J. Headspace Solid-Phase Microextraction. *Anal. Chem.* **1993**, *65*, 1843−1852.

(3) Steffen, A.; Pawliszyn, J. Analysis of flavor volatiles using headspace solid-phase microextraction. *J. Agric. Food Chem.* **1996**, *44*, 2187−2193.

(4) Ulrich, D.; Hoberg, E.; Rapp, A.; Sandke, G. Flavour analysis in plant breeding—solid-phase microextraction of strawberry aroma compounds. In *Proceedings of the 5th Warburg Aroma Symposium*, Eisenach, Germany, March 17−20, 1997; 1997; pp 283−295.

(5) Urruty, L.; Gaunt, D.; Maque, S.; Montury, M. Assessment of strawberry quality. Contribution of a multi-usage chemical analysis method. Proceedings of the 4th International Strawberry Symposium; Hietaranta, T., et al., Eds. *Acta Hortic.* **2002**, 567, ISHS 2002.

(6) Sanusi, A.; Ferrari, F.; Millet, M.; Montury, M. Pesticide vapours in confined atmospheres. Determination of dichorvos by SPME-GC-MS at the $\mu g \cdot m^{-3}$ level. *J. Environ. Monit.* **2003**, *5*, 574−577.

(7) Urruty, L.; Giraudel, J. L.; Lek, S.; Roudeillac, P.; Montury, M. Assessment of strawberry aroma through SPME/GC and ANN methods. Classification and discrimination of varieties. *J. Agric. Food Chem.* **2002**, *50*, 3129−3136.

(8) Kohonen, T. *Self-Organizing Maps*, 3rd ed.; Springer: Berlin, Germany, 2001.

(9) Dutta, R.; Kashwan, K. R.; Bhuyan, M.; Hines, E. L.; Gardner, J. W. Electronic nose based tea quality standardization. *Neural Network* **2003**, *16*, 847−853.

(10) Miller, M. E.; Stuart, J. D. Comparison of gas-sampled and SPME-sampled static headspace for the determination of volatile flavor components. *Anal. Chem.* **1999**, *71*, 23−27.

(11) Bicchi, C.; Drigo, S.; Rubilio, P. Influence of fiber coating in headspace solid-phase microextraction-gas chromatographic analysis of aromatic and medicinal plants. *J. Chromatogr. A* **2000**, *892*, 469−485.

(12) Lek, S.; Giraudel, J. L.; Guégan, J. F. Neural Networks: Algorithms and architectures for ecologists and evolutionary ecologists. In *Artificial Neuronal Networks: Application to Ecology and Evolution*; Lek, S., Guégan, J. F., Eds.; Springer: Berlin, Germany, 2000; pp 3−27.

(13) Ihaka, R.; Gentleman, R. R. A language for data analysis and graphics. *J. Comput. Graphical Statistics* **1996**, *5*, 299−314.

(14) Giraudel, J. L.; Lek, S. A comparison of self-organizing map algorithm and some conventional statistical methods for ecological community ordination. *Ecol. Modell.* **2001**, *146*, 329−339.

Received for review November 22, 2003. Revised manuscript received February 26, 2004. Accepted February 29, 2004. The Conseil Régional d'Aquitaine, the Conseil Général de la Dordogne, and the Centre Interrégional de Recherche et d'Experimentation de la Fraise (CIREF) are gratefully acknowledged for supporting this work.

JF035376L