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Determination of Volatile Organic Compound Patterns Characteristic of Five Unifloral Honey by Solid-Phase Microextraction−Gas Chromatography−Mass Spectrometry Coupled to Chemometrics

MARÍA VERÓNICA BARONI,[†] MARÍA LAURA NORES,[‡] MARÍA DEL PILAR DÍAZ,[§] GUSTAVO ALBERTO CHIABRANDO,[†] JUAN PABLO FASSANO,[||] CRISTINA COSTA,[⊥] AND DANIEL ALBERTO WUNDERLIN*,[†]

Universidad Nacional de Córdoba-CONICET, Facultad de Ciencias Químicas, Dto. Bioquímica Clínica-CIBICI, Medina Allende y Haya de la Torre, Ciudad Universitaria, 5000 Córdoba, Argentina; Facultad de Ciencias Médicas, Enrique Barros y Enfermera Gordillo, Ciudad Universitaria, 5000 Córdoba, Argentina, Facultad de Ciencias Médicas, Escuela de Nutrición, Enrique Barros y Enfermera Gordillo, Ciudad Universitaria, 5000 Córdoba, Argentina; Facultad de Ciencias Químicas, CEQUIMAP, Medina Allende y Haya de la Torre, Ciudad Universitaria, 5000 Córdoba, Argentina; Facultad de Ciencias Exactas Físicas y Naturales, Av. Vélez Sarsfield 399, 5000 Córdoba, Argentina

We report the evaluation of the floral origin of honey by analysis of its volatile organic compounds (VOCs) profile, joined with the use of combined pattern recognition techniques. Honey samples, from five floral origins, were analyzed by headspace solid-phase microextraction−gas chromatography−mass spectrometry, selecting 35 VOCs out of the entire profiles, which were analyzed by hierarchical cluster analysis (HCA), stepwise discriminant analysis (SDA), and *K*-nearest-neighbor (KNN). Both HCA and SDA were used as exploratory tools to select a group of VOCs representing similitude and differences among studied origins. Thus, six out of 35 VOCs were selected, verifying their discriminating power by KNN, which afforded 93% correct classification. Therefore, we drastically reduced the amount of compounds under consideration but kept a good differentiation between floral origins. Selected compounds were identified as octanal, benzeneacetaldehyde, 1-octanol, 2-methoxyphenol, nonanal, and 2-*H*-1-benzopyran-2-one. The analysis of VOC profiles, coupled to HCA, SDA, and KNN, provides a feasible alternative to evaluate the botanical source of honey.

KEYWORDS: Honey; floral origin; SPME; volatile organic compounds

INTRODUCTION

The floral origin of honey is an important characteristic in the evaluation of its quality. Indeed, the estimation of honey quality by consumers depends on its organoleptic characteristics, which is strongly dependent on its botanical origin and to some extent also on its geographical origin. Unifloral honey has highly characteristic aromas, presumably derived from nectar, indicating the presence of volatile components responsible for their characteristic fragrances (*1*−*3*). Sugars and water represent the main chemical constituents of honey (>95%), whereas proteins, flavors (taste and aromas), pigments, vitamins, free amino acids, and numerous volatile compounds constitute minor components. However, this small fraction of the overall composition is mainly responsible for honey's organoleptic and nutritional properties (*4*).

Melissopalynology has been traditionally used for assessing the botanical origin of honey (*5*) and remains nowadays as the reference method despite several disadvantages. Noticeably, counting, identification, and interpretation of pollen analysis require a highly trained analyst, in addition to the need of a complete pollen library. Additionally, industrial filtration also affects both the accuracy and the precision of melissopalynology. Some methods alternative to melissopalynology involve measurements of physical and chemical parameters associated with honey characteristics (*6*, *7*). Others authors have looked for honey classification through the use of chemical markers such as flavonoids, amino acids, proteins, and volatile compounds (*3*, *8*−*11*).

The aroma profile is one of the most typical features of a food product for the evaluation of both organoleptic quality and authenticity. Volatile substances are the main factors responsible for aroma, which in concert with other factors such as taste and physical factors contribute to the flavor. Because of the high number of volatile organic compounds (VOCs), its profiles represent a fingerprint of the product, which could be used to determine honey origin (*12*). Some VOCs are present in the

* To whom correspondence should be addressed. Tel/Fax: (+54)351 4334162. E-mail: dwunder@mail.fcq.unc.edu.ar.
[†] Dto. Bioquímica Clínica, Facultad de Ciencias Químicas.
[‡] Facultad de Ciencias Médicas.
[§] Escuela de Nutrición, Facultad de Ciencias Médicas.
[||] Dto. Química Orgánica, INFIQ, Facultad de Ciencias Químicas.
[⊥] Físicas y Naturales,, Facultad de Ciencias Exactas.

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nectar or honeydew collected by bees (*2*, *3*, *13*) and could be related to plant characteristics; other VOCs originate during honey processing or storage (*14*, *15*). It has already been pointed out that a careful analysis of VOCs of honey could be a useful tool for the characterization of its botanical origin (*12*, *16*, *17*).

Qualitative and quantitative determination of VOCs from a complex mixture, such as honey, is a rather difficult task. It is usually carried out by gas chromatography−mass spectrometry (GC-MS), with a previous fractionation step, which is always necessary (*18*, *19*). Solid-phase microextraction (SPME) has recently been developed as a rapid, solvent free, and less expensive technique for the fractionation of VOCs in different samples (*20*−*24*). The principle of headspace (HS) SPME involves partition and equilibrium of analytes among the coating of the fiber, the sample, and its HS (*13*). Despite its advantages, there are only few reports of the application of SPME to the classification of honey through the analysis of its VOCs (*16*, *19*, *25*−*27*).

Once VOCs have been analyzed, it is common to have a complex chromatographic profile, with multiple peaks, different intensities, and the presence of characteristic compounds mixed with noncharacteristic ones, mainly arising from impurities, industrial processes, a blend of different products, etc. From this complex profile, it is necessary to identify compounds, characteristics for a given source, as a mandatory step to assess the origin of a food product by this method. Identification of characteristic patterns can be performed by chemometrics (pattern recognition techniques), which have been widely applied to food chemistry in recent years (*28*−*31*).

There are many research papers in which the potential of VOCs analysis, in combination with chemometrics, was used to achieve a correct classification of honey samples from different origins. However, there are no reports on the use of pattern recognition techniques to evaluate correlations between different VOCs.

The main goal of this work was to assess the floral origins of different unifloral honey by evaluating the VOC profiles through headspace solid-phase microextraction and gas chromatography coupled to mass spectrometry (HS-SPME-GC-MS) coupled with chemometric techniques, looking to reduce the dimension of the original data matrix. This allowed us to identify patterns characteristic of a given floral source, thus providing us with an accurate method to verify honey floral origins alternative/complementary to melissopalynology.

MATERIALS AND METHODS

Standards. All standards were of analytical grade: octanal, benzeneacetaldehyde, 1-octanol, nonanal, 2-methoxyphenol, 2-*H*-1-benzopyran-2-one, and the mixture of *n*-C_7 to *n*-C_{21} hydrocarbons for calculation of the Kovats index were from Sigma-Aldrich Co. (United States). Stock solutions were prepared by diluting the corresponding compound with methanol (high-performance liquid chromatography grade).

Honey Samples. This study was carried out on 42 samples from different floral origins. Unifloral honey samples were provided by beekeepers, evaluated by melissopalynology according to Loveaux (*5*), and preserved at 4 °C until VOCs analysis. The floral origins of the honey used were as follows: 12 samples of *Medicago sativa* (alfalfa), six samples of *Helianthus annuus* (sunflower), five samples of *Melilotus albus* (white clover), ten samples of *Prosopis* spp. (carob), and nine samples of *Prosopis caldenia* (caldén).

SPME. A manual SPME holder (Supelco, Bellafonte, PA) was used for evaluation of VOC profiles. A 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) (Supelco) 1 cm length SPME fiber was used for fractionation of volatile and semivolatile compounds from the HS of properly conditioned samples. Prior to extraction, the fiber was conditioned for 1 h at 270 °C in the injection port of the GC. HS-SPME fractionation was carried out in according to the literature (*17*) with minor changes. Briefly, 4.5 g of honey was introduced into a 30 mL vial and sealed with a screw cap equipped with a PTFE septum. Afterward, honey samples were homogenized and heated at 60 °C for 15 min. After equilibration, the fiber was introduced into the HS through the septum and exposed to the vial HS for 45 min; the sample was magnetically stirred to improve the extraction efficiency.

GC-MS. GC analyses were performed on a Shimadzu QP5050A equipped with a split/splitless injector, quadrupole mass spectrometer (EI 70 eV), Class 5000 data software, and NIST 107 mass spectral library. The SPME fiber was desorbed at 250 °C for 5 min in the split mode (1:25). Separation was carried out on a Hewlett-Packard HP-5 fused silica capillary column (cross-linked 5% methylsiloxane) of 25 m length and 0.2 mm internal diameter, with a phase thickness of 0.25 μm. The oven temperature was programmed as follows: initial temperature 60 °C held for 5 min, heated at 6 °C/min until 200 °C, further heating at 15 °C/min until 250 °C, and held at this temperature for 5 min. The total run time was 37 min. The injector and detector temperatures were set at 250 and 280 °C, respectively. The carrier gas was helium (>99% purity) at a flow rate of 1 mL/min.

Qualitative and Quantitative Determinations. Initial identification of volatile compounds was made by comparing their mass spectra with those of the NIST spectral library. Additionally, a *n*-C_7 to *n*-C_{21} hydrocarbon mixture was used for calculation of the Kovats index, comparing obtained values with those reported in the literature. Furthermore, identification of six VOCs selected by chemometrics was also confirmed by evaluating both retention times and mass spectra with those of authentic substances.

The concentrations of honey volatiles were determined by external calibration curves. The standard solutions at the appropriate concentration were added to 4.5 g of a solution containing 35% glucose, 30% fructose, and 1% saccharose, in order to simulate the honey matrix. Then, the fractionation procedure was followed as previously described.

The limit of detection (LOD) was taken at a signal-to-noise ratio (S/N) of 3. The limit of quantification (LOQ) was taken at a signal-to-noise ratio (S/N) of 10. The reproducibility of the assay was determined by comparing calibration curves on successive days, with further evaluation of the relative imprecision expressed as the coefficient of variation (CV%) averaged over the entire calibration curve.

Data Processing. The starting data matrix contained 42 samples of five floral origins and 35 compounds on each honey. These 35 compounds were selected considering their relative abundance and ubiquitous presence on a particular floral origin, although many of these compounds are not present in all of the studied samples. Multivariate techniques such as hierarchical cluster analysis (HCA) (*32*), stepwise discriminant analysis (SDA) (*33*), and *K*-nearest-neighbor (*34*) discriminant method (KNN) were used. HCA and SDA were applied in order to obtain a set of variables (compounds) that help to discriminate among floral origins, while KNN was implemented to classify honey samples and to calculate the classification error rate. Data were analyzed using SAS System Release 8.02 (*35*) and InfoStat (2005), version 1.6 (*36*).

HCA. The aim of the HCA is to uncover some latent structure of the objects and variables in terms of groups of similar elements and, possibly, in terms of hierarchy of embedded groups (*30*). Because of its unsupervised character, HCA is commonly applied before other multivariate techniques. The procedure involves a measurement of either the distance or the similarity between objects to be clustered (*29*). The clustering is represented in a dendrogram by the junction of the corresponding branches, which is called a node of the tree.

HCA was applied to evaluate associations between VOCs. That is, we looked for natural clustering of variables (compounds) instead of honey samples, since our aim was to select a few compounds necessary to achieve discrimination of floral origins. Because of the observed lack of symmetry, we decided to transform the original data matrix considering either the presence or the absence of each compound within a given sample instead of its relative abundance. Thus, we assigned a value of one (1) if the compound was detected and a null value (0) if it was absent. We evaluated the proximity between compounds using simple matching (*37*), which measured similarity (δ) according to the

proportion agreement of 1s and 0s. The distance was calculated as $\sqrt{1-\delta}$. Then, the average linkage method was used to identify the clusters of VOCs. Some compounds representing each cluster were selected in order to reduce workspace dimension, and this set of VOCs was denoted by *A*.

SDA. Discriminant analysis is rather exploratory in nature, while classification procedures was less exploratory in the sense that they led to well-defined rules, which can be used for assigning new objects (*32*). Thus, continuing the exploratory approach, SDA was applied to the original data matrix, previously standardized, only to identify the most important compounds that explained the variability among different floral origins. We did not use SDA to calculate the classification error rate since our data were not multivariate normal; in addition, our sample sizes were small. The set of variables selected by SDA was denoted by *B*.

KNN Discriminant Method. Classification of samples was performed by a nonparametric discriminant analysis: KNN, considering data corresponding to variables selected by both HCA and SDA (that is, $A \cap B$) as input. We chose this nonparametric method since linear or quadratic discriminant analyses were not suitable because our data were not multivariate normal. The objective of applying KNN was to know the power of the selected compounds in classifying an unknown sample in terms of its VOC profile into one of the groups corresponding to the different floral origins in study.

KNN is a well-known nonparametric method, which has been previously used for food classifications (*31*, *38*, *39*). KNN assigns the tested object to the cluster, which is the most represented in the set of *k* nearest training objects, considering the Mahalanobis distance. The value of *k* should be so that it performs the minimum possible error. The goodness of this method was checked using the known cross-validation rate (*40*).

RESULTS AND DISCUSSION

Analysis of VOCs in Honey. Honey samples from five different floral origins were analyzed with the objective to identify and compare their VOC profiles. A typical honey VOC chromatogram exhibited from 30 to more than 50 peaks, which were initially identified by their retention times. Differences in total ion current (TIC) chromatographic profiles were observed when comparing honey samples from different floral origins. As it was explained in the Materials and Methods, we selected 35 peaks (evaluated by TIC) from the chromatograms corresponding to studied honey samples. Selected peaks were preliminarily assigned to chemical compounds by comparison with the NIST MS library (**Table 1**). Many of these compounds were present in all honey samples, although differences in TIC were shown due to quantitative variations in the composition of VOCs, which is characteristic of each floral origin. We did not observe a particular compound characteristic of a given floral source (floral marker) but did observe quantitative variations of various compounds among several floral origins.

From this preliminary peak assignment, we found that honey contains numerous VOCs such as aldehydes, alcohols, alkanes, norisoprenoids, and furanic derivates, which are in good agreement with previous reports. For instance, aromatic aldehydes, such as benzaldehyde and benzeneacetaldehyde, have been reported as common components of various unifloral honey at variable concentrations (*14*, *25*, *26*). We have found both of these aldehydes in the five types of unifloral honey studied, although benzeneacetaldehyde was present in major proportions in *M. sativa* and *M. albus* honey.

We have also found linalool derivatives (*3*) such as lilac aldehydes, which have been proposed as markers of citrus honey and nodding thistle honey since they are present in these types of honey in major concentrations than in other unifloral honey (*3*, *17*, *26*). We have found that lilac aldehyde isomers were present in different percentages in all of the studied honey. Thus, the previous assumption of these compounds as markers of a particular floral origin should be revised in view of the present results.

Table 1. VOCs Considered in 42 Unifloral Honey Analyzed

no.	compound	RT[a]	KI calcd[b]	ID[c]
1	furfural	5.211	800	MS, KI
2	benzaldehyde	9.017	938	MS, KI
3	octanal	10.341	1007	MS, RT, KI
4	benzeneacetaldeyde	11.653	1033	MS, RT, KI
5	1-octanol	12.429	1067	MS, RT, KI
6	furanmethanol	12.593	1063	MS, KI
7	methyl-2-pyrazinil-methanol isomer	12.678	1079	MS, KI
8	2-methoxyphenol	13.049	1086	MS, RT, KI
9	nonanal	13.436	1105	MS, RT, KI
10	phenylethylalcohol	13.758	1125	MS, KI
11	2-cyclohexen-1-one, 3,5,5-trimethyl	14.022	1135	MS, KI
12	lilac aldehyde isomer	14.633	1156	MS
13	lilac aldehyde isomer	14.888	1169	MS
14	octanoic acid	15.318	1180	MS, KI
15	decanal	16.368	1213	MS, KI
16	nonanoic acid	17.952	1308	MS, KI
17	dimethyldecane	18.862	1338	MS
18	tetradecane	21.162	1400	MS, RT, KI
19	NI 43 (100), 57 (84), 71(49)	21.361	1456	MS
20	α-farnesene	22.415	1485	MS, KI
21	2*H*-1-benzopyran-2-one	22.642	1497	MS, RT, KI
22	NI 43(100), 57 (71), 71 (47)	22.84	1504	MS
23	NI 75 (100), 41 (85), 43 (78), 165 (49)	23.198	1536	MS
24	NI 41 (100), 97 (77), 57 (55)	23.446	1541	MS
25	pentadecane-2- methyl	23.712	1543	MS, KI
26	NI 33(100), 57 (83), 71 (49)	23.852	1546	MS
27	butylated OH tolueno	24.204	1575	MS, KI
28	hexadecane	25.916	1639	MS, RT, KI
29	NI 43 (100), 57 (96), 71 (49)	28.048	1710	MS
30	NI 57 (100), 43 (87), 71 (43)	28.179	1722	MS
31	NI 97 (100), 57 (73), 41 (53)	28.479	1726	MS
32	6,7-dimethylheptadecane	29.993	1813	MS, KI
33	octadecane-2-methyl	31.449	1887	MS, KI
34	eicosane	32.768	1942	MS, RT, KI
35	eicosane-5-methyl	34.167	2052	MS, KI

[a] Retention time. [b] Kovats index. [c] Method of identification: MS, identification by comparison with mass spectrum stored in NIST library; RT, identification by comparison with retention time of authentic reference compounds; KI, identification by comparison of Kovats index with literature; NI, not identified. The intensity of MS main ions is given in parentheses.

The analyses of VOCs in honey also show the presence of norisoprenoids (carotenoid-derived compounds), which have been found as aroma contributors in honey as well as in tobacco, tea flower scents, fruit species, grapes, and wine (*2*, *3*).

Several furanic compounds like furfural and furanmethanol were also present in all samples. Furanic compounds are commonly produced by either heating or storage (*41*−*43*). The mild heating undergone by samples during SPME sampling, which is recommended to improve the extraction yield and to reduce the equilibrium time, could be partially responsible for some of them.

Chemometrics. Our aim was to select a minimum number of compounds, which were able to afford a correct classification. The main purpose of extracting some compounds from the entire chromatogram is to reduce and/or eliminate irrelevant information, evidencing the main information necessary to achieve a good discrimination among different floral origins (*29*). To do that, we started applying a HCA to the VOCs, without considering the floral origin of the corresponding honey sample, just clustering VOCs by similarities among them. Thus, we looked to evaluate if VOCs were associated responding to a

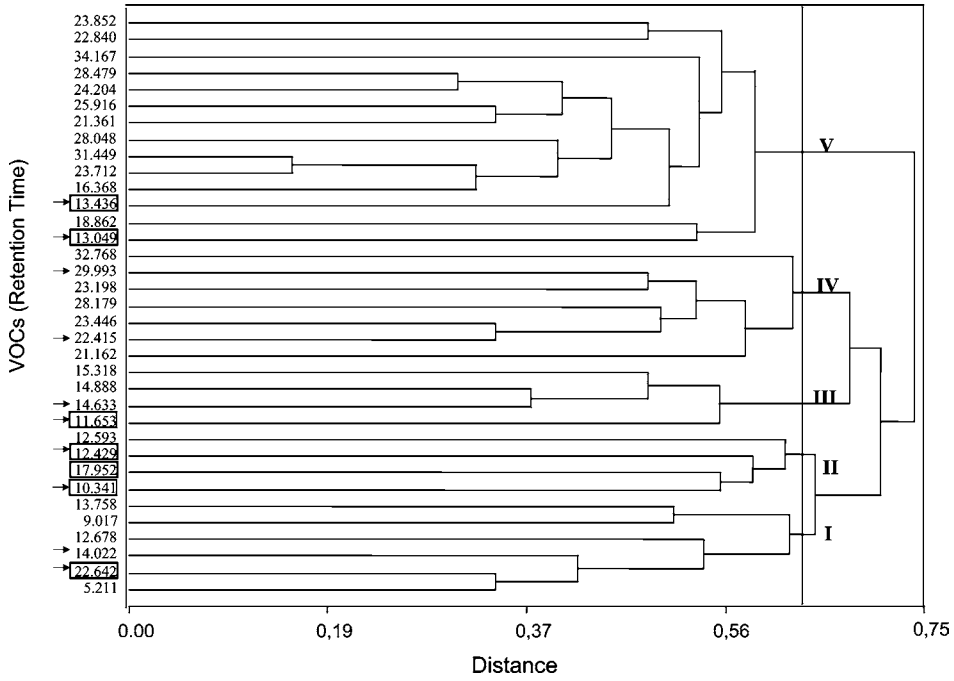

Figure 1. Dendrogram of cluster analysis of VOCs identified by its retention time in GC. Arrows and boxes indicate selected compounds by HCA and SDA, respectively.

given pattern. The dendrogram obtained is shown in **Figure 1**. From this figure, we can observe five clusters at a linkage distance of 0.635 (85% of maximal distance). Cluster I is formed mainly by VOCs that were present in *M. albus* honey and absent in *H. annuus*, *Prosopis* spp., and *M. sativa* honey. Cluster II is associated with VOCs that were present in *Prosopis* spp. and *P. caldenia* honey but absent in *M. sativa*, *H. annuus*, and *M. albus* honey. Cluster III corresponds to VOCs that were present in *M. sativa* and *M. albus* honey but absent in *Prosopis* spp. and *H. annuus* honey. Cluster IV corresponds to VOCs that were mainly present in *M. sativa* and *H. annuus* honey but absent in *Prosopis* spp., *P. caldenia*, and *M. albus* honey. Finally, cluster V corresponds to VOCs that are present in the five types of honey studied.

Thus, the characterization arising from HCA helped us to identify those compounds that could better explain the differentiation among the honey samples on the basis of their VOCs profile. Because VOCs included in a given cluster present similarity, we decided to select two compounds representative from each cluster, thus reducing the number of compounds necessary to achieve a good differentiation between floral origins. This selection was done considering that clusters I−IV were characterized by compounds that were, as a general rule, present in some floral origins and absent in others. Therefore, those compounds that better represent that characteristic (presence or absence in a given floral source) are important for discrimination and were the ones selected. On the other hand, compounds in cluster V are generally present in the five floral origins. Consequently, they are not useful for discrimination. The criteria used in this last case was to select the two compounds that less represent the cluster, that is, those compounds that are absent in some honey samples. Thus, compounds selected (identified by its retention time in GC) were 14.022, 22.642, 22.415, 29.993, 10.341, 12.429, 11.653, 14.633, 13.049, and 13.436 min (**Figure 1**).

SDA was also applied as an alternative and exploratory method to evidence which compounds were more clearly associated with honey floral origin. Thus, SDA was carried out considering the floral origin of honey as the grouping variable, with 35 selected VOCs as dependent ones. SDA pointed out seven compounds as the most representative of differences among floral origins. These compounds (identified by its GC retention time) were 10.341, 11.653, 12.429, 13.049, 13.436, 17.952, and 22.642 (**Figure 1**).

Table 2. Classification and Assignment Results of KNN Analysis

	predicted group					
actual group	*M. sativa*	*Prosopis* spp.	*P. caldenia*	*H. annuus*	*M. albus*	% correct
M. sativa	12	0	0	0	0	100
Prosopis spp.	0	10	0	0	0	100
P. caldenia	0	0	6	3	0	66.67
H. annuus	0	0	0	6	0	100
M. albus	0	0	0	0	5	100
total	12	10	6	9	5	93.33

Combining results from both HCA and SDA, we selected six VOCs as the most representative to discriminate among different floral origin of honey samples. The selected compounds (identified by its GC retention time) were 10.341, 11.653, 12.429, 13.049, 13.436, and 22.642 (**Figure 1**). These six compounds, which were tentatively identified by comparison of their mass spectra with the NIST MS library, were further confirmed by comparison of both retention times and mass spectra with pure commercial standards (**Table 1**). So, these six compounds were positively identified as octanal, benzeneacetaldehyde, 1-octanol, 2-methoxyphenol, nonanal, and 2-*H*-1-benzopyran-2-one (coumarin).

In the next step, we applied KNN ($k = 2$) to the six selected VOCs, obtaining 93% correct classification. We consider that the error rate is satisfactory; therefore, we did not look for a different configuration of variables. The classification matrix is shown in **Table 2**. Only three samples of *P. caldenia* honey were misclassified as *H. annuus*. Thus, the use of only six out of 35 initially considered VOCs (17%) was enough to afford good discrimination among the five types of unifloral honey analyzed during this study (KNN applied to all of the variables provided 100% correct classification). This result agrees with

Table 3. Concentration of Six Compounds Selected by Chemometrics in Five Analyzed Honey Types[a]

				floral origin				
compound	LOD	LOQ	CV%[b]	*Prosopis* spp.	*H. annuus*	*M. sativa*	*P. caldenia*	*M. albus*
octanal	1.5	5	12.1	11.75	<LOD	<LOD	5.85	<LOD
benzeneacetaldehyde	18	60	6.7	<LOD	<LOD	437	<LOQ	98.60
1-octanol	1.80	6	8.6	<LOD	<LOD	<LOD	8.85	<LOD
2-methoxyphenol	33	110	11.5	131	1120	810	533	120
nonanal	0.75	2.5	12.6	4.60	<LOD	5.44	<LOQ	<LOQ
2-*H*-1-benzopyran-2-one	750	2500	8.7	<LOD	<LOD	<LOD	<LOQ	4990

[a] LOD, LOQ, and concentrations in μg/kg (ppb). [b] CV% was averaged over the entire calibration curve (seven concentrations with five replicates at each one).

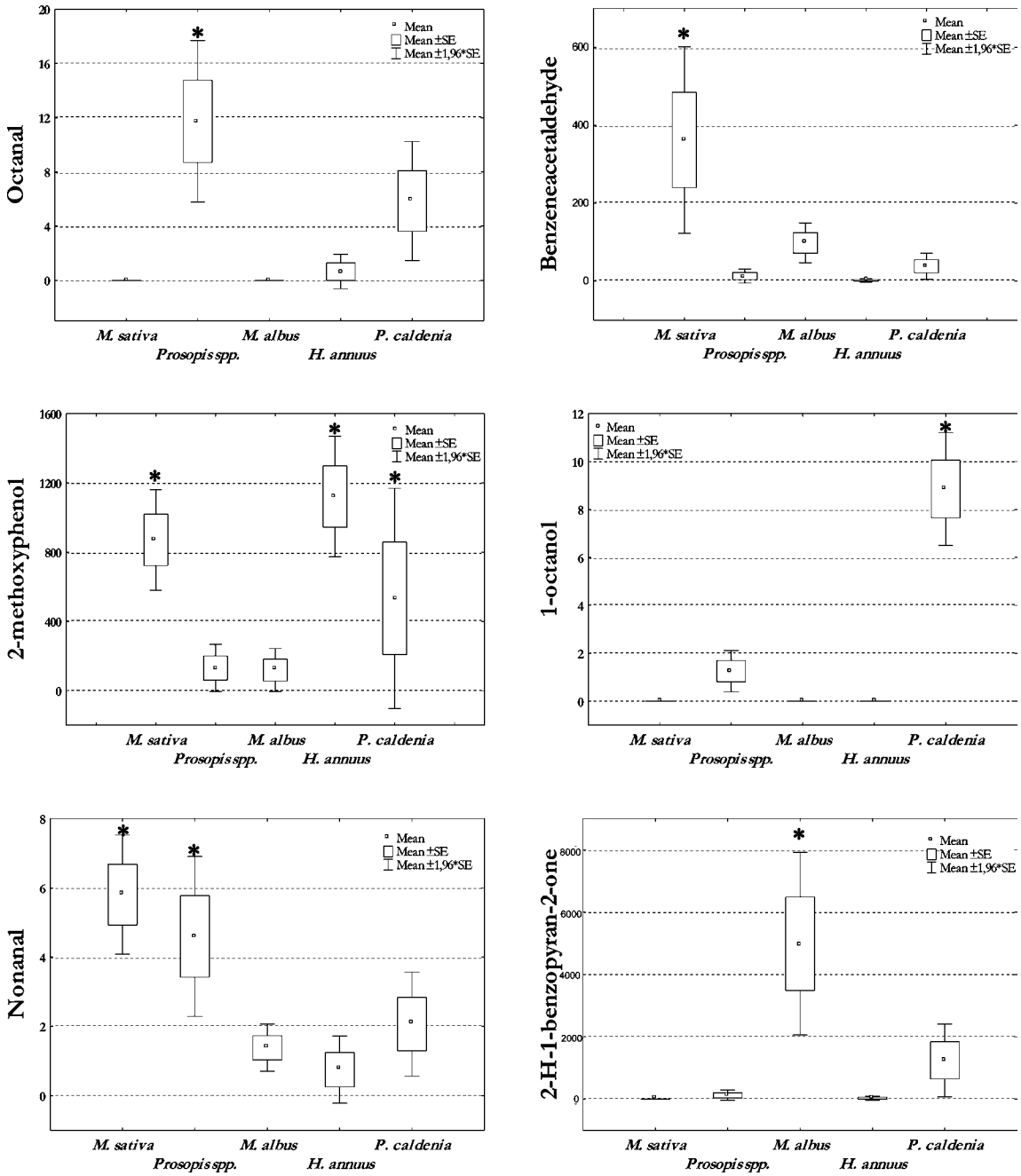

Figure 2. Box plots of the six compounds selected for floral honey discrimination (concentration expressed as μg/kg honey vs floral origin). *Significant differences ($p < 0.05$) according to the Kruskal–Wallis test.

the general concept, which indicates that only a limited number of VOCs are responsible for the overall aroma in foods. The rest of the VOCs would then act as modifiers of the aroma provided by these components (*14*).

Lineal and aromatic aldehydes such as octanal, nonanal, and benzeneacetaldehyde are common flavor components of honey, which have been previously described in the literature. However, none of these compounds have been considered as a floral

marker (*2*, *12*, *44*). 1-Octanol was found in citrus honey and multifloral honey from the south region of Italy by Alissandrakis (*3*) and Bentivenga (*44*). 2-Methoxyphenol (guaiacol) has been found by Guyot et al. (*19*) in lime tree honey. 2-*H*-1-Benzopyran-2-one is present in the shikimic metabolic pathway, resulting from phenylalanine metabolism. It has been previously found in lavender and lime tree honey (*19*, *45*).

These six selected compounds were quantified in the five floral origins. In **Table 3**, the corresponding analytical information is given. Additionally, we present box and whisker plots of the six selected VOCs (**Figure 2**) to improve the visualization of different patterns associated with honey from a particular floral origin, including an estimated concentration for those VOCs that were below the LOQ for a given floral source (**Table 3**).

From **Figure 2**, it is evident that each type of honey presents a characteristic pattern. *M. sativa* honey is characterized by the high amounts of benzeneacetaldehyde, nonanal, and 2-methoxyphenol. *Prosopis* spp. honey is better characterized by nonanal and octanal. *M. albus* honey is characterized mostly by the presence of 2-*H*-1-benzopyran-2-one. In *H. annuus* honey, the presence of 2-methoxyphenol with low levels of the other selected VOCs is typical. Finally, *P. caldenia* honey is mainly characterized by the presence of 1-octanol.

HS-SPME-GC-MS affords good results in the analysis of honey aromas, providing typical VOC profiles for several floral origins. This technique allowed us to detect numerous volatile substances, many of them previously reported in the literature as floral makers for other origins, while some of them were never reported as markers.

The application of combined pattern recognition techniques, applied to the chemical data set (partial aroma profiles), allowed us to extract useful information, pointing out the most significant VOCs in differentiating honey aromas. Thus, we were able to classify honey from five different floral origins using only six VOCs, which shows how powerful the complementary use of statistical techniques applied during this study is. The use of KNN, combined with HCA and SDA, provides us with an interesting mixture of exploratory and formal multivariate statistical methods. Its implementation allows an important data reduction, keeping a mathematically correct procedure, considering that most of the analyzed variables presented non-normal distribution.

Analysis of VOCs profile of honey provides us with a feasible alternative to melissopalynology in the evaluation of botanical sources of honey. We obtained better results by studying VOC profiles instead of looking for a particular marker for each floral origin. This last observation reinforces the need of reviewing compounds previously reported as chemical markers of a given source, changing the concept of analyzing a particular marker for the combined use of several VOCs, associated to a profile analyzed by pattern recognition techniques, thus providing us with a more complex fingerprint, which could be specific of a particular botanical or geographical origin of honey and probably applicable to other foodstuffs.

ABBREVIATIONS USED

LOD, limit of detection; LOQ, limit of quantification; CV%, coefficient of variation; VOCs, volatile organic compounds; HS-SPME-GC-MS, headspace solid-phase microextraction and gas chromatography coupled to mass spectrometry.

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