

## Inductively Coupled Plasma Optical Emission Spectrometric Determination of Minerals in Thyme Honeys and Their Contribution to Geographical Discrimination

ANASS TERRAB,<sup>†</sup> DOLORES HERNANZ,<sup>§</sup> AND FRANCISCO J. HEREDIA\*<sup>‡</sup>

Departamento de Biología Vegetal y Ecología, Universidad de Sevilla, Apartado 1095, 41080 Sevilla, Spain; Departamento de Química y Ciencia de los Materiales, Facultad de Ciencias Experimentales, Universidad de Huelva, 21007 Huelva, Spain; and Área de Nutrición y Bromatología, Facultad de Farmacia, Universidad de Sevilla, c/ P. García González 2, 41012 Sevilla, Spain

Twenty-four Spanish thyme honey samples were analyzed using inductively coupled plasma optical emission spectrometry (ICP-OES). Twenty-four minerals were quantified for each honey. The elements Al, As, Ba, Ca, Cr, Cu, Fe, K, Li, Mg, Mn, Na, P, Pb, S, Se, Si, Sr, and Zn were detected in all samples; seven elements are very abundant (Ca, K, Mg, Na, P, S, and Si), and six are not abundant (Al, Cu, Fe, Li, Mn, and Zn). Eleven of them are trace elements (As, Ba, Cd, Co, Cr, Ni, Mo, Pb, Se, Sr, and V) at  $<1 \text{ mg kg}^{-1}$ . Classification of thyme honeys according to their origin (coast, mountains) was achieved by pattern recognition techniques on the mineral data. By means of principal component analysis, a good separation by geographical origin is obtained when scores for the two first principal components are plotted. Classification functions of 11 metals (Al, As, Cr, Cu, K, Li, Mg, Na, P, S, and V) were obtained using stepwise discriminant analysis and applied to classify correctly  $\sim 100\%$  of the honey samples.

**KEYWORDS:** ICP-OES; minerals; honey; thyme; cluster analysis (CA); principal component analysis (PCA); stepwise discriminant analysis (SDA)

### INTRODUCTION

Honey is one of the most complex foodstuffs produced by nature, and certainly the only sweetening agent that can be used by humans without processing. Honey is produced by honey bees from carbohydrate-containing exudates produced by plants and contains all of the trace minerals that are essential to health: iron, copper, manganese, silica, chlorine, calcium, potassium, sodium, phosphorus, aluminum, magnesium, etc. The mineral content is closely related to the floral type, mineral resources in the soil, and environmental or seasonal factors (1).

The mineral content of honey has been the subject of many studies. A flow injection analysis coupled with atomic spectroscopy in order to determine the mineral elements of honey was used (2). The mineral content in some honeys from Galicia (Spain) was analyzed using flame atomic absorption spectrometry (3). High-performance ionic chromatography was used to quantify minerals in some unifloral honeys of Italian origin (4). The mineral content in Moroccan honey was also studied using inductively coupled plasma atomic emission spectrometry (5).

In addition, the mineral composition has also been employed for the purpose of geographical origin discrimination (6). The

mineral content of honey reflects the presence of specific minerals within the forage area of the hive (7–9). The application of principal component analysis (PCA) and stepwise discriminant analysis (SDA) was assessed to differentiate honeys from 10 Canadian provinces (10). Some minerals of Hungarian acacia honeys were determined, and a relationship with those of plants and soils was reported (11); in addition, the mineral content has been employed in an attempt to differentiate between honeys produced both inside and outside Galicia (northwestern Spain), using several statistical methods (12).

In the Mediterranean area, thyme honeys are mainly produced in Greece, Italy, Morocco, and Spain (13). Although there are some melissopalynological studies (14–17), the mineral characterization of this honey type is unknown, contrary to the various studies carried out on other honey types, such as acacia, chestnut, eucalyptus, lavender, and orange (4, 18–20).

Thus, the goal of the present work is the study of the mineral content of the thyme honey produced in Spain, using inductively coupled plasma optical emission spectrometry (ICP-OES). In addition, multivariate statistics, such as PCA and SDA, were applied in an attempt to differentiate between thyme honeys produced in coastal and mountain regions.

### MATERIALS AND METHODS

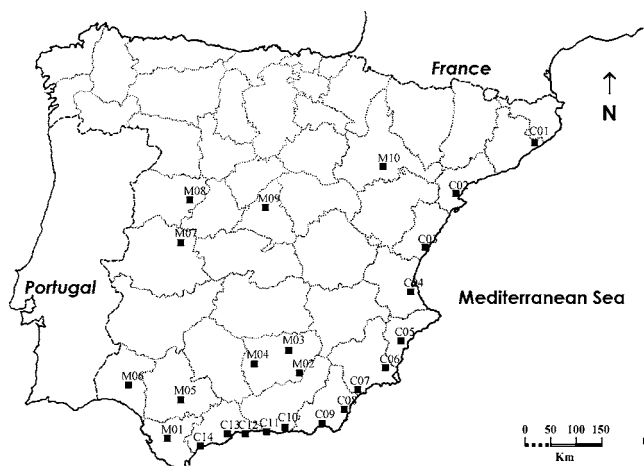
**Samples.** The present study made use of 24 thyme honey samples from *Thymus* spp. (mainly *T. mastichina* and *T. capitatus*), collected in Spain, between 2002 and 2003, 14 belonging to the coastal region

\* Corresponding author [telephone (34) 954556761; fax (34) 954557017; e-mail heredia@us.es].

<sup>†</sup> Departamento de Biología Vegetal y Ecología, Universidad de Sevilla.

<sup>§</sup> Departamento de Química y Ciencia de los Materiales.

<sup>‡</sup> Área de Nutrición y Bromatología, Universidad de Sevilla.



**Figure 1.** Map of distribution of the honey samples: C01–C14, honey samples produced on the coast; M01–M10, honey samples produced in the mountains.

**Table 1.** Operational Parameters for ICP-OES Measurements

parameter	
power (kW)	1.2
radio frequency (MHz)	27
plasma gas flow rate (L min <sup>-1</sup> )	2
auxiliary gas flow rate (L min <sup>-1</sup> )	2
nebulizer gas flow rate (L min <sup>-1</sup> )	0.02
nebulizer pressure (bar)	1
rinsing time (s)	35
rinsing pump speed	high
transfer time (s)	60
stabilization time (s)	20
transfer pump speed	high

and 10 to mountain regions. The samples were taken directly from the beekeepers, all professionals, and the extraction of honey from combs was made by centrifugation. For the site of collection, see **Figure 1**. All samples were unpasteurized and were taken no more than 3 months after extraction, stored in holders, and immediately transferred to the laboratory and kept at 0 °C. Analyses were made within a 6-month time period after harvesting.

**Pollen Analysis.** The botanical origin of the honeys was studied using the techniques described by Maurizio (21) and Erdtman (22). Slides were prepared with acetolysis by centrifuging 10 g of honey dissolved in 20 mL of diluted sulfuric acid (5 g of H<sub>2</sub>SO<sub>4</sub>/L) for 10 min at 2500 rpm. The supernatant was decanted, and the sediment was washed twice with 10 mL of distilled water and then centrifuged. The sediment was extended on a slide, dried at 70 °C, and then mounted with stained glycerine gelatin. The number of the pollen grain (NPG) was counted in four different slides for each honey sample. For the identification of the pollen grains, the general key to pollen types was used (23), and at least 500 pollen grains were identified among four different slides for each honey sample. A minimum of 15% pollen from *Thymus* spp. was required to consider these honeys as unifloral from a mellissopalynological point of view.

**Apparatus.** A Jobin-Yvon Ultima 2 ICP optical emission spectrometer and an Ultrasonic nebulizer (U6000 AT<sup>+</sup>, Cetac) were used for metal determination. The instrument was operated in the following conditions (**Table 1**).

**Reagents and Solutions.** Distilled, deionized water of 18 MΩ cm<sup>-1</sup> resistivity, obtained from a Milli-Q system (Millipore), was used to prepare all solutions. A 10% v/v solution of nitric acid (Panreac, Barcelona, Spain) was used for digestion of the samples. Spex plasma standard (1000 mg L<sup>-1</sup>) was used to prepare Al, As, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, S, Se, Si, Sr, V, and Zn reference solutions.

**Procedures.** The ashes were obtained by calcinations (600 °C) of ~5 g samples of honey to constant weight (24). Nitric acid (0.1 M, 5

**Table 2.** Emission Wavelength, Linear Working Range, and Correlation Coefficient of the Calibration, for Each Mineral Determined

mineral	wavelength (nm)	correlation coefficient	linear range (mg L <sup>-1</sup> )
Al	266.039	0.99996	0–20.7
As	189.042	0.99993	0.018–5.20
Ba	455.403	0.99998	0.001–0.53
Ca	422.673	1	0–51.3
Cd	228.802	0.99999	0–0.52
Co	228.616	1	0–0.52
Cr	267.716	1	0–0.51
Cu	213.598	1	0.003–5.10
Fe	262.167	0.99991	0.022–5.10
K	766.490	0.99985	2.83–518
Li	670.784	0.99999	0.009–5.10
Mg	382.935	1	0.05–210
Mn	259.373	0.99995	0.001–0.52
Mo	204.598	0.99997	0.001–0.51
Na	589.592	0.99998	0.374–204
Ni	221.647	1	0–0.52
P	255.493	0.99897	2.16–102
Pb	220.353	0.99999	0–0.52
S	181.978	0.99369	2.01–41.5
Se	196.026	0.99996	0.016–5.10
Si	212.412	0.99919	0.724–49.9
Sr	407.771	0.99993	0.001–0.53
V	311.838	0.99995	0–0.52
Zn	334.502	0.99969	0.086–5.20

mL) was added to the resultant ashes, and the mixture was stirred on a heating plate to almost complete dryness. Then 10 mL of the same acid was added, and the mixture was brought up to 25 mL with distilled water. The emission wavelengths used, the correlation coefficients for the calibration straight line, and the working linear ranges found for each metal in the ICP-OES determination are presented in **Table 2**. Results are expressed as milligrams of metal per kilogram of honey.

**Statistical Analysis.** The multivariate analysis statistical treatments were carried out using Statistica (25). Multivariate analysis involves cluster analysis (CA), PCA, and SDA.

The search for natural groupings in the samples is one of the main ways to study the structure of the data. CA describes the similarity between honey samples included. It can also represent multidimensional space by mapping in two dimensions (26).

PCA is a display method to visualize the data structure in a lesser dimensional space. The original descriptors are rotated in such a way that the new variables, called principal components (PCs), are not correlated and the first ones explain the major data variability. For a two-dimensional perspective, the data samples are projected onto the plane formed by the first two PCs (score plot) (27).

SDA is a well-known classification technique based on building new axes called discriminant functions obtained by linear combination of the selected descriptors that leads to the maximum ratio of between-classes sum of squares and within-classes sum of squares (28).

## RESULTS AND DISCUSSION

The results of pollen analysis of the sediment for the honey samples used in this work are briefly summarized. The NPG in 10 g of honey ranges between 27500 and 640000; ~80% of the samples presented NPG values >80000.

The most frequent families found are Cistaceae, Fabaceae, and Lamiaceae (present in 100% of the samples), Asteraceae (95%), Boraginaceae and Resedaceae (90%), Myrtaceae (80%), Oleaceae and Scrophulariaceae (75%), Brassicaceae (70%), Fagaceae (55%), and Apiaceae and Salicaceae (50%).

The only pollen type present in all of the samples along with *Thymus* sp. is *Genista* f. Percentages are always referred to pollen from nectar plants. Thyme honeys contained between 15 and 73% pollen of *Thymus*, according to under-representing the presence of this pollen type in the honeys. The most

**Table 3.** Descriptive Statistical Data for Mineral Content in Thyme Honey

mineral	mean (mg kg <sup>-1</sup> )	range	standard deviation
Al	17.53	5.26–23.61	4.25
As	0.20	0.14–0.26	0.03
Ba	0.63	0.15–0.84	0.15
Ca	185.30	68.49–260.7	46.08
Cd	<0.01	0–0.01	0
Co	0.03	0–0.09	0.01
Cr	0.23	0.02–0.40	0.10
Cu	2.44	0.34–5.81	1.55
Fe	6.19	3.65–11.52	1.93
K	716.68	175.13–1380.3	313.21
Li	8.04	2.25–15.6	4.29
Mg	78.48	37.91–139.9	29.69
Mn	1.20	0.37–6.17	1.17
Mo	<0.01	0–0.04	0.01
Na	388.70	98.45–502.5	86.35
Ni	0.13	0–0.51	0.10
P	52.49	12.82–96.7	22.50
Pb	0.16	0.04–0.26	0.04
S	32.80	12.87–75.4	15.47
Se	0.05	0.03–0.07	0
Si	106.36	20.63–239.4	51.59
Sr	0.66	0.17–2.52	0.54
V	0.03	0–0.13	0.03
Zn	1.78	0.75–2.48	0.50

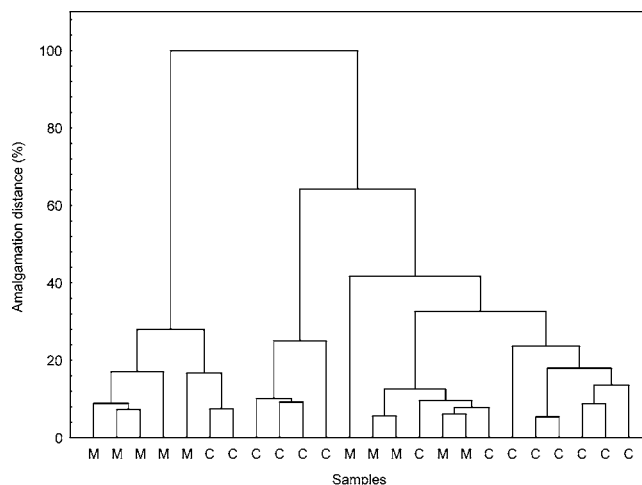
characteristic accompanying species are Leguminosae (*Calicotome villosa*, *Cytisus baeticus*, *Genista hirsuta*, and *Ulex borgiae*), present in 100% of the samples, followed by *Echium* sp. and *Reseda luteola* (90%) and *Eucalyptus* sp. (80%). Our results agree with data reported by other authors (17), who analyzed the pollen content of seven thyme honeys from Valencia (eastern Spain).

The results of the 24 metals determined in thyme honey samples are summarized in **Table 3**, where we can differentiate three mineral groups: elements that are very abundant, elements in a medium concentration, and trace elements.

The first group is composed of seven minerals (Ca, K, Mg, Na, P, S, and Si), the potassium being quantitatively the most important; it accounts for 45% of the total minerals quantified, which coincides with most authors, who consider this mineral to be the most abundant in honey (29). The sodium content is very similar to that reported in Spanish eucalyptus honeys (30), but higher than that found in Italian eucalyptus and Spanish lavender honeys (4, 20), the mean value being 388 mg kg<sup>-1</sup>, which represents ~24% of the total mineral content. The third most abundant mineral was calcium, with a average value of 185 mg kg<sup>-1</sup>; this value is slightly less in sunflower and heather honeys (2). Silicon is present in average quantities (106 mg kg<sup>-1</sup>), a value much higher than that found in some Spanish commercial honeys. Finally, with quantities never greater than 78 mg kg<sup>-1</sup>, magnesium, phosphorus, and sulfur are present, which agrees with the results found by the same authors (3).

The second mineral group is composed of six elements (Al, Cu, Fe, Li, Mn, and Zn), aluminum being the most abundant, with 17.53 mg kg<sup>-1</sup> as a mean, whereas the other minerals are found with values of <8 mg kg<sup>-1</sup>.

The third mineral group is composed of As, Ba, Cd, Co, Cr, Mo, Ni, Pb, Se, Sr, and V, always present in values of <1 mg kg<sup>-1</sup>. It is worth pointing out the low levels of cadmium and lead, minerals appropriate for testing the contamination of the environment (31), which show the low levels of contamination in the atmosphere and water in the area where the thyme honey is produced (32).

**Figure 2.** Dendrogram for honeys: complete linkage with Euclidean distances.

The average total mineral content of thyme honey is ~1600 mg kg<sup>-1</sup>, a value that is above those of light to amber-light unifloral honeys such as acacia, citrus, or lavender; it is on the same level as that of brown honeys such as eucalyptus and is fairly lower than that of dark honeys such as avocado, heather, or honeydew (2, 4, 11, 18–20, 30).

In an attempt to establish differences between Spanish thyme honey produced in coastal and mountain regions, some chemometric tools have been applied to the mineral data. Thus, CA, PCA, and SDA were applied as indicated in the previous section.

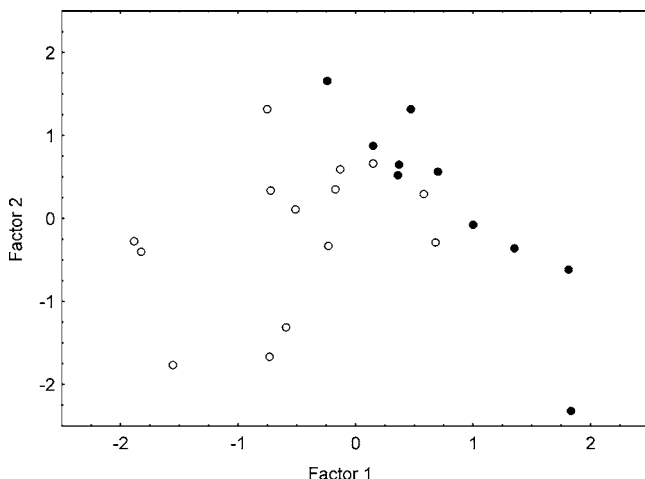
For the CA, a similarity matrix  $S_{24 \times 24}$  was constructed from the autoscaled data. The elements of this similarity matrix were the Euclidean distance of one object from the rest. The clustering procedure used was the complete linkage method with the Euclidean distances between objects. The results obtained showed the presence of honey clusters; the data of minerals contained significant information to achieve a two-category classification between thyme coast honeys and thyme mountain honeys. The results of CA are shown in a dendrogram (**Figure 2**). At a similarity level of ~30%, two clusters were found for several coast honey samples. Nevertheless, no specific groupings for mountain honey samples were detected.

PCA was performed to provide a data structure study in a reduced dimension, covering the maximum amount of information present in the data. PCA represents the original data matrix as a product of two matrices, the score matrix and the loading matrix. This corresponds to the projection of the data matrix onto a few-dimensional space. **Table 4** shows the factor-loading matrix obtained for the two factors and the variance explained by each of them. The first principal component accounts for 33.19% of the variance and the second for 20.50%, the former being strongly correlated with Ca, Cu, Li, Mg, S, and V and the latter very specifically with Al, Cr, and Na, the cumulative variance being 53.7%. **Figure 3** represents the graphic distribution of the samples according to their factor scores, showing a perfect separation between the thyme honey produced on the coast and in the mountains, except for one sample, with higher values for the two factor scores, which separates it from both honey origins.

With respect to the SDA, a forward iterative inclusion of variables was performed to choose the parameters with a higher discriminant power. A tolerance of 0.01 was stated to eliminate the variables that provide superfluous information at a 99% level along with those previously included in the model. Taking into account the results obtained in the PCA, the elements selected

**Table 4.** Results of Principal Component Analysis

	factor 1	factor 2
Al	-0.481527	0.683102
As	-0.675058	-0.356985
Ba	-0.637410	0.571862
Ca	-0.804693	0.340022
Cd	-0.171240	-0.178841
Co	-0.277559	0.631170
Cr	0.158132	0.883681
Cu	-0.797716	-0.362910
Fe	-0.019618	0.449948
K	-0.684830	-0.093728
Li	-0.779900	-0.465160
Mg	-0.786452	0.325682
Mn	-0.206308	0.393315
Mo	-0.597184	-0.458477
Na	-0.460335	0.693512
Ni	-0.616649	0.151920
P	-0.648654	-0.185918
Pb	-0.624024	0.288653
S	-0.781996	-0.066948
Se	-0.347028	-0.383098
Si	-0.470179	0.626358
Sr	-0.560770	-0.273715
V	-0.704463	-0.447808
Zn	-0.443689	-0.449511
% total variance	33.19	20.50
cumulative variance (%)	33.19	53.70

**Figure 3.** Plot of the first factor versus the second factor, for the classification of honeys according to their origin: (○) coast; (●) mountain.

by stepwise discriminant analysis were as follows: Al, As, Cr, Cu, K, Li, Mg, Na, P, S, and V.

The discriminant algorithm calculates the canonical correlations between the variables entered and the dummy variables representing the two honey classes (coast and mountain) and the coefficients for the canonical variables. The first canonical variable (which accounts for the highest variability between honey classes) is that which best discriminates between the honey types. The second is the next best linear function orthogonal to the first canonical variable and accounts as well as possible for differences between honey classes not shown by this one, etc. The method extracts  $n - 1$  canonical variables,  $n$  being the number of groups to discriminate between. Therefore, **Table 5** lists the cumulative proportion of total dispersion, and the standardized coefficients for the unique canonical variable. The higher the absolute value of a standardized coefficient is, the more significant is the related selected variable in the canonical variable. Lithium (mean = 10 mg kg<sup>-1</sup> in coast thyme honeys and mean = 5 mg kg<sup>-1</sup> in mountain

**Table 5.** Cumulative Proportion of Total Dispersion and Standardized Coefficients for Canonical Variable Obtained by SDA

	root 1
Al	-1.78536
As	-1.96360
Cr	-2.20497
Cu	-2.30567
K	2.87442
Li	4.31861
Mg	2.06617
Na	2.44585
P	-5.06625
S	-1.71728
V	-1.68342
eigenvalue	7.74729
cumulative proportion	1.00000

**Table 6.** Classification Functions for Thyme Honeys Obtained by SDA

	honey origin	
	coast	mountain
Al	8.395	6.015
As	2430.515	2028.697
Cr	376.798	263.138
Cu	29.817	19.904
K	-0.281	-0.224
Li	-28.945	-22.423
Mg	-1.226	-0.826
Na	-0.564	-0.410
P	6.121	4.753
S	2.809	2.135
V	797.897	500.577
constant	-274.615	-181.615

**Table 7.** Classification Matrix for Thyme Honeys Obtained by SDA

	% correction	coast	mountain
coast	100	14	0
sierra	100	0	10
total	100	14	10

thyme honeys) and phosphorus (mean = 61 mg kg<sup>-1</sup> in coast thyme honeys and mean = 39 mg kg<sup>-1</sup> in mountain thyme honeys) appear to be the minerals that account for most of the discrimination between the two honey classes.

Classification functions are linear combinations of the variables selected by the statistical method; the coefficients and constants for these functions are shown in **Table 6**. By applying these functions to the samples, their validity can be verified according to the percentages of accuracy of each case in its corresponding group (**Table 7**). It can be observed that all honey samples were correctly classified into their previously established classes (coastal, mountain) (100%).

## CONCLUSIONS

The thyme honeys produced in Spain are characterized by a total average mineral content of ~1600 mg kg<sup>-1</sup>, potassium, sodium, calcium, silicon, magnesium, phosphorus, and sulfur being the most abundant elements, representing >97% of the total mineral; this content places them between very dark and amber honeys, which coincides with their intrinsic golden/dark amber color. On the other hand, thyme honeys from the continental regions (mountains) are characterized by their low mineral levels (mean = 1300 mg kg<sup>-1</sup>) and those from coastal regions by their high mineral levels (mean = 1900 mg kg<sup>-1</sup>).



This concurs with the results obtained in Canadian honeys (10), showing that the mineral content of the honey is independent of the floral origin, the geographical origin (maritime or continental) and the extent of precipitation being the principal reasons for these differences.

#### LITERATURE CITED

- (1) Guizelan, G.; Guizelan, R. Données concernat les éléments minéraux dans quelques sortes de miel. *Proc. 23rd International Congress on Apiculture*, Moscow, Apimondia Publishing: Bucharest, Romania, 1971; pp 592–593.
- (2) Salinas, F.; Montero, V.; Osorio, E.; Lozano, M. Determinación de elementos minerales en mieles de diversos orígenes florales por análisis de inyección en flujo acoplada a la espectroscopía atómica. *Rev. Esp. Cienc. Tecn. Aliment.* **1994**, *34*, 441–449.
- (3) Rodríguez-Otero, J. L.; Paseiro, P.; Simal, J.; Cepeda, A. Mineral content of the honeys produced in Galicia (north-west Spain). *Food Chem.* **1994**, *49*, 169–171.
- (4) Poiana, A.; Fudo, S.; Manzin, E.; Postarino, S.; Mincione, B. Ricerche sui mieli commercializzati in Italia: la componente minerale. *Ind. Aliment.* **1996**, *35*, 522–530.
- (5) Terrab, A.; González, A. G.; Díez, M. J.; Heredia, F. J. Mineral content and electrical conductivity of the honeys produced in north-west Morocco and their contribution to the characterisation of unifloral honeys. *J. Sci. Food Agric.* **2003**, *83*, 637–643.
- (6) Anklam, E. A review of the analytical methods to determine the geographical and botanical origin of honey. *Food Chem.* **1998**, *63*, 549–562.
- (7) Prat, C. R.; Sikorski, R. S. Lead content of wildflowers and honey bees (*Apis mellifera*) along a roadway: possible contamination of a simple food chain. *Proc. Pa. Acad. Sci.* **1983**, *9*, 151–152.
- (8) Free, J. B.; Williams, I. H.; Pinsent, R. J. F. H.; Townshend, A.; Basi, M. S.; Graham, C. L. Using foraging honey bees to sample an area for trace metals. *Environ. Entomol.* **1983**, *9*, 9–12.
- (9) Crane, E. Bees, honey and pollen as indicators of metals in the environment. *Bee World* **1984**, *65*, 47–49.
- (10) Féller-Demalsy, M. J.; Vincent, B.; Beaulieu, F. Teneur en minéraux et origin géographique des miel du Canada. *Apidologie* **1989**, *20*, 77–91.
- (11) Varju, M. Mineral content of Hungarian acacia honeys and its relationship with the plant and soil. *Z. Lebensm. Unters. Forsch.* **1970**, *144*, 308–312.
- (12) Latorre, M. J.; Peña, R.; García, S.; Herrero, C. Authentication of Galician (N. W. Spain) honeys by multivariate techniques based on metal content data. *Analyst* **2000**, *125*, 307–312.
- (13) Ricciardelli D'Albore, G. *Mediterranean Melissopalynology*; Institute of Agricultural Entomology, University of Perugia: Perugia, Italy, 1998.
- (14) Damblon, F. Miels de thym du Maroc. II. Étude palynologique des sources mellifères. *Al Biruniya* **1987**, *3*, 51–75.
- (15) Oddo, L. P.; Piazza, M. G.; Sabatini, A. G.; Accorti, M. Characterization of unifloral honeys. *Apidologie* **1995**, *26*, 453–465.
- (16) Thrasyvoulou, A.; Manikis, J. Some physicochemical and microscopic characteristics of Greek unifloral honeys. *Apidologie* **1995**, *26*, 441–452.
- (17) Roselló Caselles, J.; Burgaz Moreno, E.; Mateu Andrés, I.; Gómez Ferreras, C. Espectro polínico de mieles de Labiadas valencianas. *Bot. Macaronésica* **1996**, *23*, 155–166.
- (18) Terrab, A.; Díez, M. J.; Heredia, F. J. Palynological, physicochemical and colour characterisation of Moroccan honeys. I. River red gum (*Eucalyptus camaldulensis* Dehnh) honey. *Int. J. Food Sci. Technol.* **2003**, *38*, 379–386.
- (19) Terrab, A.; Díez, M. J.; Heredia, F. J. Palynological, physicochemical and colour characterisation of Moroccan honeys. II. Orange (*Citrus* sp.) honey. *Int. J. Food Sci. Technol.* **2003**, *38*, 387–394.
- (20) Serra Bonvehí, J. Propietés physico-chimiques, composition et spectre pollinique des miels de *Lavandula latifolia* Med, produits en Espagne. *Sci. Aliments* **1988**, *8*, 295–307.
- (21) Maurizio, A. Microscopy of honey. In *Honey—A Comprehensive Survey*; Crane, E., Ed.; Heinemann: London, U.K., 1979; pp 240–257.
- (22) Erdtman, G. The acetolysis method A revised description. *Svensk Bot. Tidskr.* **1960**, *54*, 561–564.
- (23) Díez, M. J. Clave general de tipos polínicos. In *Atlas Polínico de Andalucía Occidental*; Valdés, B., Díez, M. J., Fernández, I., Eds.; Instituto de Desarrollo Regional y Excma Diputación de Cádiz: Sevilla, Spain, 1987; pp 23–61.
- (24) AOAC. *Official Methods of Analysis*, 15th ed.; Association of Official Analytical Chemists: Arlington, VA, 1990.
- (25) Statsoft. *Statistica for Windows* [computer program manual]; Tulsa, OK, 1999.
- (26) Jacobsen, T.; Gunderson, R. W. *Applied Cluster Analysis in Statistical Procedures in Food Research*; Elsevier Applied Science Publishers: London, U.K., 1986.
- (27) Manly, B. F. J. *Multivariate Statistical Methods. A Primer*, 2nd ed.; Chapman and Hall: London, U.K., 1986.
- (28) Morrison, D. F. *Multivariate Statistical Methods*, 3rd ed.; McGraw-Hill: New York, 1990.
- (29) White, J. W. Honey. *Adv. Food Res.* **1978**, *24*, 287–375.
- (30) Serra Bonvehí, J. Características físico-químicas Composición de la miel de eucalipto (*Eucalyptus* sp) producida en España. *Ann. Bromatol.* **1989**, *41*, 41–56.
- (31) Przybyłowski, P.; Wilcznska, A. Honey as an environmental marker. *Food Chem.* **2001**, *74*, 289–291.
- (32) Stocker, H. S.; Seager, S. L. *Química Ambiental: Contaminación del Aire y del Agua*; Blume Publishing: Barcelona, Spain, 1981.

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