Linking Soil, Water, and Honey Composition To Assess the Geographical Origin of Argentinean Honey by Multielemental and Isotopic Analyses

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Ⓢ Supporting Information

ABSTRACT: The objective of this research was to investigate the development of a reliable fingerprint from elemental and isotopic signatures of Argentinean honey to assess its geographical provenance. Honey, soil, and water from three regions (Córdoba, Buenos Aires, and Entre Ríos) were collected. The multielemental composition was determined by ICP-MS. $\delta^{13}C$ was measured by isotopic ratio mass spectrometry, whereas the $^{87}Sr/^{86}Sr$ ratio was determined using thermal ionization mass spectrometry. The data were analyzed by chemometrics looking for the association between the elements, stable isotopes, and honey samples from the three studied areas. Honey samples were differentiated by classification trees and discriminant analysis using a combination of eight key variables (Rb, K/Rb, B, U, $^{87}Sr/^{86}Sr$, Na, La, and Zn) presenting differences among the studied regions. The application of canonical correlation analysis and generalized procrustes analysis showed 91.5% consensus between soil, water, and honey samples, in addition to clear differences between studied areas. To the authors' knowledge, this is the first report demonstrating the correspondence between soil, water, and honey samples using different statistical methods, showing that elemental and isotopic honey compositions are related to soil and water characteristics of the site of origin.

KEYWORDS: *honey, trace elements, isotopic composition, geographical origin, authenticity, traceability, fingerprint*

INTRODUCTION

In these past years, authenticity and traceability of foodstuffs became a major requirement for consumers and producers worldwide. Food products have been linked to a specific geographical origin; however, such links have disappeared over time mostly because of the globalization of the food industry[1] Food is becoming more regulated, with the increase in global trade and free markets. That is why there is an increased need for developing new methods and techniques to discriminate different food origins, sources, or farming systems.[2]

Honey is a high value-added product, which is of high interest for assessing its authenticity (both geographical origin and floral sources). Additionally, the detection of disallowed substances, such as syrups or sugars, contributes to honey authenticity and safety.[3,4] According to the European legislation (Council Directive 2001/110), the country where the honey was collected has to be declared. Honey with geographical and botanical origins declared are differently valued and, for that reason, subjected to frauds, leading to doubtful labeling.[3]

Honey chemical composition is generally associated with its botanical origin and, to a lesser degree, with the geographical

area where it was collected, because environment (soil and climate) determine the melliferous flora.[5,6] For botanical origin classification, conventional methods (physical characteristics, chemical composition, pollen analysis, and sensory parameters) are mainly used.[3,7−10]

The multielemental content of food reflects the type of soil and environment conditions.[11−13] Honey contains between 0.04 and 0.2% (w/w) of trace elements. The soil composition of the origin, determined by geochemical and geological features (i.e., regional geology and climatic changes), affects the composition and concentration of metals in honey.[14,15] The botanical origin (nectar, pollen, and floral type of honey plants) are also responsible for the metal content of honey.[14,16] Furthermore, trace element contents in honey could also serve as indicators of environmental pollution.

On the other hand, the analysis of stable isotopes has gained importance in the determination of the geographic origin of food. Stable isotope measurements were first introduced within the European wine industry to ensure authenticity and to detect adulteration using carbon, oxygen, and hydrogen, mostly, but also strontium.[17] These measurements have been extended to many other foods (e.g., milk, meat, honey, juices, spirits, and flavors).[9,11−13,18,19] Small but significantly different ratios of stable isotopes can be correlated with the botanical−metabolic and/or geographical−geological origin of a product. Isotope ratios may add to the element fingerprint information on the plant type and the geographical−geological origin.[20]

Even though there are many studies focusing on the elemental profile of honey,[6,21−23] or on stable isotopes,[9,19,24] there are few that combine both parameters.[9] In addition, there are also few studies that consider the association with soil and water from places where honey is made.[19] Furthermore, there has been little research on the determination of both the content of elements and stable isotopes in Argentinean honey.[14,21,25] In this paper, for the first time, we present the results of the determination of 33 elements and carbon and strontium isotopes in Argentinean honey from three different regions (Buenos Aires, Córdoba, and Entre Ríos). Additionally, we were interested in demonstrating the link between honey, soil, and water by applying different chemometric tools. To our knowledge, this is the first approach in Argentina on the combined use of Sr and C isotope ratios and multielement analysis of soil, water, and honey to assess the geographical origin.

MATERIALS AND METHODS

Reagents and Materials. Ultrapure water (<5 μg L^{-1} total organic content) was obtained from an Arium 61316-RO purification system combined with an Arium 611 UV (Sartorius, Germany). The certified ICP-MS multielement Merck VI CertiPUR standard solution was obtained from Merck Química Argentina (Buenos Aires, Argentina). Nitric acid (63.7%), sub-boiling grade, was prepared from analytical grade acid (Merck Química Argentina) using a distiller (Figmay sub-boiling distiller, Córdoba, Argentina).

The purity of nitric acid was verified by ICP-MS. Filters (0.45 μm, HAWG04756) were obtained from Millipore (São Paulo, Brazil). Trace element certified reference material (CRM) wheat flour 1567a was obtained from the National Institute of Standards and Technology (Gaithersburg, MD, USA). The best estimate values for constituent elements in CRM 1567a are detailed in the accompanying certificate of analysis provided by the manufacturer. Ion exchange chromatography Sr Spec resin was obtained from Eichrom Technologies (USA). Strontium isotopic standard (NBS SRM 987) was employed to determine mass discrimination during the isotopic analyses by thermal ionization mass spectrometry (TIMS). All other analytical grade reagents were used.

Sampling. Soil, water, and honey samples were collected from selected producing areas from three provinces of Argentina: Buenos Aires, Córdoba, and Entre Ríos. These provinces are the most important honey-producing areas in Argentina (Figure 1). The sampling area in Buenos Aires is located between 37°25′ and 37°38′ S and between 59°10′ and 59°14′ W; the Córdoba sampling area is located between 30°30′ and 31°08′ S and between 63°34′ and 64°08′ W; and the Entre Ríos sampling area is located between 32°49′ and 32°53′ S and between 58°32′and 58°34′ W. The altitude varies from 460 m above sea level in Córdoba to 240 m above sea level in Buenos Aires to 30 m above sea level in Entre Ríos. Soil, water, and honey samples were collected from different beekeepers during three harvests (2007, 2008, and 2009): seven in Córdoba, five in Buenos Aires, and six in Entre Ríos. Soil and water samples were collected and processed according to the method of Baroni et al.[11] A total of 79 honey samples

Figure 1. Map of the three regions where honey samples were collected.

were collected: 27 samples were collected in Buenos Aires, 25 in Córdoba, and 27 in Entre Ríos. Five hundred grams of honey was collected in plastic recipients, transported to the laboratory within 48 h, and stored at 4−8 °C until analysis.

Elemental Analyses. Five hundred milligrams of honey sample was mineralized with 8 mL of concentrated nitric acid using a microwave oven (Anton Paar Multiwave 3000, Vienna, Austria) following methods described by Podio et al.[13] CRM 1567a was processed and measured in the same way used for honey. Values obtained for the latter were between 85 and 105% of certified values.

Thirty-one elements were quantified in honey samples: Al, B, Ba, Ca, Cd, Ce, Co, Cs, Cu, Eu, Ga, K, La, Li, Lu, Mg, Mn, Mo, Na, Nd, Ni, Pb, Rb, Se, Sm, Sr, Tl, U, V, Yb, and Zn. The analysis was carried out by quadrupole inductively coupled plasma mass spectrometry (QICP-MS) for all elements except sodium as stated in Baroni et al.[11]

Stable Isotope Analysis. *Carbon Isotope Analysis.* Carbon isotope ratios were determined on whole honey and protein fraction. Protein isolation and purification were done according to AOAC Official Method 998.12 (repetitive washing procedure), using tungstic acid, sodium salt, and sulfuric acid. Both honey and protein samples were placed in quartz tubes, with added copper oxide. Tubes were prepared for combustion by evacuation on a vacuum line and sealed with a torch. Combustion was performed at 550 °C over 8 h, after which the tubes were allowed to cool at room temperature inside the oven. Following combustion the tubes were cracked open to extract pure CO_2 on the vacuum line using a cryogenic trap to retain water and non-CO_2 gases. The purified CO_2 was collected with a liquid nitrogen trap in a glass collection tube. The purified CO_2 was measured in a Multiport Dual Inlet IRMS (Finnigan MAT Delta S), using CO_2 gas as reference.

The isotope ratios were normalized using laboratory working standards, calibrated with international standards supplied by the International Atomic Energy Agency (NBS 22, NBS18). The $^{13}C/^{12}C$ ratios are expressed in the delta notation, $\delta^{13}C$ as the deviation per mil (‰) from the Vienna Pee Dee Belemnite (V-PDB) standard. The uncertainty of carbon isotope determinations was ±0.2‰.

Strontium Isotope Analysis. Bulk samples were processed by dry ashing technique in a muffle furnace. More than 100 g of sample was placed in porcelain crucibles and heated at 550−600 °C. The furnace was programmed to ramp up to the ashing temperature over a period of 8 h. Complete ashing was ensured by maintaining this temperature for 10 h. Residues were treated with concentrated HNO_3 on a hot plate and then transferred again to the muffle until white ashes appeared. The residues were dissolved in HNO_3, and subsequently Sr was separated by ion chromatography on a Sr-specific resin (Sr-spec). Sample manipulation was carried out in a clean environment (Class 100 laboratory). To prevent contamination, all laboratory-ware was soaked in 50% (v/v) nitric acid for at least 24 h, rinsed several times with ultrapure water, and dried in a Class 100 laminar flow exhaust hood.

Table 1. Means and Standard Deviations of Measured Elements (Micrograms per Kilogram) and Isotopic Ratios Corresponding to Honey According to Region of Origin and the Contribution of Two Spoons of Ingested Honey to DDA of Nutritional Elements[a]

variable	Buenos Aires	Córdoba	Entre Ríos	contribution to DDA (%)
Li	163 ± 112a	174 ± 183a	495 ± 159b	
B	$(84 ± 18) × 10^3$c	$(39 ± 15) × 10^3$a	$(66 ± 16) × 10^3$b	
Na	$(12 ± 52) × 10^3$a	$(16 ± 11) × 10^3$a	$(46 ± 20) × 10^3$b	0.1
Mg	$(177 ± 174) × 10^3$a	$(359 ± 209) × 10^3$b	$(496 ± 238) × 10^3$c	2.1
Al	$(4.63 ± 4.67) × 10^3$a	$(10.93 ± 8.12) × 10^3$a	$(22 ± 35) × 10^3$b	
K	$(339 ± 105) × 10^3$a	$(1041 ± 528) × 10^3$b	$(1575 ± 760) × 10^3$c	2.1
Ca	$(698 ± 542) × 10^3$a	$(862 ± 453) × 10^3$a	$(1298 ± 1009) × 10^3$b	5.4
V	1431 ± 1287a	1798 ± 1450a	1038 ± 747a	
Mn	$(2.05 ± 1.08) × 10^3$a	$(9.88 ± 4.33) × 10^3$b	$(21.78 ± 16.88) × 10^3$c	12
Co	28 ± 22a	40 ± 19a	67 ± 42b	
Ni	847 ± 1786a	617 ± 1647a	955 ± 862a	
Cu	1290 ± 480a	1202 ± 607a	5863 ± 3629b	3.97
Zn	$(12.16 ± 21.89) × 10^3$a	$(12.72 ± 8.73) × 10^3$a	$(30.76 ± 21.88) × 10^3$b	5.25
Ga	12 ± 13a	25 ± 16b	17 ± 9a	
Se	392 ± 302a	642 ± 287b	431 ± 251a	
Rb	$(3.95 ± 2.19 × 10^3$a)	$(1.75 ± 0.73 × 10^3$a)	$(31.62 ± 8.32 × 10^3$b)	
Sr	1760 ± 1099a	3883 ± 1479b	5292 ± 2337c	
Mo	28 ± 30a	57 ± 31a	112 ± 79b	
Cs	113 ± 145a	84 ± 140a	134 ± 192a	
Ba	0.87 ± 0.83a	6.71 ± 6.41b	5.97 ± 10.58b	
La	6.70 ± 20.93a	<LOD	116 ± 197b	
Ce	3.47 ± 7.72a	20.50 ± 33.87a	39.02 ± 55.37b	
Nd	3.65 ± 4.43a	4.63 ± 5.41a	12.55 ± 7.84b	
Sm	6.39 ± 5.34b	6.30 ± 4.04b	2.73 ± 2.30a	
Eu	0.54 ± 0.69a	0.66 ± 0.80a	1.40 ± 0.64b	
Yb	1.08 ± 1.53a	2.70 ± 2.11a	1.58 ± 1.01b	
Lu	0.20 ± 0.24a	0.21 ± 0.18a	4.78 ± 4.92b	
Tl	<LOD	<LOQ	<LOD	
Pb	21 ± 51a	209 ± 512a	191 ± 714a	
U	1.70 ± 2.99a	3.25 ± 2.56a	22.46 ± 19.95b	
K/Rb	120 ± 78a	617 ± 234b	48 ± 16a	
Ca/Sr	430 ± 182b	232 ± 113a	245 ± 83a	
$^{87}Sr/^{86}Sr$	0.7125 ± 0.0015a	0.7172 ± 0.0023c	0.7160 ± 0.0024b	
$\delta^{13}C$ (‰) bulk	−25.7 ± 1a	−24.1 ± 2.4b	−25.7 ± 0.4a	
$\delta^{13}C$ (‰) protein	−26.1 ± 0.3a	−24.7 ± 0.7b	−26.1 ± 0.5a	

[a]<LOD, below limit of detection; <LOQ, below limit of quantification; IDL ($\mu g\ kg^{-1}$), La (1.0) Tl (0.5); IQL ($\mu g\ kg^{-1}$), Tl (1.67). Different letters in the same row indicate significant differences ($p < 0.05$).

Strontium isotope ratios were measured following the method described by Baroni et al.[11]

Soil and Water Analysis. The methodology and the results of soil and water samples in the regions under study were published by Baroni et al.[11]

Statistical Analysis. Data were analyzed using the statistical package Statistica 8.0 from StatSoft Inc. (2007) and the Infostat software package.[26]

Analysis of Variance. Results were analyzed by ANOVA, and significant differences between mean values were determined by DGC test ($p < 0.05$).

Classification Tree Analysis (C&RT). Classification trees are used to predict membership of cases or objects in the classes of a categorical dependent variable from their measurements on one or more predictor variables. These methods consist of algorithms based on rule induction, which is a way of partitioning the data space into different class subspaces. Basically, the data set is recursively split into smaller subsets, where each subset contains objects belonging to as few categories as possible. A test set, containing objects not used in the training and also with known class memberships, serves to test the performance of the classifier.[27] The training set contains 75% of total honey samples. The subsets were selected randomly for each class

separately. A leave-one-object out cross-validation procedure was adopted to optimize models constructed. The efficiency of the model was used to characterize quality of model prediction, which is the total percentage of a correctly classified test sample.

Discriminant Analysis (DA). DA was performed in stepwise mode to discriminate honey samples according to their geographical origin. Most significant variables were selected according to the F value. A cross-validation test was used to evaluate the robustness of the classification model.

Generalized Procrustes Analysis (GPA). GPA is a useful statistical method to evaluate the correspondence between different data from the same object. In this case, we evaluate correspondence of elemental and isotopic data from soil, water, and honey from each geographical origin.[11]

Canonical Correlation Analysis (CCA). CCA was used to find relationships between concentrations of elements and isotopes in soil, water, and honey. This analysis allows us to investigate the relationship of two sets of variables, honey versus soil and honey versus water.

RESULTS AND DISCUSSION

Multielement Contents. Table 1 shows mean values and standard deviations of element data for honey samples from the
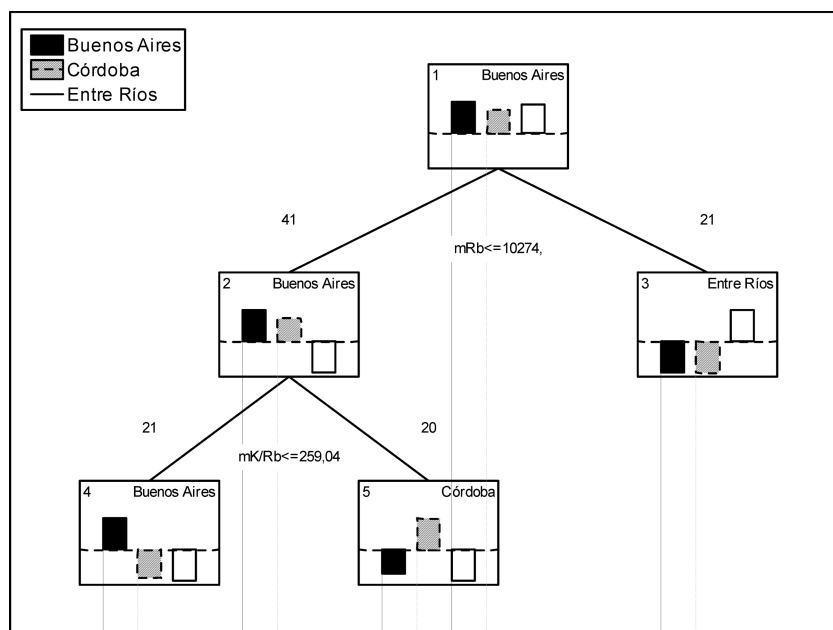
Figure 2. Classification tree of honey samples according to their type using the classification tree method.

three studied regions. Furthermore, K/Rb and Ca/Sr ratios were included. The K/Rb ratio can greatly differ among various types of rocks and soils.[28] On the other hand, the Ca/Sr ratio has been used as a chemical tracer in geochemistry, hydrogeochemistry, and bioavailability studies.[29]

Tl was the only element analyzed that fell below the detection level in honey. In line with most previous studies,[21−23,27] potassium (K) was the most abundant element in studied honey, followed by Ca, Mg, and Na. B, Al, Mn, Zn, Rb, and Ba were present in intermediate concentrations (Table 1). The other analyzed elements were below 1 ppm.

We observed some differences between the geographical origins despite honey samples being multifloral in majority causing certain variability in the elemental composition. Twenty-five elements were significantly different at least in one region ($p < 0.05$), demonstrating that honeys from different regions have a characteristic elemental profile. Five variables (B, Mg, K, Mn, and Sr) were significantly different among the three studied regions. Conversely, V, Ni, Yb, and Pb did not show significant differences between the three studied areas. Ba presented lowest values in Buenos Aires, whereas Córdoba and Entre Ríos had similar higher values. Additionally, Li, Na, Al, Ca, Co, Cu, Zn, Rb, Mo, Ce, Nd, Eu, Yb, and Lu presented highest values in honey from Entre Ríos, whereas Buenos Aires and Córdoba presented similar lower values. Conversely, Sm presented lower values in Entre Ríos and similar higher values in Córdoba and Buenos Aires.

The ratios Ca/Sr and K/Rb also showed variations. Ca/Sr was higher in Buenos Aires and had similar lower values in Córdoba and Entre Ríos, whereas K/Rb was higher in Córdoba and presented lower values in Buenos Aires and Entre Ríos.

The contents of nutritionally important elements were calculated in a daily intake of 42.8g of honey (two spoons) and compared to their daily dose allowance (DDA) in the human body, taken from Seiler et al.[30] The ingestion of two spoons of honey represents between 0.1 and 12% of the DDA (Table 1).[31]

Apart from mineral nutrients, the analysis of the elemental composition of honey may also give information about environmental pollution. The contents of trace elements in the Argentinean honey were comparable with the contents in honey from uncontaminated areas.[22,23,27] This emphasizes the good quality of Argentinean honey, especially with regard to the contents of toxic trace elements, such as Cd, Pb, and U. All of the honey samples presented a concentration of heavy metals below maximum levels for food set by Commission Regulation (EC) No. 1881/2006 (EC, 2006) amended by EC 629/2008 (EC, 2008).[32] With respect to cadmiun content, samples from Córdoba showed the highest values (600 ppb), even though these values are below the maximum levels set. From previous studies, it can be noted that industry, mining, automobile exhaust gases, etc., may be responsible for cadmium contamination in honey.[33]

Carbon Isotopes. The carbon isotopes in plants and resulting products are mainly influenced by metabolism (carbon fixation), which is why the $\delta^{13}C$ values can be used to detect honey adulterated with C4 plant sugars, such as high fructose corn syrup (HFCS).[6,30,31] On the other hand, carbon isotopes are related to humidity and temperature (climate) and can be useful for food geographical origin assignment.[34]

On the basis of $\delta^{13}C$ values obtained, samples of this study were derived from C3 plants and have not been adulterated, at least with respect to C4 plant addition (corn or cane) (Table 11).[35,36] In previous studies[11,13] we found differences in meat and wheat $\delta^{13}C$ values from the same geographical origin, so we expected to find similar differences in honey samples. Córdoba showed the highest $\delta^{13}C$ value, whereas Buenos Aires and Entre Ríos had similar lower values. The difference between the highest (Córdoba) and lowest (Buenos Aires and Entre Ríos) $\delta^{13}C$ mean values is 1.6‰. $\delta^{13}C$ values of honey increase with increasing temperatures, increasing amount of sun exposure, and decreasing humidity.[19] As noted for wheat,[13] the highest $\delta^{13}C$ values from Córdoba honey samples could be related to an evaporation effect due to its warm and dry growing conditions with respect to Buenos Aires and Entre Ríos. This is so because the cultivation area of Córdoba is located close to a semiarid region. The difference between the climate in Córdoba and Buenos Aires−Entre Ríos is sufficient to influence the $\delta^{13}C$

values of the honey and make this parameter useful to discriminate the geographic origin of food.[19] However, one has to bear in mind that there is variability also within each sampling area. Observed variations could be explained by different temperature ranges at harvest times of each honey, leading to different $\delta^{13}C$ values.

Strontium Isotopes. The strontium isotope composition of plants, and the animals that eat them, is related to the $^{87}Sr/^{86}Sr$ signature of the bioavailable strontium derived from water and soil (bedrock). Once a $^{87}Sr/^{86}Sr$ database of one crop has been constructed, it can be applicable to new crops from the same geographical origin. For example, it has been reported that $^{87}Sr/^{86}Sr$ values of water could be used to estimate the geographic origin of honey and wheat.[37] Therefore, the Sr isotope ratio inherits the geological character of a production area.[38]

We found statistically significant differences for $^{87}Sr/^{86}Sr$ between honeys from Córdoba, Entre Ríos, and Buenos Aires. Cordoba had the highest values, followed by Entre Ríos and Buenos Aires. Similar results were found in our previous studies for meat and wheat from the same geographical origin.[11,13] This shows the importance of including strontium isotopes in the determination of the geographical origin of foods.

Honey Geographical Classification. Analyzing only one isotopic element gives us an incomplete view regarding the interpretation of the origin of food in general and honey in particular. A multielement evaluation is essential to enhance the reliability of origin classification of food. To get greater origin resolution, the first choice will be to use multivariate statistical methods. In this case, classification trees and lineal discriminant analysis were applied.

Classification and regression tree (C&RT) analysis is a nonparametric methodology that segments populations into subgroups of increasing group homogeneity. The results are formed into a tree structure (decision tree), according logical rules "if—then". C&RT selects those variables that are most important for predicting classification without concern for the possible relationships between variables.[27]

Variables selected by C&RT as most important were Rb and K/Rb, which have 100 and 95% prediction power, respectively. The content of Rb allows discriminating Entre Ríos honey from the others, whereas K/Rb content separated Buenos Aires honey from Córdoba honey samples (Figure 2). The results are shown in Table 2. In the training set none of the samples were misclassified, whereas in the test set two samples of Buenos Aires were misclassified (13.4%).

Linear Discriminant Analysis. Eight of 35 original variables were pointed out by stepwise discriminant analysis as the most important for geographical discrimination, Rb being the most significant variable, followed by K/Rb, B, U, $^{87}Sr/^{86}Sr$, Na, La, and Zn. With these eight elements and isotopes, 98.7% of samples were classified correctly according to its origin. Two different independent discriminant functions were obtained by linear combination of these variables. The combination of the two canonical variables explained 100% of variability (Figure 3A). As shown in Figure 3, Rb was the most important variable in root 1, separating Entre Ríos samples from Buenos Aires and Córdoba; meanwhile, B and K/Rb improved the discrimination between Córdoba and Buenos Aires in root 2.

Rb, Na, Zn, and La presented higher values in Entre Ríos and lower values in Buenos Aires and Córdoba (Figure 3D). Additionally, $^{87}Sr/^{86}Sr$ shows higher values in Córdoba, intermediate values in Entre Ríos, and lowest values in Buenos

Table 2. Classification Tree Analysis: Recognition and Prediction Ability[a]

		Córdoba	Entre Ríos	Buenos Aires	total correct classification (%)
training set N = 62	Córdoba (N = 19)		0	0	
	Entre Ríos (N=21)	0		0	
	Buenos Aires (N = 22)	0	0		
% correctly classified		100	100	100	**100**
test set N = 17	Córdoba (N = 6)		0	0	
	Entre Ríos (N = 6)	0		0	
	Buenos Aires (N = 5)	2	0		
% correctly classified		60	100	100	**86.6**

[a]Number of samples in the training and test set and efficiency (in percentage) of the model.

Aires (Figure 3C). The K/Rb ratio shows the highest values in Córdoba and lower values in Entre Ríos and Buenos Aires (Table 1). Finally, B is capable of differentiating the three honey origins (Figure 3B).

Elements and isotopes selected by discriminant and classification tree analysis were also pointed out in previous studies as capable of differentiating diverse food matrices from the same Argentinean regions. For example, wheat samples from Córdoba, Buenos Aires, and Entre Ríos were classified by K/Rb, Na, $^{87}Sr/^{86}Sr$, and Zn;[13] meat samples from the same provinces were characterized by $\delta^{13}C$, Rb, $\delta^{15}N$, Ca/Sr, and $^{87}Sr/^{86}Sr$.[11] Furthermore, wine samples from different Argentinean regions were classified by B, Na, Zn, and $^{87}Sr/^{86}Sr$.[12] It is worth noting that $^{87}Sr/^{86}Sr$ is not capable of discriminating wine variety, but it is always associated with geographical origin. Voerkelius et al.[37] noted that $^{87}Sr/^{86}Sr$ serves as an indicator of geographical origin; in fact, they found a good correlation between honey strontium isotopes values and surface water values.

Furthermore, in various papers honey was also discriminated by the same elements. Chua et al.[23] found that Na, between other elements, was capable of discriminating the geographical origin of honey. Pisani et al.[39] showed that some geological and geochemical features appeared to affect honey chemistry composition, Na being one of the most important. Pellerano et al.[14] also classified Argentinean honey but from other locations using La and Zn as the most important elements.

Correlation between Soil, Water, and Honey Chemical Composition. Taking into account the previous background where it is known that metals in honey derived from soil and to some extent also from nectar and pollen, we were interested in evaluating the correlation between geographical origin (soil and water) and honey.[38–41] Characterization (elemental profile and isotopes ratios) of soil and water from the three regions of origin has been previously published (see the Supporting Information).[11]

GPA was applied to evaluate the correspondence between the elemental profile and isotopes ratios of honey, soil, and water. One hundred percent of the variability between samples could be explained by first and second principal axes (Figure 4). Furthermore, multielement and isotope concentrations from
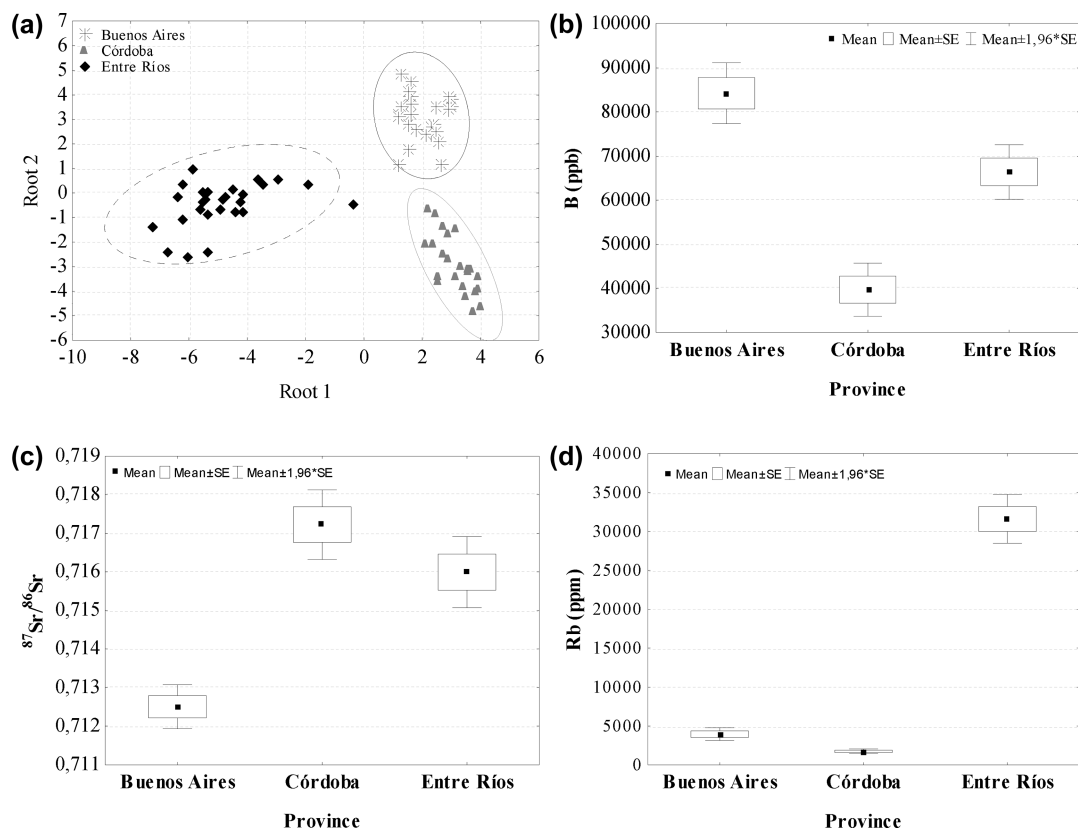
Figure 3. Discriminant analysis (canonical roots) and some selected parameters differentiating honey.
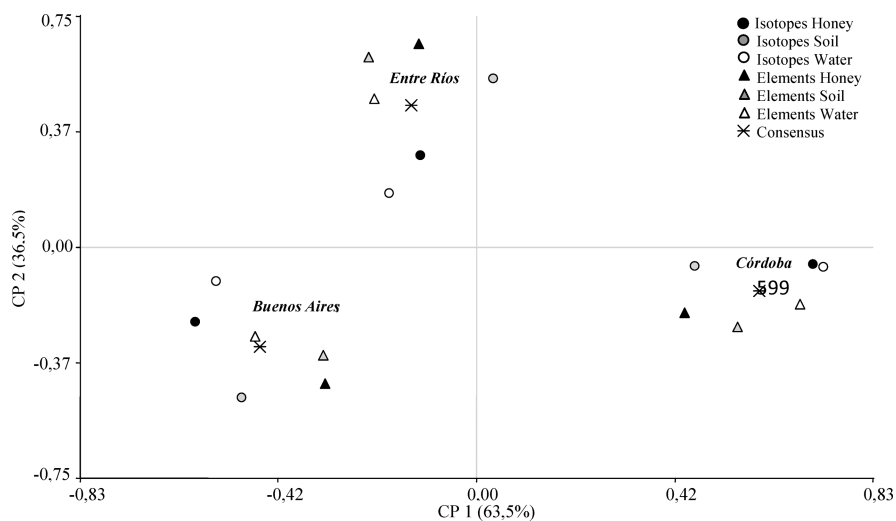

Figure 4. Consensus space from Generalized Procrustes analysis: plot in the plane defined by the first two dimensions.

soil, water, and honey samples allow discrimination of the three geographical origins. Honey chemical data have a significant consensus (91.5%) with those obtained from soil and water, as three data sets project the different geographical origins in the same way onto the plane defined by its first and second principal axes. GPA results show evidence on the correspondence between the three studied matrices.

Finally, we applied CCA to assess the correspondence between soil, water, and honey composition. The first CCA showed significant correlation ($r^2 = 0.99732$; $p < 0.001$) between soil and honey data sets. Contents of Co, Ni, Sr, and K/Rb in soil as well as Mn and Rb in honey were pointed out

as the variables that mainly contribute to the correlation between soil and honey. The CCA between water and honey data sets also showed a significant correlation ($r^2 = 0.9899$; $p < 0.001$). Ca, B, and Ca/Sr contents in water as well as Rb and Mg contents in honey were the variables that mainly contribute to the correlation between water and honey. Therefore, both GPA and CCA coincide, showing the influence of environment (soil and water) on honey composition.

In this work, geographical discrimination of honey through a suitable multivariate statistical analysis was accomplished because differences between elemental concentrations and isotope ratios were found. Linear discriminant analysis enabled

correct classification of honey samples from different Argentinean regions when the isotopic ratio $^{87}Sr/^{86}Sr$ as well as elements such as Rb, La, U, B, Na, Zn, and the K/Rb ratio were considered. Moreover, we were able to demonstrate correspondence between soil, water, and honey samples by appropriate statistical methods, such as Generalized Procrustes analysis and canonical correlations.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Table showing element and isotope concentrations in soil and water samples from studied regions. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jf5060112.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

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