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Characterization of honey from the Canary Islands: determination of the mineral content by atomic absorption spectrophotometry

O.M. Hernández, J.M.G. Fraga, A.I. Jiménez, F. Jiménez, J.J. Arias *

Departamento de Química Analítica, Nutrición y Bromatología, Facultad de Química, Universidad de La Laguna, E-38071 La Laguna, Tenerife, Spain

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Abstract

In this work, different types of honey produced in the Canary Islands were characterized on the basis of their mineral contents. Overall, 10 metals were determined in 116 samples, 81 of which were from the Canaries and 35 from various other places. Iron, copper, zinc, magnesium, calcium and strontium were determined by atomic absorption spectrophotometry, and potassium, sodium, lithium and rubidium by atomic emission spectrophotometry. A flow injection manifold was employed to analyse those samples requiring dilution. The chemometric processing of the spectroscopic results by various techniques (including principal component analysis, cluster analysis, discriminant analysis and logistic regression) allowed the accurate classification of the honey samples according to origin.

Keywords: Honey; Metals; Multivariate analysis

1. Introduction

Honey is a natural product obtained by bees from flower nectar or secretions from other living parts of plants or brought onto them by other insects; bees suck the nectar, combine it with specific substances and store it in hive cells for ripening. The composition and properties of the end-product depend on the botanical origin of the nectar or secretion used. All types of honey share certain characteristics, including a moisture content below 20%, a reducing sugar content of 60–65% and a bulk sucrose content of 5–10% (Ministerio de Sanidad y Consumo, 1985), so these major components cannot be used to distinguish samples. Rather, it is minor components that are usually employed to discriminate food samples and detect potential fraud in their stated origin.

The floral and geographic origin of honey is usually determined using the melisso-palynological technique, application of which, however, requires some expertise (Anklam, 1998). A number of papers have confirmed the possibility of characterizing honey samples by quantifying selected chemical parameters (Anklam & Radovic, 2001; Anupama, Bhat, & Sapna, 2003; Conte, Miorini, Giomo, Bertacco, & Zironi, 1998; Devillers, Morlot, Pham-Delègue, & Doré, 2004; Paradkar & Irudayaraj, 2001; Pérez Arquillué & Herrera Marteache, 1987; Serrano, Villarejo, Espejo, & Jodral, 2004); a number of methods for this purpose use gas chromatography–mass spectrometry (Bianchi, Careri, & Musci, 2005; Radovic et al., 2001; Soria, Martinez-Castro, & Sanz, 2003) or an electronic nose (Lammertyn, Veraverbeke, & Irudayaraj, 2004). However, determining inorganic compounds in honey, with a view to its characterization, can be much more simple, expeditious and economic.

Honey has a rather low mineral content (typically 0.1–0.2% in floral honey and 1% or higher in mellate

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* Corresponding author. Tel.: +34 922 318 076; fax: +34 922 318 003.

E-mail address: jjarias@ull.es (J.J. Arias).

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honey) that varies widely depending on the particular botanical origin, pedoclimatic conditions and extraction technique. The dominant element in honey is potassium, followed by chlorine, sulphur, sodium, phosphorus, magnesium, silicon, iron and copper (La Serna Ramos, Méndez Pérez, & Gómez Ferreras, 1999). Any marked deficiency of soil, rocks or water in any particular element is reflected in the mineral composition of plants and hence that of nectar and pollen (J., A., & J., 1987). Therefore, the contents of metal ions in honey, which can be determined by various techniques (Buldini, Cavalli, Mevoli, & Sharma, 2001; López-García, Viñas, Blanco, & Hernández-Córdoba, 1999; Rodríguez-Otero, Paseiro, Simal, & Cepeda, 1994; Sanna, Pilo, Piu, Tapparo, & Seeber, 2000; Viñas, López-García, Lanzón, & Hernández-Córdoba, 1997; Yilmaz & Yavuz, 1999), can help identify its geographical origin as this is consistent with the environmental conditions (Przybylowski & Wilczynska, 2001).

In fact, the mineral content of honey has been successfully correlated with its origin by using various statistical techniques, including principal component analysis (PCA), partial least-squares (PLS) regression, neural networks, cluster analysis, linear discriminant analysis (LDA), the K nearest neighbour (KNN) and/or independent modelling of class analogy (SIMCA) (Latorre, Peña, García, & Herrero, 2000; Latorre et al., 1999; Rashed & Soltan, 2004).

The Canary Islands produce many types of farmhouse honey, some of which are presumably monofloral. Canary honey varieties span a wide range of originating flora and microclimatic and atmospheric conditions. This substantially increases their market value and raises the need to detect potential frauds in origin or even adulteration (La Serna Ramos et al., 1999).

In recent years, a pressing need has arisen to find parameters allowing some products (particularly foods) to be distinguished in terms of origin. In the Canary Islands, this has been especially so with wines since their specific designations of origin were established (Barbaste, Medina, Sarabia, Ortiz, & Pérez-Trujillo, 2002; Frías, Conde, Rodríguez-Bencomo, García-Montelongo, & Pérez-Trujillo, 2003; González & Peña-Méndez, 2000; Pérez-Trujillo, Barbaste, & Medina, 2003), and recently with other products (Peláez Puerto, Fresno Baquero, Rodríguez Rodríguez, Darías Martín, & Díaz Romero, 2004).

Previous studies on honey varieties from the Canaries and other places revealed that the former, by virtue of the special climate and vegetation of the Islands, exhibit chemical and physico-chemical characteristics (e.g. pH, optical activity, conductivity, total and lactone acidity, hydroxymethylfurfural contents) that facilitate their discrimination from honey produced in other regions or even on different islands or different areas on the same island, as the prevailing local vegetation is to a great extent dictated by height (Fraga, Hernández, Jiménez, Jiménez, & Arias, 2000; Fraga, Hernández, Jiménez, Jiménez, & Arias, 2000).

In this work, alkaline (Na and K), alkaline-earth (Ca, Mg and Sr) and trace elements (Fe, Cu, Zn, Li and Rb) were determined in honey samples from the Canaries and various other places with a view to identifying their origin.

2. Materials and methods

2.1. Apparatus

Measurements were made on a Varian SpectrAA-10 Plus atomic absorption spectrophotometer equipped with a deuterium lamp for background correction and hollow-cathode lamps for each of the elements studied.

The continuous-flow manifold used included a Gilson Minipuls-2 peristaltic pump fitted with PVC tubes. All other tubing and connectors were made of Teflon.

2.2. Reagents

Standard stock solutions of the different metal ions at a 1000 μg/ml concentration were prepared from Panreac atomic absorption spectroscopic grade chemicals and used to make working solutions by appropriate dilution.

Reagent-grade nitric acid, ultrapure de-ionized Milli-Q water and the surfactant Extran (Merck) were used.

2.3. Glassware cleaning

All glassware was washed with Extran, immersed in 0.1 N nitric acid for 48 h and rinsed with ultrapure Milli-Q water prior to use in order to avoid potential contamination.

2.4. Samples

An overall 116 samples of monofloral and multifloral honey, 81 of which were from the Canary Islands (62 from Tenerife, 8 from La Palma, 9 from Gomera and 2 from Hierro) and the other 35 from various other places, were studied. The samples were either directly purchased on the market, supplied by Casa de la Miel (Tenerife) or obtained from beekeepers.

2.5. Sample preparation

2.5.1. Procedure 1 (47 samples)

About 5 g of honey were placed in a previously weighed porcelain crucible and heated (first in a stove and then in a furnace), following the temperature programme depicted in Fig. 1. The resulting white ash

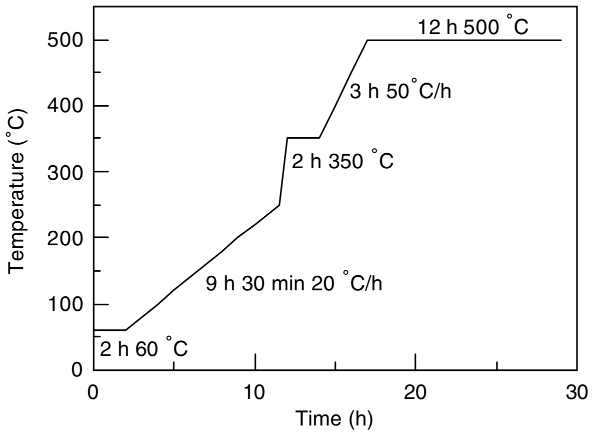

Fig. 1. Temperature programme used in the samples treatment.

was weighed, dissolved in 3 ml of concentrated nitric acid and diluted with de-ionized water in a 25 ml calibrated flask. The solution was used to determine Fe, Cu, Zn, K, Na, Mg, Ca, Sr, Li and Rb.

2.5.2. Procedure 2 (proposed by the authors; 116 samples)

About 2.5 g of honey were dissolved in 0.1 M nitric acid and diluted to 25 ml with more nitric acid in a calibrated flask. Metal ion contents were directly determined in the solution.

2.6. Determination of metal ions

The metal ions studied were determined by comparing the atomic spectroscopic signal for each with that for a standard solution of the same ion.

The determination of Ca and Mg by flame atomic spectroscopy is usually subject to some chemical interferences that can be avoided by using a higher-energy flame for Ca and by adding La_2O_3 as an Mg releasing agent. The determination of Na, K, Li and Rb by this technique is confounded by ionization interferences that entail the use of some suppressor; thus, the samples used to determine Na, Li, Rb were supplied with KCl, and those used to determine K with NaCl. Because these elements were present at concentrations outside the linear range of the atomic spectroscopic techniques used, the samples had to be diluted. In order to solve both problems simultaneously, the manifold of Fig. 2 was employed. The sample and carrier streams were merged in a conical vessel from which they were aspirated by the nebulizer of the atomic absorption spectrophotometer, excess sample being aspirated and sent to waste by the peristaltic pump. The sample and carrier flow-rates were set in such a way as to avoid interferences and allow the final concentrations of the elements to fall within the linear metal concentration-measured signal ranges.

Table 1 shows the composition and flow-rate of the carrier, as well as the values of the other operating parameters of interest. The flame composition, wavelength, lamp intensity and slit width used were those recommended for each metal ion (Varian, 1989). The measurement delay time was based on the time taken by the samples to give a steady signal upon reaching the flame and the measurement time was chosen so as to ensure that the standard deviation of the measurements would not exceed 2%. As can be seen, both times were longer with the FIA system as signals tended to oscillate over longer periods. Each determination was performed five times.

2.7. Data processing

The results of the above-described determinations were processed chemometrically, using various multivariate techniques, as implemented in the statistical software package SPSS v. 10.0 for Windows. Each sample was characterized in terms of 10 variables (the 10 metal ions studied). This provided a 116×10 matrix with honey types as rows and the metal ions as columns.

Prior to chemometric processing, data were autoscaled by subtracting their mean values and dividing into the standard deviation. A preliminary statistical analysis was conducted on all samples and variables in order to identify outliers (i.e. samples with a unique combination of characteristics enabling their easy distinction from the others). Such samples must be

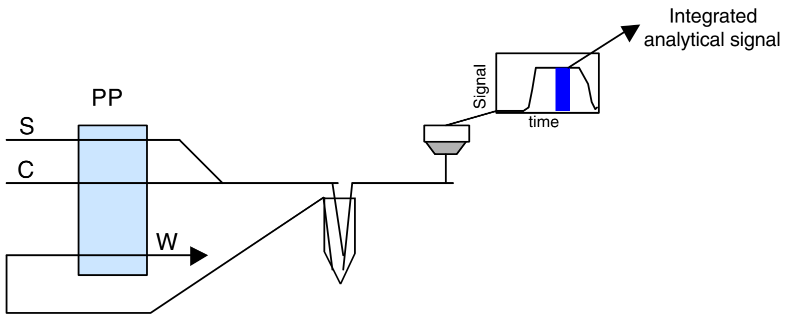

Fig. 2. Manifold used in the AAS and AES determinations of metal ions. PP, peristaltic pump; S, sample channel; C, carrier channel; W, waste.

Table 1
Instrumental operating conditions used in the determination of metal ions

Ion	Method	Flame composition	Wavelength (nm)	Lamp intensity (mA)	Split (nm)	Flow system			Measurement	
						Sample flow (ml/min)	Carrier			
							Composition	Flow (ml/min)	Delay (s)	Time (s)
Fe	AAS	Air/CH_2CH_2	248.3	5	0.2	–	–	–	1.0	1.0
Cu	AAS	Air/CH_2CH_2	324.7	4	0.5	–	–	–	1.0	1.0
Zn	AAS	Air/CH_2CH_2	213.9	5	1.0	–	–	–	1.0	1.0
K	AES*	Air/CH_2CH_2	766.5	–	1.0	1.0	5 g/l NaCl	10.5	30.0	2.0
Na	AES*	Air/CH_2CH_2	589.0	–	0.5	1.0	2.5 g/l KCl	10.5	30.0	2.0
Mg	AAS*	Air/CH_2CH_2	285.2	4	0.5	0.6	1 g/l La_2O_3 + 10 ml/l HNO_3	11.8	30.0	2.0
Ca	AAS*	NO_2/CH_2CH_2	422.7	10	0.5	0.6	2.5 g/l KCl	11.8	30.0	2.0
Sr	AAS	NO_2/CH_2CH_2	460.7	10	0.5	–	–	–	1.0	1.0
Li	AES*	Air/CH_2CH_2	441.7	–	0.2	1.0	2.5 g/l KCl	10.5	30.0	2.0
Rb	AES*	Air/CH_2CH_2	780.0	–	0.2	1.0	2.5 g/l KCl	10.5	30.0	2.0

* Flow injection system was used in determinations.

analysed separately as their behaviour may or may not be a group trend not reflected in the body of samples and thus require elimination.

3. Results and discussion

3.1. General

Metal ions in honey are usually determined after ashing. This is a labour-intensive, time-consuming process, so an alternative procedure was sought. To this end, preliminary tests were conducted to determine the metal ion contents in 47 samples of variable origin, using solutions obtained from the ashing residue or prepared by dissolving the honey in 0.1 M nitric acid. The results obtained with both were highly correlated, so we chose to use nitric solutions in subsequent tests.

3.2. Data reduction

We first examined the contents in the different metal ions on an individual basis. The values of the univariate statistical descriptors thus obtained are listed in Table 2. As can be seen, the samples from Hierro differed clearly from the rest; however, there were too few to allow their separate characterization. Also, the Rb contents were highly disperse, with no clear-cut differences between groups. Therefore, Rb contributed little information of use for distinction purposes, so it was excluded from subsequent studies.

Outliers, in multivariate methodology, were identified using the Mahalanobis distance, which is a measure of the distance of each sample in a multidimensional space from the mean centre for the body of samples. All variables except Rb – for the above-described reasons – were used to this end. As can be seen in Fig. 3, three samples exhibited rather a long distance relative to the rest.

Based on the results of the previous univariate and multivariate analyses, the following samples were excluded on account of their low reliability: (a) both samples from Hierro, which differed markedly from all others, (b) the three samples with the longest Mahalanobis distances, which were purchased as Canary but were of dubious origin based on their physical properties (colour and odour) and on the abnormal values they had previously exhibited in parameters other than those studied in this work (e.g. acidity, moisture content) (Fraga et al., 2000) and (c) five samples labelled as eucalyptus honey that differed clearly from those in this group in the preliminary multivariate analysis.

Once the database was purged by deleting outliers, variables were re-typified and multivariate analyses were performed with a twofold purpose, namely: characteriz-

Table 2
Values for metals studied in honey according to their geographical origin

	N	Statistics	Fe (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	K (mg/kg)	Na (mg/kg)	Mg (mg/kg)	Ca (mg/kg)	Sr (mg/kg)	Li (mg/kg)	Rb (mg/kg)
All	116	Mean	4.85	0.37	1.57	1088	70.0	41.0	74.8	0.39	12.18	534
		SD	8.76	0.25	2.22	675	53	31.5	34.2	0.27	15.97	670
		Minimum	0.40	0.10	0.18	214	9.42	7.25	20.7	0.03	0.00	0.00
		Maximum	52.51	1.73	19.1	3166	258	165	193	1.45	110	3193
Tenerife	62	Mean	3.78	0.44	1.65	122	89.6	49.6	74.4	0.37	8.53	606
		SD	7.59	0.28	2.90	724	57.8	33.0	37.5	0.25	8.42	625
		Minimum	0.41	0.13	0.25	214	11.3	7.70	20.7	0.10	0.00	0.00
		Maximum	47.54	1.73	19.1	3166	258	157	193	1.21	35.4	3193
Palma	8	Mean	3.41	0.36	1.18	1353	86.1	35.2	82.0	0.89	11.1	1423
		SD	2.27	0.17	0.46	257	25.3	12.7	24.6	0.35	6.37	603
		Minimum	1.22	0.22	0.74	984	52.4	17.2	33.4	0.44	3.77	338
		Maximum	7.01	0.76	2.08	1664	108	50.5	104	1.45	21.7	2144
Hierro	2	Mean	6.91	0.20	2.43	555	51.8	30.6	57.3	0.33	11.96	228.41
		SD	8.37	0.06	2.40	63.2	8.29	8.67	18	0.07	1.62	323
		Minimum	0.98	0.16	0.74	510	45.9	24.4	44.5	0.28	10.8	0.00
		Maximum	12.83	0.23	4.13	60.0	57.6	36.7	70.1	0.38	13.1	457
Gomera	8	Mean	1.52	0.32	1.89	1022	74.4	38.6	76.0	0.38	9.08	123
		SD	0.30	0.23	1.75	462	31.7	25.6	34.8	0.30	5.61	119
		Minimum	0.98	0.13	0.42	235	35.3	7.25	30.5	0.10	1.19	0.00
		Maximum	1.90	0.87	4.85	1852	129	97.4	152	1.09	15.9	357
Non-Canary	36	Mean	7.64	0.27	1.40	792	32.7	28.4	74.5	0.33	19.41	322
		SD	11.63	0.19	0.81	598	30.6	29.7	31.5	0.17	24.9	660
		Minimum	0.40	0.10	0.18	254	9.42	7.37	27.0	0.03	0.00	0.00
		Maximum	52.51	0.94	4.26	2454	155	165	147	0.90	110	2363

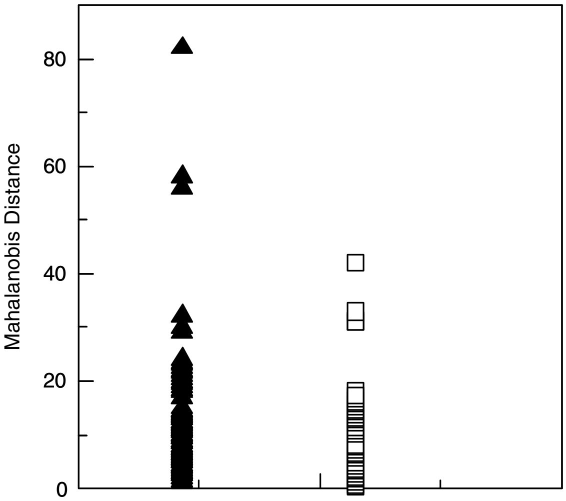

Fig. 3. Mahalanobis distances: ▲, Canary; □, non-Canary.

ing and classifying of the honey samples. Characterization was based on cluster analysis and principal component analysis, and classification on discriminant analysis and logistic regression.

3.3. Characterization of the honey samples

3.3.1. Cluster analysis

Because of its unsupervised nature, cluster analysis is frequently used to screen data for clustering of samples. This method was applied to the purged database (106 samples in all) and the nine variables, using various criteria for the amalgamation rule and similarity measurement. Thus, the hierarchical method, with full bonding or the farthest neighbour, and the squared Euclidian distance, which is less sensitive to outliers, failed to expose distinct groups owing to the high scatter in the data for the non-Canary honey samples. Fig. 4 shows the dendrogram obtained by using the Ward method and the squared Euclidian distance. Although no clear-cut class structure is apparent, the samples clustered into two groups: one on the right comprising mainly Canary samples and the other on the left, including Canary and non-Canary samples. Most of the Canary samples, however, fell on the right. More careful inspection of the samples in each group reveals that the non-Canary samples in the group on the right were mainly labelled "mountain", "holm oak", "linden" or "heather" honey and only one was multifloral. Also, the Canary samples in the group on the left came from specific areas of the islands. Therefore, the botanical factor had a stronger weight than the geographical factor.

A multivariate cluster analysis of variables exposed the presence of two distinct groups: one comprising Fe, Zn and Li and the other consisting of Cu, K, Na, Mg, Ca and Sr. This suggests a potential relationship between these two groups of variables and sample origin (Fig. 5).

3.3.2. Principal component analysis

Principal component analysis was used to search for data trends; in fact, by combining the original variables, this multivariate technique provides a partial view of the data in a space with a reduced number of dimensions while preserving most of their variability. In our case, three principal components (PCs) accounted for 69.07% of the total variance; the first accounted for 39.80%, the second for 15.09% and the third for 14.18%.

As can be seen from Table 3, which lists the loadings of the original variables in the first three PCs, the variables with the greatest weights in the third component also had a substantial weight in the first; this suggests that the two components are closely related, so a plot of the first two PCs should suffice to identify any differences between Canary and non-Canary samples. As can

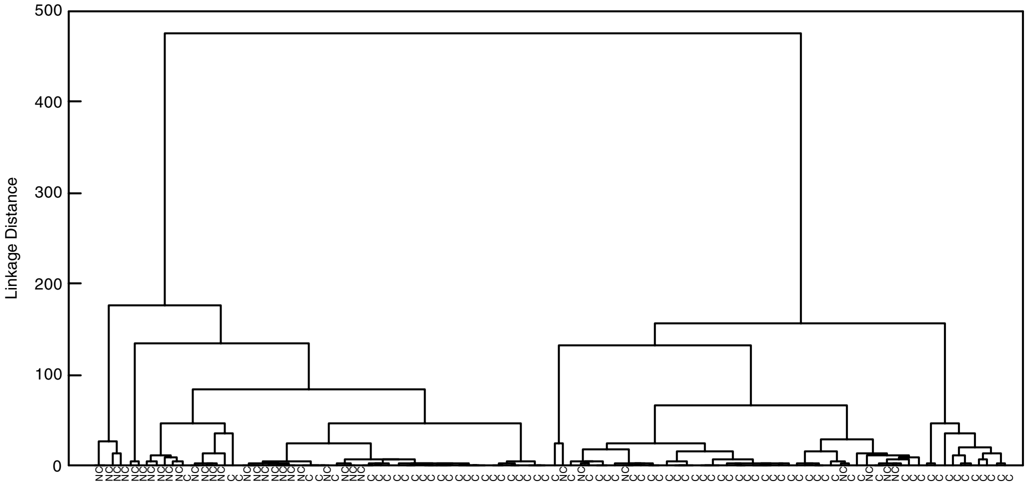

Fig. 4. Dendrogram of cluster analysis (Ward's method). C, canary; NC, non-Canary.

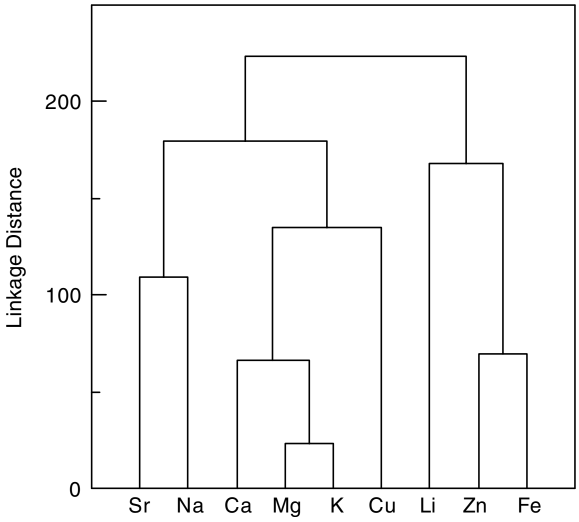

Fig. 5. Dendrogram of cluster analysis for variables.

Table 3
Loadings of variables in the first three principal components

Analyte	Component		
	1	2	3
Fe	−0.036	0.816	0.082
Cu	0.538	−0.047	−0.744
Zn	0.227	0.671	−0.181
K	0.938	−0.092	−0.167
Na	0.620	−0.263	0.471
Mg	0.906	−0.044	−0.271
Ca	0.818	0.055	0.193
Sr	0.651	0.074	0.544
Li	0.252	0.388	0.167

be seen from Fig. 6, a plot of PC1 against PC2, most of the Canary samples were characterized by the former; also, most of a non-Canary origin were – to a lesser extent – characterized by the latter. Therefore, the Canary samples are similar in their Cu, K, Na, Mg, Ca ad Sr contents (PC1), whereas the non-Canary ones are characterized by similar Fe, Zn and Li contents (PC2). As expected from the peculiarities of the Canary Islands, their honey contains larger amounts of the ions in marine aerosol (Na and K) and volcanic soil (Mg and Sr).

A comparison of the variables encompassed by the first two PCs with the groups exposed by the cluster analysis revealed a relationship between such groups and the origin of the samples; in fact, the elements in the groups coincided with those having the greatest weights in the second and first PC, respectively.

As can also be seen from Table 3, PC1 and PC3 were complementary; the variables with the greatest weights in PC3 were also present (with smaller weights, however) in PC1. A plot of PC3 vs PC2 (Fig. 7), again revealed that the second PC characterized the non-Canary samples, whereas the third contributed no new information with a view to further distinguishing samples.

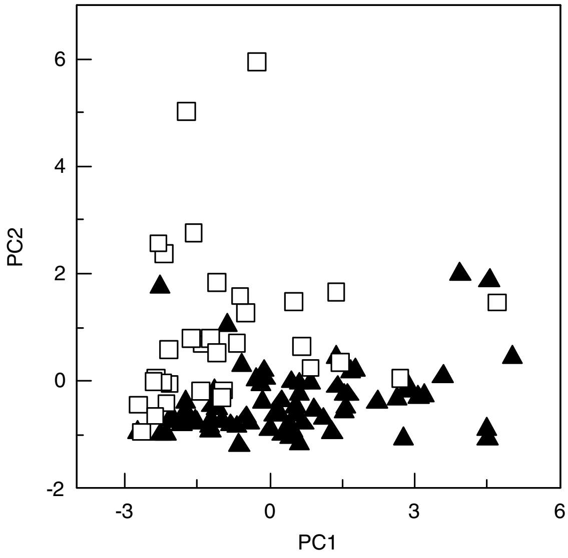

Fig. 6. Score plot of honey samples (PC2 vs PC1): ▲, Canary; □, non-Canary.

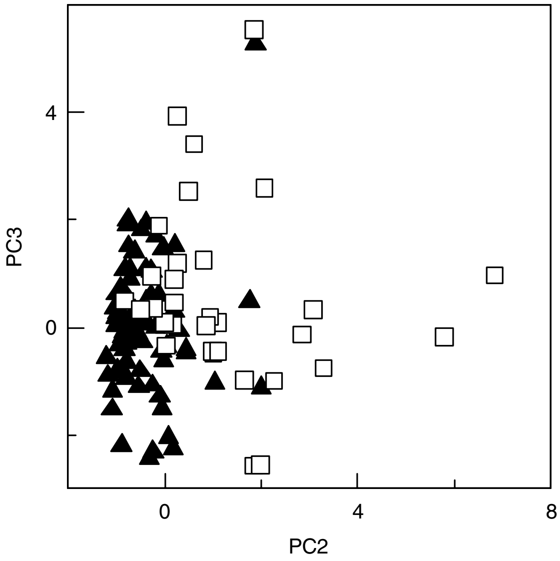

Fig. 7. Score plot of honey samples (PC3 vs PC2): ▲, Canary; □, non-Canary.

3.4. Classification of samples

3.4.1. General classification

Discriminant analysis and logistic regression are two appropriate techniques for statistical processing of data when the dependent variable is categorical (i.e. nominal or non-metric) and the independent variables are metric (Hair, Anderson, Tatham, & Black, 1999). In our case, the dependent variable encompassed two groups or classes, namely Canary honey and non-Canary honey.

3.4.2. Discriminant analysis

The discriminant analysis technique is used to find a theoretical value (i.e. a linear combination of two or more variables) resulting in the best possible discrimination between a priori established groups. Discrimination relies on weighting the theoretical values for each variable in such a way as to maximize between-group variance with respect to within-group variance. Discriminant analysis models comprise sets of equations that are linear combinations of the independent variables, resulting in the maximum possible separation between groups. The number of equations to be used is the minimum between the number of independent variables and that of groups minus 1. The discriminant function for k sample groups and p independent variables will be of the form

$$Y_{ij} = b_0 + b_1 X_{1ij} + b_2 X_{2ij} + \cdots + b_{\mathrm{p}} X_{pij},$$

where Y_{ij} is the value of the discriminant function for case j in group i, X_{kji} is that of the discriminant variable X_k for case j in group i, and b_p are the coefficients ensuring fulfilment of the conditions of the discriminant analysis.

One of the requirements for correct application of discriminant analysis is normality in the variables. The histogram for our typified variables was somewhat non-normal; this, which was confirmed by the Kolmogorov–Smirnov test, required the transformation of the variables Fe, Cu, Zn, Na, Mg, Sr and Li, which exhibited a non-normal distribution. The transformation used was of the logarithmic type, $\ln(x + 1)$, which is recommended for asymmetric distributions exhibiting very small values (Molinero, 2003).

The model was constructed from 74.5% of the honey samples, which were randomly chosen within each group; the remaining 25.5% were used to validate it. Therefore, the learning matrix consisted of 70 samples (55 Canary and 24 non-Canary) and the validation matrix of 10 Canary and 8 non-Canary samples. The discriminant function obtained using all the variables was

$$\begin{aligned} Y_{ij} = {} & -0.553 \times \mathrm{Fe}_{\mathrm{trans}} + 0.861 \times \mathrm{Cu}_{\mathrm{trans}} - 0.059 \\ & \times \mathrm{Zn}_{\mathrm{trans}} + 1.492 \times \mathrm{Na}_{\mathrm{trans}} - 0.061 \times \mathrm{Mg}_{\mathrm{trans}} \\ & + 0.359 \times \mathrm{Sr}_{\mathrm{trans}} - 0.873 \times \mathrm{Li}_{\mathrm{trans}} - 0.075 \times K \\ & - 0.444 \times \mathrm{Ca}. \end{aligned}$$

The Wilks lambda value for the model was calculated to be 0.203 (i.e. significant enough for the discriminant function to allow two distinct groups to be established).

As can be seen from Table 4, the model exhibited good fitting. Only one non-Canary sample in the learning matrix was misclassified, and none in the validation matrix.

Table 4
Recognition and prediction ability for model with discriminant analysis (25% of samples in validation set)

Set	Predicted group		
	C	NC	% correct
Training			
C	55	0	100
NC	1	23	95.8
% global			98.7
Validation			
C	19	0	100
NC	0	8	100
% global			100

3.4.3. Logistic regression

Logistic regression, also known as logit analysis, is a special type of regression used to predict and explain a binary categorical variable (i.e. two groups) instead of a metric dependent measurement. It has the advantage over discriminant analysis that it is less markedly affected by unfulfilment of the basic requisites (particularly normality in the variables). The form of the theoretical value of a logistic regression is similar to that of the theoretical value in a multiple regression. Thus, the theoretical value represents a unique multivariate relation with coefficients similar to those of a regression that are a measure of the relative influence of the predictor (Hair et al., 1999); as a result, they allow the likelihood of a sample falling into a state to be estimated on the basis of various individual characteristics.

Logistic regression is used mainly to analyse dichotomous variables or phenomena. The ensuing model should allow (a) the potential relationship of one or more independent variables (X_j) and the dependent variable to be confirmed or denied, (b) the magnitude of any such relationship to be measured and (c) the likelihood of an event defined as $Y = 1$ to be estimated from the values of the independent variables.

Logistic regression models are formally different from multiple linear regression models; in fact, they are based on the odds ratio, which is ratio of the probability in state 1 to that in state 0:

$$\frac{P(Y=1)}{1-P(Y=1)} = e^{B_0 + B_1 X_1 + \cdots + B_p X_p},$$

e being the base of the natural logarithm, B_0 a constant, B_j ($j = 1,2\ldots,p$) the weights of the predictors included in the model, Y the dependent variable and X_j the independent variables.

If P is taken to be $P(Y = 1)$ and the two sides of the previous function are expressed in logarithmic form, then

$$\ln(P/(1-P)) = B_0 + B_1 X_1 + \cdots + B_n X_n,$$

which is linear and similar to the expression for multiple linear regression.

The model selects the body of variables best predicting changes in odds ratio by using the principles of maximum likelihood for estimation. Unlike multiple linear regression, the logistic regression model does not require

Table 5
Recognition and prediction ability for model with logistic regression (25% of samples in validation set)

Set	Predicted group		
	C	NC	% correct
Training			
C	55	0	100
NC	0	24	100
% global			100
Validation			
C	18	1	94.7
NC	0	8	100
% global			96.3

fulfilment of the normality, homoscedasticity or linearity criteria.

Logistic regression is applied in much the same way as multiple regression: a base model is initially constructed that is used as a reference for comparison. In multiple regression, the base model is established from the mean and sum of squares; in logistic regression, the mean is used to calculate the logarithm of the likelihood rather than the sum of squares.

Our logistic regression model provided a Negerlkerke R^2 value – which is equivalent to the actual Cox and Snell corrected R^2 value (0.707) – of 1, so the model was accurately fitted. The final mean for the fitted model (i.e. the result of the Hoster–Lemeshow test, the null hypothesis for which is equal actual and predicted values) also indicates that fitting was perfect as the null hypothesis was verified at a significance level of $p = 1.000$.

Finally, the goodness of the model was checked by using it to classify the samples employed in the learning and validation processes. As can be seen from Table 5, the results of the fitting were very good: every sample in the learning matrix was accurately classified and only one in the validation matrix (a sample from the Gomera island) was misclassified.

4. Conclusions

In this work, various multivariate techniques were applied to metal ion contents in honey samples of different origin with a view to establishing a potential correlation between the two variables. This can be an appropriate method for detecting fraud, adulteration or faking without the need to use sophisticated techniques.

Based on the results, the multivariate techniques used allow both the characterization and classification of Canary honey samples. Thus, principal component analysis and cluster analysis revealed that Canary samples can be characterized in terms of their Na, K, Sr, Mg, Ca and Cu contents. Also, discriminant analysis and logistic regression allow Canary honey to be distinguished from non-Canary honey on the basis of its metal content.

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