Emerging approach for analytical characterization and geographical classification of Moroccan and French honeys by means of a voltammetric electronic tongue

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Abstract

Moroccan and French honeys from different geographical areas were classified and characterized by applying a voltammetric electronic tongue (VE-tongue) coupled to analytical methods. The studied parameters include color intensity, free lactonic and total acidity, proteins, phenols, hydroxymethylfurfural content (HMF), sucrose, reducing and total sugars. The geographical classification of different honeys was developed through three-pattern recognition techniques: principal component analysis (PCA), support vector machines (SVMs) and hierarchical cluster analysis (HCA). Honey characterization was achieved by partial least squares modeling (PLS). All the PLS models developed were able to accurately estimate the correct values of the parameters analyzed using as input the voltammetric experimental data (i.e. $r>0.9$). This confirms the potential ability of the VE-tongue for performing a rapid characterization of honeys via PLS in which an uncomplicated, cost-effective sample preparation process that does not require the use of additional chemicals is implemented.

Keywords

Analytical methods, Voltammetric electronic tongue, Chemometrics, PLS models, Classification, Food control.

1. Introduction

Honey is a natural and pure substance, produced by *Apis mellifera* bees from the nectar of plants, which the bees collect, transform and leave in honeycombs to ripen and mature (Codex Alimentarius commission, 2001). In the long human tradition, honey has been used not only as a nutrient but also as a therapeutic product, depending on the presence of various antioxidant components, like polyphenols, amino and organic acids, enzymes and proteins (Oryan, Alemzadeh, & Moshiri, 2016). This composition is highly dependent on the floral source, the geographical region of production and with external factors, such as environmental conditions, processing and storage methods (Alzahrani et al., 2012); therefore, the geographical origin play an important role in quality control, which requires monitoring.

In some traditional approaches, the geographical origin of honey was discriminated based on microscopic examination of its pollen (melissopalynology analysis), as pollen in honey reflects the vegetation type where the nectar has been collected by the bees (Corvucci, Nobili, Melucci, & Grillenzoni, 2015). However, in the case of the geographical proximity of the samples, discrimination becomes more difficult (Bogdanov & Martin, 2002a); hence the use of alternative analytical studies for the same purpose.

Several analytical methods were found in literature for the geographical discrimination of honeys, such as mid-infrared spectroscopy (Ruoff et al., 2006), near-infrared spectroscopy (Woodcock, Downey, Kelly, & O'Donnell, 2007), FT-Raman spectroscopy (Corvucci, Nobili, Melucci, & Grillenzoni, 2015), gas chromatography-mass spectroscopy (Karabagias, Badeka, Kontakos, Karabournioti, & Kontominas, 2014), nuclear magnetic resonance (Zheng, Zhao, Wu, Dong, & Feng, 2016), isotope ratio mass spectrometry (Dinca, Ionete, Popescu, Costinel, & Radu, 2015), ultra-performance liquid chromatography (Jandrić, Frew, Fernandez-Cedi, & Cannavan, 2015) and fluorescence spectroscopy (Lenhardt, Bro, Zeković, Dramićanin, & Dramićanin, 2015). Recently, various studies have dealt the physical properties and chemical

composition of honey from different countries (de Sousa et al., 2016; Roshan et al., 2016; Solayman et al., 2016; Mignani et al., 2016).

Generally, all these methods give good discrimination capabilities, accuracy and reliability, but they are bulky, expensive and time-consuming for sample preparation and measurement processes, making them inappropriate for in situ monitoring. In order to overcome these drawbacks, alternative simpler methodologies have been introduced; one of the most important in this field is the electronic tongue as an effective and practical tool in food quality control. Unlike in standard analytical methods, the initial electronic tongues qualitatively analyzed and classified fingerprints of the food products, without quantifying their compounds. However, more recently the application of such devices in the rapid, quantitative determination of food constituents is receiving increasing attention (Peres et al., 2011; Nuñez, Cetó, Pividori, Zanoni, & Del Valle, 2013; Tahri et al., 2015; De Sá, Cipri, González-Calabuig, Stradiotto, & del Valle, 2016). Several sensing approaches can be used in electronic tongues, including electrochemical methods (e.g., potentiometry or voltammetry), optical methods, mass change-sensing techniques based on some principles like quartz crystal microbalances and surface acoustic wave devices (Ha et al., 2015). Voltammetry is often preferred as this technique offers various advantages such as versatility, good sensitivity, simplicity, robustness and good signal to noise ratio (Winquist, 2008).

Some research groups have dealt with the use of electronic tongues for the qualitative analysis of honeys, in particular to discriminate them according to their botanical origin, allowing different monofloral and/or polyfloral samples to be distinguished. (Escriche, Kadar, Domenech, & Gil-Sánchez, 2012; Tiwari, Tudu, Bandyopadhyay, & Chatterjee, 2013). In some cases the discrimination according to their different geographical origins has been addressed too (Wei & Wang, 2014; Bougrini et al., 2016). Recently, a commercial potentiometric electronic tongue (PE-tongue) was applied for botanical classification and

physicochemical characterization of honey samples, by taking as parameters: electrical conductivity, acidity, water content, invert sugar and total sugar (Major et al., 2011). Another PE-tongue with a matrix of five electrodes (Ag, Ni, Co, Cu and Au) was used to differentiate and only predict the total antioxidant capacity of honey samples (Juan-Borrás, Soto, Gil-Sánchez, Pascual-Maté, & Escriche, 2016).

The present work is substantial advancement of our previous study on the use of voltammetric electronic tongue (VE-tongue) system in the detection of adulteration in honeys combined with their geographical and botanical origin classification (Bougrini et al., 2016). Herein, we aimed further to examine the use of this system in the analytical characterization of honey samples, by setting up a reliable, rapid and simple technique, allowing a correlation between the results given by the VE-tongue based on cyclic voltammetry and those resulting from analytical approaches. The interest of using such an approach is that a single measurement with the VE-tongue could be used to obtain quantitative information about key constituents and characteristics of honeys, avoiding the different sample preparation procedures associated to multiple standard analytical methods and, significantly shortening analysis time and cost. The markers used were color intensity (ABS_{450}), the titratable acidity, namely free, lactonic and total acidity, proteins, phenols, hydroxymethylfurfural content, sucrose as well as reducing and total sugars. The interpretation of complex datasets produced by the VE-tongue signals is performed by using multivariate statistics including principal component analysis (PCA), support vector machines (SVMs), hierarchical cluster analysis (HCA) and partial least squares (PLS). As far as the authors know, this is the first time that a VE- tongue has been used to characterize honeys according to various analytical properties; this could be an alternative to the classic analytical methods used so far.

2. Experimental

2.1 Chemicals and reagents

Folin-Ciocalteu reagent was purchased from Solvachim, Morocco. Sodium carbonate, sodium hydroxide, sodium chloride, coper (II) sulfate, potassium sodium tartrate were purchased from Acros organics, Morocco. Bovine serum albumin (BSA) was purchased from Polysciences, Inc. USA. Gallic acid (GAE), 3,5-dinitrosalicylic acid (DNSA), glucose, potassium ferrocyanide, zinc acetate and sodium bisulphite were from Sigma-Aldrich, St. Louis USA. Methanol and hydrochloric acid were purchased from VWR BDH Prolabo, France.

2.2 Honey samples

For this study, 14 polyfloral honeys from the 2016 spring harvest were produced by local beekeepers settled in different geographical areas of Morocco and France (Fig. 1). Honey samples were collected and provided by "Secrets d'Apiculteur" and "Apia" cooperatives based in France and Morocco respectively. All samples were produced by *Apis mellifera* bees, unpasteurized and obtained by centrifugation. All samples were from a matrix of acacia, thyme, eucalyptus and chestnut plants in order to fix the variable of the floral origin and to study just the influence of the geographical origin. Samples were stored at room temperature in the darkness until processing. Analyses were performed less than 6 months after harvesting.

2.3 Analytical characterization

2.3.1 Color intensity: ABS_{450}

The color intensity of honey samples was described by (Beretta, Granata, Ferrero, Orioli, & Facino, 2005). Partly it reflects the content of pigments with antioxidant properties (flavonoids, carotenoids, etc.). Briefly, the honey samples were diluted to 50 % (w/v) in distillated water, heated to 50 °C to dissolve sugar crystals, and filtered through a 0.45 µm filter. The absorbance was determined at 450 nm and 720 nm by *ANACHEM instruments*

UV220 spectrophotometer and the difference in absorbance was expressed as mAU by applying the equation (1):

$$ABS_{450} = (Abs_{450} - Abs_{720}) \times 1000 \quad (1)$$

Where:

Abs_{450}: absorbance at 450 nm;

Abs_{720}: absorbance at 720 nm.

2.3.2 pH, free, lactonic and total acidity

The pH was measured using *Milwaukee pH51* pH meter for a 10 % (w/v) solution of honey prepared in distillated water. Free, total and lactonic acidity were determined by a titrimetric method (Bogdanov, Martin, & Lullmann, 2002b): 10 g of each honey samples (W) were dissolved in 75 mL of CO_2-free water. The solutions were titrated with 0.05 N NaOH until pH 8.3 (V) under magnetic stirring. Immediately, 10 mL of 0.05 N NaOH was added and without delay back titrated with 0.05 N HCl to pH 8.30 (V'). The results were expressed as milliequivalents of sodium hydroxide required to neutralize 1 kg of honey (meq/kg) by applying the following equations (AOAC, 2002):

- $\text{Free acidity} = \frac{V \times 50 \times 1000}{W}$ (2)
- $\text{Lactonic acidity} = \frac{(10 - V') \times 50 \times 1000}{W}$ (3)
- $\text{Total acidity} = \text{free acidity} + \text{lactonic acidity}$ (4)

Where:

W: weight of honey samples;

V: added volume of NaOH;

V': added volume of HCl.

2.3.3 Total protein content

The determination of protein content in honey was performed by the Lowry's assay. Briefly, honey samples (0.1 g) were dissolved in 100 µL of NaOH (2 N) solution and hydrolyzed at 100 °C for 10 min in a boiling water bath. The protein extracts were then added to 1 mL freshly mixed complex-forming reagent [20 mL of 2 % (w/v) sodium carbonate in distilled water + 0.2 mL of 2 % (w/v) sodium-potassium tartrate in distilled water + 0.2 mL of 1 % (w/v) copper sulfate in distilled water] and immediately mixed. After 10 min, 100 µL of Folin-Ciocalteu phenol reagent was added and the samples were thoroughly mixed, then the absorbance of the developed blue color, which depends partly on the tyrosine and tryptophan content was measured at 550 nm. The protein content was determined by comparing to the standard curve of BSA (0‑2000 µg/mL) (Lowry et al., 1951).

2.3.4 Total phenolic content

The total phenolic content was established by the Folin-Ciocalteu method as described in (Saxena, Gautam, & Sharma, 2010). A volume of 2.4 mL of each honey solution (1.25 mg/mL) was mixed with 150 µL of 0.2 N Folin-Ciocalteu reagent. The solutions were thoroughly stirred and incubated for 2 min at ambient temperature. The reaction mixtures were then incubated with 450 µL of sodium carbonate solution (0.2 g/mL) for 2 h at ambient temperature, and the absorbances were measured at 765 nm. A standard curve was prepared using gallic acid (0-400 mg/L). Three replicates were performed and expressed as mg of gallic acid equivalents (mg GAE)/100 g of honey.

2.3.5 Hydroxymethylfurfural content (HMF)

HMF measurement was made in order to evaluate the quality of fresh honey. Generally, this compound was formed during acid-catalyzed dehydration of hexoses and its content increases during heat conditioning used to prevent crystallization or fermentation (Tosi, Ciappini, Re, & Lucero, 2002). The HMF content of different honey samples was

established using the method described by White (White Jr, 1957). For that, 0.5 mL of Carrez solution I (15 g potassium ferrocyanide in 100 mL of water) was added to 5 g of each honey sample and the volume was completed to 25 mL with distilled water. The mixture was added to 0.5 mL of Carrez solution II (30 g zinc acetate in 100 mL of water) and then completed to 50 mL with distillated water. The solutions were then filtered through 0.45 µm membrane filter, and 5 mL of water was added to each remaining filtrate. After this, the solutions were vortexed and the absorbance was read at 284 nm, and 336 nm against a blank aliquot treated with 0.2 % sodium bisulphite, which remove the carbonyl bond in HMF. The HMF content was calculated using the following formula:

$$\text{HMF (mg/kg honey)} = (A_{284} - A_{336}) \times 149.7 \quad (5)$$

Where

A_{284}: absorbance at 284 nm;

A_{336}: absorbance at 336 nm;

149.7: a factor corresponding to the molecular weight of HMF and the mass of the sample.

2.3.6 Reducing and total sugars

The amount of reducing sugars was determined as already described in (Saxena et al., 2010) by using 3,5-dinitrosalicylic acid (DNSA). The honey solutions (0.1 g/mL) were diluted 100 times with distillated water. 1 mL aliquot of these solutions was mixed with 1 mL of DNSA and incubated in boiling water bath during 10 min. After cooling in room temperature, the solutions were mixed with 7.5 mL of distillated water and then the absorbance was read at 540 nm. A calibration curve was obtained by using the standard solutions of glucose. The determination of total sugar was performed by the inversion of sucrose to a reducing sugar and measuring its concentration as previously discussed. Briefly, the honey solutions (0.1 g/mL) were diluted 33 times with distillated water and an aliquot of 1

mL was mixed with concentrated hydrochloric acid to achieve a final concentration of 2 N. Afterward, the mixture was incubated at 68 °C during 8 min in order to complete the reaction of sucrose inversion to a reducing sugar. After cooling, the acidic solution was neutralized by addition of sodium hydroxide, and then the total volume was adjusted to 2 mL with distillated water. Then, 0.5 mL aliquot was taken from the mixture in order to determine the total sugar amount as described above. Measurements were carried out in triplicate. The concentration of sucrose in honey samples was calculated using the equation:

$$\text{Sucrose}\ (\%) = (\text{total sugar} - \text{total reducing sugar}) \times 0.95 \quad (6)$$

A higher sucrose content found in a given honey sample can be associated to overfeeding of honeybees with sucrose syrup, adulteration, or an early harvest of honey, wherein sucrose has not been totally converted into glucose and fructose.

2.4 VE-tongue analysis

In order to accomplish the classification and characterization of honey samples, a VE-tongue was employed as already described in our previous work (Bougrini et al., 2016). The measurements of cyclic voltammetry were performed in a standard three-electrode electrochemical system and in triplicate. The electrode matrix was connected via a relay box to a portable potentiostat (*PalmSens BV*, the Netherlands). A *PSTrace 3.0* software was used to connect the VE-tongue to a measuring computer. The software automatically collects and stores the outputs of the sensors.

2.5 Feature Extraction

Three representative features from the cyclic voltammograms of each sensor were extracted:

- $\Delta I = I_{max} - I_{min}$, the current change as the difference between the cathodic and anodic values of the current ;

- S_{ox}: the maximum slope of the current curve in the oxidation shape;
- S_{rd}: the maximum slope of the current curve in the reduction shape.

Each voltammetric measurement was described by 21 variables, as there were 7 working electrodes inside the array.

2.6 Data analysis and chemometric procedures

The data were analyzed by *Matlab R2010a* software according to the relevant programs. Principal components analysis (PCA), hierarchical cluster analysis (HCA), support vector machines (SVM), and partial least squares (PLS) were used for multivariate statistical modeling of the input data.

Principal component analysis (PCA) was mainly used to achieve a reduction of dimension, i.e., to fit a K-dimensional subspace to the original p-variate ($p > K$) objects and permit a primary evaluation of the between-category similarity. However, in hierarchical cluster analysis (HCA), the squared Euclidian distance and coefficient of similarity were used to group the cases in clusters in terms of their nearness or similarity by using Ward's clustering method. Support vector machines (SVM) was also applied in order to classify different honey samples, by using an algorithm from the machine learning community. This algorithm was developed by (Vapnik, 1998), it determines the hyperplane able to separate two classes and maximizes the distance between the decision plane and the closest samples of the training set, which are called support vectors (SVs). Furthermore, a PLS-toolbox was used to model the relationship between the array of dependent variables Y (voltammetric measurements) and the array of independent variables X (analytical measurements). The aim of PLS is to find the components of the matrix of input (X) that describe, as much as possible, relevant variations in the input variables and at the same time provide the highest correlation with the objectives (Y), giving minor weight to the variations that are irrelevant or relate to noise.

3. Results and discussion

3.1 Analytical characterization

The results of honey characterization based on their analytical parameters are presented in Table 1. The color intensity ABS_{450} is presumed to be associated to pigments (carotenoids, flavonoids etc.), which are also known to have antioxidant properties (Alzahrani et al., 2012). The ABS_{450} values for the samples ranged from 103 ± 3 mAU to 879 ± 2 mAU for H-M and H-S respectively, which explain their light and dark color respectively.

Respecting to pH values, all the analyzed honey samples were found to be acidic in character, with a pH value ranged from 3.4 to 4.9. It has been shown that the pH values of honeys during extraction and storage have a considerable importance, since acidity can influence its stability, texture, and shelf life (Terrab, Recamales, Hernanz, & Heredia, 2004). The free acidity of all honey samples ranged from 4 ± 2 meq/kg to 27 ± 1 meq/kg, thus all honey samples fell within the permitted range of no more than 50 meq/kg of honey proposed by (Codex Alimentarius commission, 2001). The presence of free acids in honey can be explained by the fermentation of sugars by yeasts, during which, glucose and fructose are converted into carbon dioxide and alcohol. In the presence of oxygen, the resulting compounds are hydrolyzed to acetic acid contributing then to the level of free acidity in honey. In addition, the lactone contents ranged from 10 ± 2 meq/kg to 43 ± 2 meq/kg. This variation in acidity among different honey samples can be attributed to the variation in these constituents according to the season of extraction. A simple comparison between lactonic and total acidity revealed that the honey sample with the higher lactone content (H-T) had also the higher total acidity. These observations clearly support the view that lactonic acidity is among the main contributors to the total acidity in honey.

Moreover, in this study the protein content ranged from 516 ± 33 µg/g to 2596 ± 33 µg/g for H-O and H-G respectively, which was determined using the BSA as standard (R^2 = 0.993).

The honey proteins are mainly in the form of enzymes, which were introduced by bees or in some case can be derived from the nectar. Glucose oxidase and catalase were the most important of them, which regulate the production of hydrogen peroxide H_2O_2 that is one of the anti-bacterial factor in honey.

The total phenolic content was determined by a standard curve of gallic acid (R^2 = 0.992). For the honey samples, phenolic content was found to lie in a range between 29 ± 1 mg GAE/100 g of honey and 70 ± 1 mg GAE/100 g of honey related to H-T and H-L samples, which explain respectively, the lower and the higher antioxidant activity.

The presence of HMF, an indicator of the thermal treatment of honey samples, was tested as already described. The HMF content of the fourteen honey samples was found to be in the range of 0.1 mg/kg to 12 mg/kg for H-Py and H-Ch respectively, which is lower than the internationally recommended limit of 80 mg/kg (Codex Alimentarius commission, 2001). This indicates that the studied honeys have not undergone heat treatment during their processing. Further, the reducing and total sugars in honey samples ranged from 34.0 % to 67 % and from 35 % to 72 % respectively. Sucrose levels for every sample were below 5 %, which is the maximum limit prescribed by *Codex Alimentarius*. These results confirm that reducing sugars are the major part of sugars present in honey samples.

2.1 VE-tongue response

A series of tests on the fourteen Moroccan and French honeys were carried out using the seven working electrodes. Fig. S1 (Supplementary material), shows the typical evolution of the signals generated by the silver electrode. Clear response variability was observed in the voltammograms of the electronic tongue due to the differences in the concentration of the electrochemically active compounds of honeys from different geographical origins

The principal component analysis (PCA) which is an unsupervised method was established using 14 samples to determine the capability of the electronic tongue to distinguish between different types of honey from different geographical areas, taking as variable the variation in current ΔI, and the oxidation slope S_{ox} of different voltammograms. Fig. 2 shows a PCA score plot of the measurements performed by the electronic tongue, in which the first three principal components explain nearly 77 % of the total variance. As observed, it is possible to discriminate clearly among the different honey types. The scores plot reveals a separation among all honey samples, which can be attributed to the differences in the chemical composition of honeys according to their geographical origin. According to our previous work (Bougrini et al., 2016) this VE-tongue is able to discriminate the subtle differences in honeys from different geographical areas, regardless the floral origin of honey.

SVMs, which is as supervised classification method, was used to develop the classifier model. Due to the small number samples available, a leave-one out cross-validation procedure was carried out to estimate the true success rate in classification. This assumes that, with the given "n" measurements, the model was formed n times using "n-1" forming vectors. The efficiency of the obtained model was estimated as the medium efficiency over n tests. The confusion matrix shown in Table S1 (Supplementary material) includes information about the actual and predicted classes realized by the SVMs method. It can be observed that every Moroccan and French honeys was correctly identified, leading to a 100 % success rate in the classification of their geographical origin.

In order to confirm the outcome obtained, an HCA method was applied. The dendrogram obtained is shown in Fig. 3. It is important to underline that the branch length in the dendrogram is related to the distances between the various clusters and hence, is a measure of their similarity. In the present study, sample similarities were calculated on the basis of the Euclidean distance and Ward method. Therefore, two similar clusters are

represented by two connected small branches, and so, have a high similarity index. As shown in Fig. 4, each sample of same grade level was closely clustered but fully distinct from each other in the dendrogram, with no misclassifications. Employing a similarity index of approximately 6, fourteen clusters can be visualized. This separation is in good agreement with the PCA results, in which all honey samples are totally distinguished according to their geographical origin.

2.1 PLS analysis

In order to examine if the measurements taken with the electronic tongue could be useful to predict the biochemical and physicochemical parameters, a PLS analysis were carried out. The PLS models were created with the values of analyzed parameters and the voltammetric experimental data obtained from the seven electrodes. Fig. 4 shows the PLS graphs in which measured vs. predicted values of all the analyzed parameters (color intensity, free, lactonic and total acidity, proteins, phenols, HMF, sucrose, reducing and total sugars) are shown by fitting the experimental points to a linear model with three latent variables. Because of the small number of honey samples available, it was not practical to divide them into calibration and validation sets (i.e. to implement a fold validation). Consequently, leave one out cross validations were used for checking models' performance.

A good correlation was observed for most of the analyzed parameters, the best results being for sucrose content with a correlation coefficient (r) of 0.999, then lactonic acidity (0.998), phenols (0.997), HMF content (0.996), total acidity (0.991), reducing sugars (0.988), color intensity (0.983), total sugars (0.982), proteins (0.969). The weaker correlation was observed for the prediction of free acidity (0.906). Therefore, the predicted values obtained using PLS technique, by correlating the features extracted from the VE-tongue responses and the findings of the different parameters estimated via standard analytical methods, showed, in overall, a good correlation among them. These results indicated that this new approach could

be successfully applied as an alternative to standard analytical methods, for several essential parameters quantification in honey samples.

The normalized root-mean-square error (NRMSE) of prediction was calculated for each studied parameters. As shown in Table 2, the VE-tongue system was able to predict all the analyzed parameters with errors that ranged between 0.015 and 0.184. The prediction capacity of the model obtained was also evaluated with the ratio of performance to deviation (RPD), which is the ratio of the standard error in prediction to the standard deviation of the samples. When the RPD value is higher than 2.5 the model has a good ability of prediction (Mouazen & Al-Walaan, 2014). The resulted models for measured parameters have RPD values above this threshold, except for the free acidity (RPD = 2.306), which explain its instability over time.

3. Conclusion

In conclusion, chemometric analysis demonstrated that the VE-tongue system, presented by seven metal electrodes, is capable of differentiating between the fourteen types of Moroccan and French honeys according to their geographical origins. In fact, PCA results explained 76.6 % of the total variance, while SVMs and HCA represented 100 % of success rate. Furthermore, a notable correlation was found between the VE-tongue response and analytical parameters with a correlation coefficient ranging between 0.999 and 0.906 for sucrose and free acids respectively. This finding confirmed that the system based on metallic voltammetric electrodes could be useful as a replacement tool to the traditional analytical methods employed to this regard. However, the results obtained up to here are adequately encouraging as a starting point for the development of new electronic tongue systems for application in the quality control of honey, as well as exhibiting a low cost, continuous usage and low execution time.

Conflict of interest

The authors declare that they have no conflicts of interest.

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Figure captions:

Fig. 1 Location map of the fourteen honey samples.

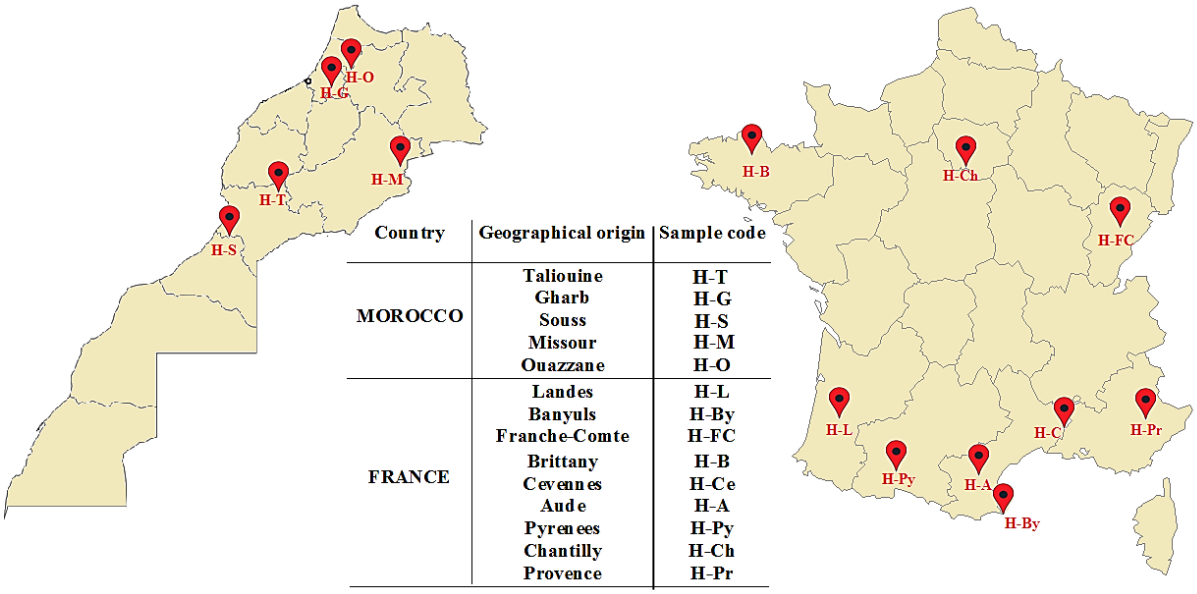
Fig. 2 PCA plot analysis for geographical honey classification by using the VE-tongue system.

Fig. 3 Tree diagram from cluster analysis in Euclidian distance for fourteen kinds of honeys.

Fig. 4 Predicted versus measured values of (a): protein, (b): color intensity, (c): phenol, (d): lactonic acidity, (e): total acidity, (f): free acidity, (g): HMF, (h): reducing sugars, (i): total sugars and (j): sucrose contents given by PLS models with three latent variables.

Figures

Fig. 1



Country	Geographical origin	Sample code
MOROCCO	Taliouine	H-T
	Gharb	H-G
	Souss	H-S
	Missour	H-M
	Ouazzane	H-O
FRANCE	Landes	H-L
	Banyuls	H-By
	Franche-Comte	H-FC
	Brittany	H-B
	Cevennes	H-Ce
	Aude	H-A
	Pyrenees	H-Py
	Chantilly	H-Ch
	Provence	H-Pr

Fig. 2

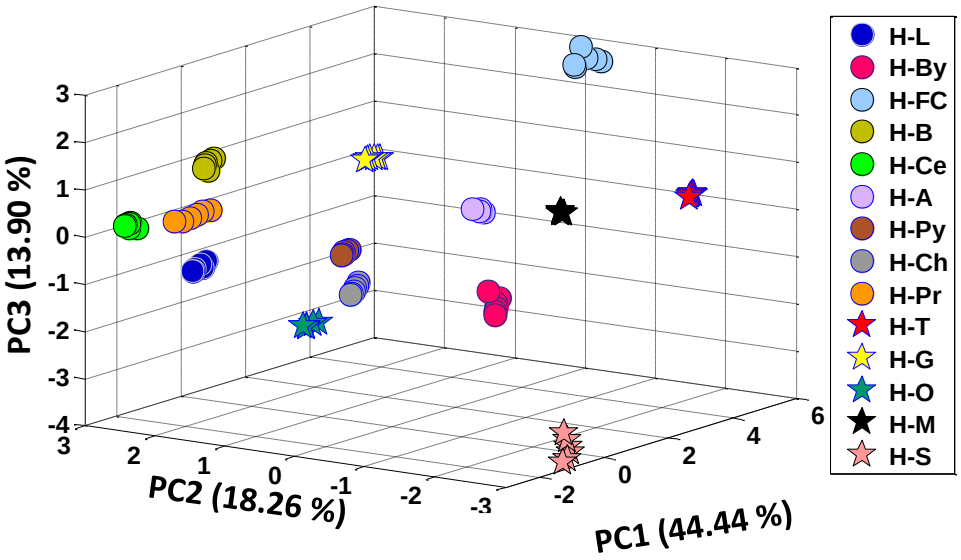

PC3 (13.90 %)
PC2 (18.26 %)
PC1 (44.44 %)
H-L
H-By
H-FC
H-B
H-Ce
H-A
H-Py
H-Ch
H-Pr
H-T
H-G
H-O
H-M
H-S

Fig. 3

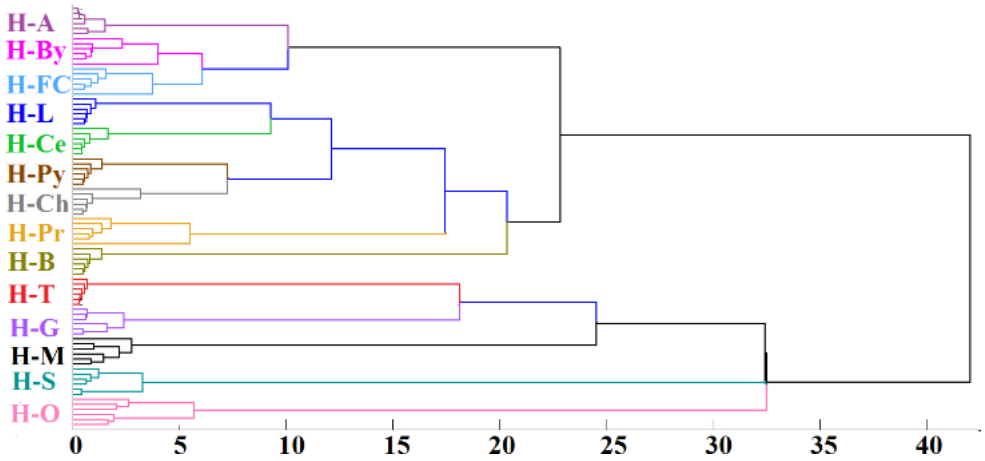
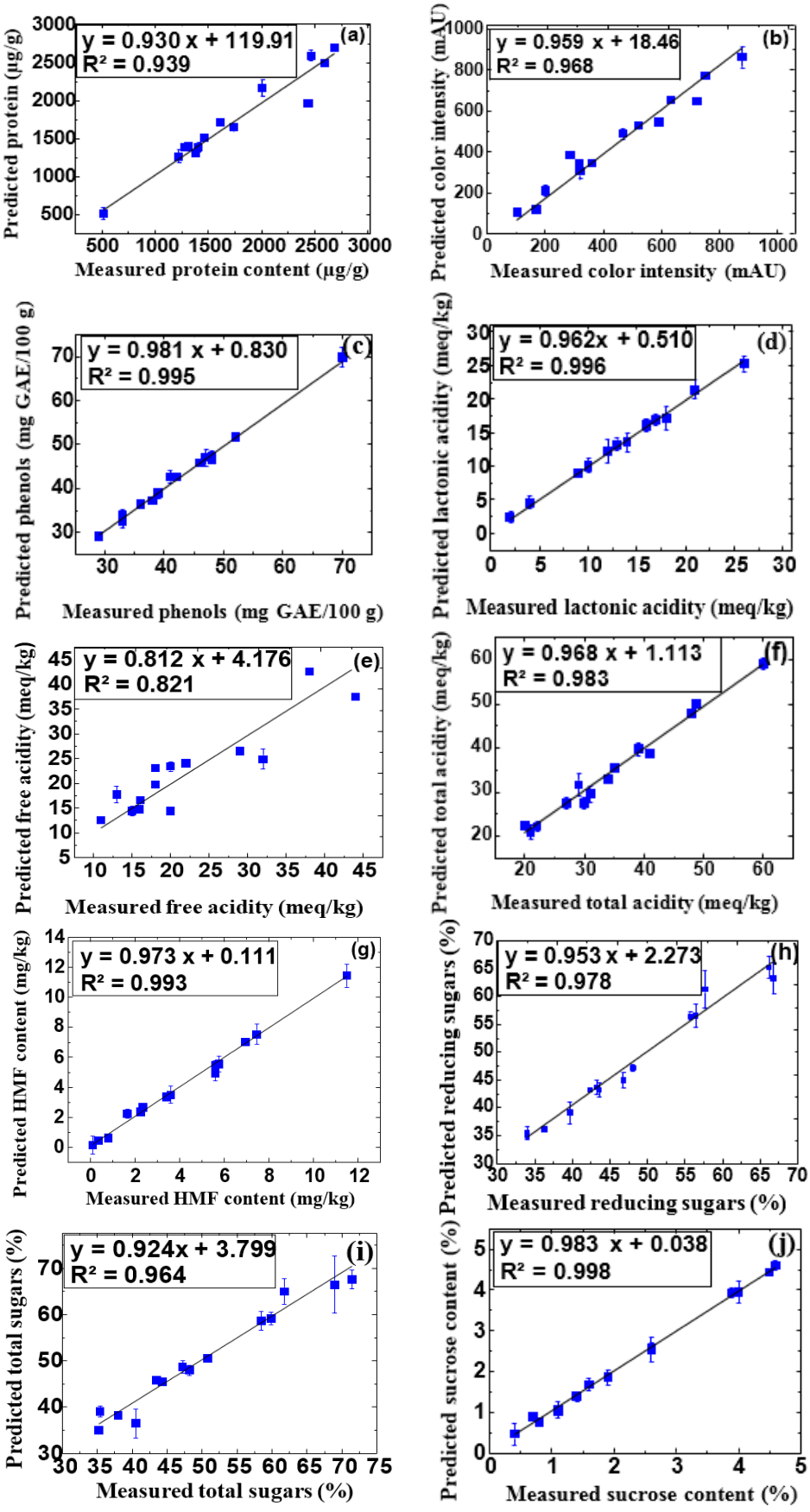

H-A
H-By
H-FC
H-L
H-Ce
H-Py
H-Ch
H-Pr
H-B
H-T
H-G
H-M
H-S
H-O
0
5
10
15
20
25
30
35
40

Fig. 4


(a)
y = 0.930 x + 119.91
R² = 0.939
Predicted protein (µg/g)
Measured protein content (µg/g)
(b)
y = 0.959 x + 18.46
R² = 0.968
Predicted color intensity (mAU)
Measured color intensity (mAU)
(c)
y = 0.981 x + 0.830
R² = 0.995
Predicted phenols (mg GAE/100 g)
Measured phenols (mg GAE/100 g)
(d)
y = 0.962x + 0.510
R² = 0.996
Predicted lactonic acidity (meq/kg)
Measured lactonic acidity (meq/kg)
(e)
y = 0.812 x + 4.176
R² = 0.821
Predicted free acidity (meq/kg)
Measured free acidity (meq/kg)
(f)
y = 0.968 x + 1.113
R² = 0.983
Predicted total acidity (meq/kg)
Measured total acidity (meq/kg)
(g)
y = 0.973 x + 0.111
R² = 0.993
Predicted HMF content (mg/kg)
Measured HMF content (mg/kg)
(h)
y = 0.953 x + 2.273
R² = 0.978
Predicted reducing sugars (%)
Measured reducing sugars (%)
(i)
y = 0.924x + 3.799
R² = 0.964
Predicted total sugars (%)
Measured total sugars (%)
(j)
y = 0.983 x + 0.038
R² = 0.998
Predicted sucrose content (%)
Measured sucrose content (%)

Tables:

Table 1 Comparative analytical parameters of analyzed honeys

Sample code	ABS_{450} (mAU)	pH	Free acidity (meq/kg)	Lactonic acidity (meq/kg)	Total acidity (meq/kg)	Proteins (µg/g)	Phenols (mg GAE/100 g)	HMF (mg/kg)	Reducing sugars (%)	Total sugars (%)	Sucrose (%)
H-L	362 ± 2.2	4.4	11 ± 0.9	10 ± 1.4	41 ± 1.2	1409 ± 49.9	70 ± 0.6	6.9 ± 0.2	55.7 ± 0.8	59.9 ± 0.4	4.0 ± 0.2
H-By	200 ± 2.6	4.1	7 ± 0.9	15 ± 0.9	22 ± 1.6	2209 ± 82.2	48 ± 0.6	5.6 ± 0.5	66.8 ± 1.1	71.5 ± 0.4	4.5 ± 0.4
H-FC	320 ± 4.1	4.0	9 ± 0.9	21 ± 0.9	31 ± 0.9	2436 ± 32.7	41 ± 0.8	1.6 ± 0.2	57.7 ± 0.6	61.8 ± 0.2	3.9 ± 0.2
H-B	522 ± 3.9	3.8	18 ± 1.2	17 ± 0.4	36 ± 1.6	1743 ± 18.9	33 ± 0.8	0.8 ± 0.3	43.3 ± 1.1	48.2 ± 0.7	4.6 ± 0.2
H-Ce	285 ± 2.2	4.7	15 ± 0.9	16 ± 0.3	30 ± 1.1	1463 ± 49.9	52 ± 1.0	3.4 ± 0.1	46.8 ± 1.5	47.2 ± 0.2	0.4 ± 0.7
H-A	316 ± 1.4	3.8	27 ± 0.9	14 ± 0.8	21 ± 2.1	2463 ± 82.2	33 ± 1.0	2.2 ± 0.6	34.0 ± 1.1	35.4 ± 0.3	1.4 ± 0.4
H-Py	753 ± 1.3	4.7	12 ± 1.6	28 ± 1.7	40 ± 3.1	1316 ± 32.7	46 ± 0.8	0.1 ± 0.1	34.0 ± 0.8	35.2 ± 0.5	1.1 ± 0.2
H-Ch	724 ± 5.9	4.2	13 ± 0.9	20 ± 0.9	34 ± 1.6	1276 ± 32.7	48 ± 2.2	11.5 ± 0.4	43.6 ± 0.9	44.3 ± 0.3	0.7 ± 0.3
H-Pr	592 ± 1.3	4.8	10 ± 0.8	24 ± 3.3	34 ± 2.4	1383 ± 37.7	36 ± 0.5	3.6 ± 0.3	39.7 ± 0.6	40.5 ± 0.4	0.8 ± 0.1
H-T	634 ± 1.3	3.4	17 ± 0.9	43 ± 1.9	59 ± 2.5	1609 ± 18.9	29 ± 0.6	0.3 ± 0.5	36.3 ± 0.7	38.0 ± 0.8	1.6 ± 0.1
H-G	169 ± 1.7	3.8	4 ± 1.6	18 ± 1.6	22 ± 2.8	2596 ± 32.7	42 ± 1.7	2.3 ± 0.6	42.4 ± 0.6	43.5 ± 0.7	1.1 ± 0.1
H-S	879 ± 1.3	3.7	17 ± 0.9	33 ± 0.1	49 ± 1.0	1223 ± 37.7	39 ± 0.8	5.6 ± 0.3	48.1 ± 0.9	50.8 ± 0.3	2.6 ± 0.3
H-M	103 ± 2.6	4.9	5 ± 0.5	16 ± 0.0	21 ± 0.5	1143 ± 18.9	38 ± 0.8	5.7 ± 0.5	66.2 ± 1.0	69.0 ± 0.3	2.6 ± 0.4
H-O	469 ± 0.0	3.6	10 ± 0.5	39 ± 1.9	50 ± 2.4	516 ± 32.7	47 ± 0.8	7.4 ± 0.1	56.4 ± 1.4	58.5 ± 0.6	1.9 ± 0.4

Table 2 PLS model performance for the measured parameters

Parameters	r*	NRMSE**	RPD***
Sucrose	0.999	0.015	2.797
Lactonic acidity	0.998	0.089	5.130
Phenols	0.997	0.031	7.658
HMF	0.996	0.125	6.070
Total acidity	0.991	0.057	5.584
Reducing sugars	0.988	0.048	4.592
Color intensity	0.983	0.103	4.949
Total sugars	0.982	0.063	3.637
Proteins	0.969	0.093	3.791
Free acidity	0.906	0.184	2.306

r*: coefficient of correlation; **NRMSE****: normalized root mean square error; **RPD*****: ratio of performance to deviation.