Ruben Ramirez

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Honey

Geographical Origins,
Bioactive Properties and
Health Benefits

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HONEY

GEOGRAPHICAL ORIGINS, BIOACTIVE PROPERTIES AND HEALTH BENEFITS

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HONEY

GEOGRAPHICAL ORIGINS, BIOACTIVE PROPERTIES AND HEALTH BENEFITS

RUBEN RAMIREZ

EDITOR

NOTICE TO THE READER

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CONTENTS

PREFACE

According to its chemical composition, honey is a complex mixture of over 70 ingredients, which enter honey in a variety of ways. Not only do the different types of honey differ, but the honey within each species differs in its composition depending on its herbal and geographical origin, climatic conditions, the type of bees, and the work of the beekeeper. In this book, Chapters One provides an assessment of geographical origins of honey by the use of chemometrics. Chapter Two presents the profile of physicochemical parameters of 40 samples of the black locust honey from Krapina-Zagorje region monitored by the season. Chapter Three examines the prebiotic and probiotic properties of honey. Chapter Four gives an updated review of traditional and modern uses of honey. Chapter Five explore the actual knowledge on honey's properties, in terms of cellular and molecular effects induced by the bee's products on skin care and wound management.

Chapter 1 – The assessment of geographical origin of honey represents high interest around the world, because is related to the authenticity analysis of raw materials and labeled products. The cost of honey in the international market is strongly associated to the geographical origin, so is very important to find the newest and more trustworthy methods to determine geographical provenance. On the other hand, chemometric includes statistical tools which have been successfully used to determine the geographical origin of several foods, inlcuding honey samples. The geographical classification of honey can be obtained by means of the combination of chemometrics with different analytical methods to analyze both organic and inorganic compounds, which can include major, minor and trace elements, amino acids profile or other components. For that, this chapter covers the last reported chemometric

methods used to classify honey according to geographical origin, as well as the description of the analytical methods used as matrix data.

Chapter 2 - According to its chemical composition, honey is a complex mixture of over 70 ingredients, which enter honey in a variety of ways. Not only that the different types of honey differ, but the honey within each species differs in its composition depending on its herbal and geographical origin, climatic conditions, the type of bees, and the work of the beekeeper.

A number of European countries have the national legislation and the reference methods used for local quality control, but they are often specific for a particular country and cannot always be applied in the commercialization of honey for the international sales. Therefore, the profession in accordance with expert associations – such as the International Honey Commission (IHC) – seeks to harmonize the reference methods and standards for proving the authenticity of botanical origin of monofloral type of honey.

Leading experts involved in this issue combine melissopalynological determination of the type and number of pollen with sensory analysis and physicochemical analysis such as determining the water content, HMF (hydroxymethylfurfural), electrical conductivity, diastasis activity, pH values, and the sugar content (fructose, glucose, sucrose, erlose, raffinose, melesitose)

The purpose of this paper is to present the profile of physicochemical parameters of 40 samples of the black locust honey from Krapina-Zagorje region monitored by the season. Morevoer, it will point out their differences within each testing season.

Botanical origin of all the samples, as declared by the manufacturer, was confirmed using the melissopalynological analysis-black locust. By conducting pollen analysis in the samples of black locust honey during both seasons pollen grains of 36 plant species were found. The most common are those of the family of *Robinia* (black locust family), *Rosaceae* (rose family), *Brassicaceae* (cabbage family), and *Fabaceae* (bean family)

Every tested sample meets the requirements set by the Regulations on the quality of monofloral honey regarding physicochemical parameters: water (15,21-19,28%); free acid (6,1-14,00 mEq/1000g); electrical conductivity (0,08-0,22 mS/cm); reducing sugars (61,57-71,20 g/100 g); sucrose (0,10-2,58 g/100g); diastase (7,15-17,19 DN) and HMF (0,97-11,20 mg/kg). The analysis of variance gained an insight into the significance of differences (p-values) within the physicochemical parameters for water and electrical conductivity given the season black locust honey is from (p < 0,05).

Based on the results obtained, the following can be concluded:

- The confirmation of botanical origin of all the honey samples, as declared by the manufacturer, was conducted using
  - melissopalynological or pollen analysis,
  - and by confirming the conformity of physicochemical parameters (water, free acid, electrical conductivity, reducing sugars, sucrose, diastase and HMF)
- All the tested samples meet the requirements of the Regulations on the quality of unifloral honey
- Using data analysis of variance an insight has been gained into the significance of differences (p-values) among physicochemical parameters – water and electrical conductivity – given the season that black locust honey comes from ($p < 0.05$).

Chapter 3 - Laboratory studies have shown that honey contains olygosaccharides and low molecular weight polysaccharides exhibiting prebiotic properties. Like the well-known commercial prebiotics the honey oligosaccharides are not digested in the upper part of gastrointestinal tract but are fermented by beneficial microflora in large intestine of human and animals and stimulate its growth and vital activity. It is emphasized that prebiotic properties of honey depend on its plant origin. It is shown that fresh honey also contains probiotics - the microorganisms beneficial for human and animals that inhibit the growth and development of pathogenic and conditionally pathogenic microflora, and can also be a source of biologically active substances with antimicrobial activity. Bifidobacteria and lactobacilli inhabiting honey stomach can survive in honey within 2-3 months after its harvest. The microflora composition of honey stomach and fresh honey may depend on the botanical origin of honey, as well as habitat and subspecies of honey bees. The probiotic microorganisms are involved in the development of honey bee resistance to adverse environmental factors directly inhibiting the growth of pathogens, and stimulating components of the immune system. Antagonistic activity of probiotic bacteria against a broad spectrum of pathogenic microorganisms enable their application for prophylaxis and treatment of honey bee diseases, and in human and veterinary medicine.

Chapter 4 - Honey has been primarily used as a natural sweetener as well as a famous component of traditional medicine in several ancient civilizations to treat various ailments. It is still a fundamental component of traditional forms of medicines throughout the world. The use of honey as an alternative and complementary medicine namely apitherapy has led to a recent renewed interest amongst consumers. Concomitantly, several studies have established the beneficial effects of honey. Its antioxidant capacity has been well

established and attributed to its polyphenolic content namely flavonoids and phenolic acids. Additionally, the use of honey in wound healing in medical setting has been linked to its antimicrobial potential. Other therapeutic properties of honey including its anti-inflammatory, anti-ulcerous, anti-cancerous and antiviral effects have been identified. While scientific evidence of the medicinal properties of honey is rapidly growing, the present review attempts to summarize some of the recent therapeutical potential of honey based on current *in vitro*, *in vivo* and clinical studies. Additionally, some proposed mechanisms of action of honey are overviewed herein.

Chapter 5 - Honey has a long and intriguing history that is intertwined with the evolution of man and his culture.

Today we know honey mostly for its characteristic food uses, but in our history honey has been used for other purposes, as a natural remedy to cure wounds and injuries. Besides, its role as an anti-bacterial compound, honey possess some interesting properties providing an opportunity for honey to be re-established as a broad-spectrum agent for human use.

In this chapter, the authors explore the actual knowledge on honey's properties, in terms of cellular and molecular effects induced by this bee's products on skin care and wound management.

Chapter 1

ASSESSMENT OF GEOGRAPHICAL ORIGIN OF HONEY BY CHEMOMETRICS

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ABSTRACT

The assessment of geographical origin of honey represents high interest around the world, because is related to the authenticity analysis of raw materials and labeled products. The cost of honey in the international

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market is strongly associated to the geographical origin, so is very important to find the newest and more trustworthy methods to determine geographical provenance. On the other hand, chemometric includes statistical tools which have been successfully used to determine the geographical origin of several foods, inlcuding honey samples. The geographical classification of honey can be obtained by means of the combination of chemometrics with different analytical methods to analyze both organic and inorganic compounds, which can include major, minor and trace elements, amino acids profile or other components. For that, this chapter covers the last reported chemometric methods used to classify honey according to geographical origin, as well as the description of the analytical methods used as matrix data.

Keywords: honey, geographical origin, chemometrics

LIST OF ABBREVIATIONS OF CHAPTER

AAS	Atomic Absorption Spectrometry
ANN	Artificial Neural Network
ANN-MLP	Artificial Neural Network Based on the Multilayer Perceptron
ANOVA	Analysis of Variance
AVQANN	Vector Quantization Classification Systems Artificial Neural Networks
BA	Bayesian Analysis
CA	Cluster Analysis
CART	Classification And Regression Trees
CDA	Canonical Discriminant Analysis
CCA	Canonical Correlation Analysis
CE	Capillary Electrophoresis
DA	Discriminant Analysis
D-PLS	Discriminant Partial Least Squares
ETAAS	Electrothermal Atomic Absorption Spectroscopy
FDA	Forward Discriminant Analysis
FTIR	Fourier Transform Mid Infrared Spectroscopy
FTIR-ATR	Fourier Transform Mid Infrared Spectroscopy by Attenuated Total Reflectance
GC×GC–TOF-MS	Two Dimensional Gas Chromatography–Time of Flight Mass Spectrometry

CG/IRMS	Gas Chromatography/Isotope Ratio Mass Spectrometry
GC-MS	Gas Chromatography Mass Spectrometry
GFAAS	Graphite Furnace Atomic Absorption Spectrometry
GPA	Generalized Procrustes Analysis
HCA	Hierarchical Cluster Analysis
HMF	Hydroxymethylfurfural
HPLC	High Performance Liquid Chromatography
HS-SPME	Head-Space Solid-Phase Microextraction
ICP-AES	Inductively Coupled Plasma Atomic Emission\ Spectroscopy
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
INAA	Instrumental Neutronic Activation Analysis
IRMS	Isotope Ratio Mass Spectrometry
KNN	k-Nearest Neighborhood
LC/IRMS	Liquid Chromatography/Isotope Ratio Mass Spectrometry
LDA	Linear Discriminant Analysis
LogR	Logistic Regression
LVQ-ANN	Learning Vector Quantization Artificial Neural Networks
MALDI TOF MS	Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry
MANOVA	Multivariate Analysis of Variance
MLF-ANN	Multilayer Feed-Forward Artificial Neural Networks
MLP	Multilayer Perceptron Network
NIR/NIRS	Near-Infrared Spectroscopy
NMR	Nuclear Magnetic Resonance
NNA	Neural Network Analysis
OPLS-DA	Orthogonal Partial Least Squares Discriminant Analysis
PCA	Principal Components Analysis
PDO	Protected Designation of Origin
PGI	Protected Geographical Indication
PLS-DA	Partial Least Square Discriminant Analysis
PLSR	Partial Least Square Regression
RF	Random Forests
SDA	Step-Wise Discriminant Analysis
SIMCA	Soft Independent Modelling of Class Analogy

SPA-LDA	Successive Projections Algorithm-Linear Discriminant Analysis
SPME	Solid Phase Micro Extraction
SPA-LDA	Successive Projections Algorithm-Linear Discriminant Analysis
SNIF-NMR	Site-Specific Natural Isotopic Fractionation Measured by Nuclear Magnetic Resonance
SNV	Standard Normal Variate
SVM	Support Vector Machine
TXRF	Total Reflection X-Ray Fluorescence Spectrometry
UPLC-QToF MS	Ultra-Performance Liquid Chromatography-Quadrupole Time of Flight Mass Spectrometry
VE-tongue	Voltammetric Electronic Tongue
VQBCP-ANN	Vector Quantization Based Classification Procedure Artificial Neural Networks

INTRODUCTION

The authentication of geographical origin in honey samples is an important goal for food quality control, since the prices in the international honey market can vary strongly according to the geographical origin. On the other hand, honeys labeled as bulk honey –that mean, without label- will have low prices in comparison to identified honeys, because they are used mainly in the food industry as raw material (Bogdanov & Gallmann, 2008). However, in several countries, foodstuffs -including honey- are identified by their location through regulatory rules, as the Protected Geographical Indication (PGI) and the Protected Designation of Origin (PDO) (Consonni & Cagliani, 2010).

The search of the determination of the geographic origin in terms of fingerprints is an interesting area in chemometric classification, because the measurement of few variables on honey samples will not be enough to provide information about geographical origin. For this reason, authentication of honey samples requires, besides of the selected analytical methods, the use of multivariate tools to be reached. Several pattern recognition techniques have been widely applied in food chemistry as criterion for geographical identification of honey. The most frequently used chemometric tools, include not supervised methods such as principal component analysis (PCA), cluster analysis (CA), factor analysis (FA), etc; as well as supervised methods, linear discriminant analysis (LDA), sequential discriminant analysis (SDA), partial

least square discriminant analysis (PLS-DA), support vector machine (SVM), artificial neuronal networks (ANN), *k*-nearest neighborhood (KNN), etc. On the other hand, several analytical methods have been used to obtain the data matrix; these methods include the study of physcochemical parameters and instrumental analysis as gas chromatography mass spectrometry (GC-MS), atomic absorption spectroscopy (AAS), inductively coupled plasma atomic emission spectroscopy (ICP-AES), high performance liquid chromatography (HPLC), electronic nose, near infrared spectroscopy (NIRS), etc. For this reason, this chapter is divided according to the type of studied compound and the corresponding analytical methods, as well as the chemometric tools used to identify the geographical origin of honey.

ASSESSMENT OF GEOGRAPHICAL ORIGIN OF HONEY

Elemental Analysis

The elemental profile determined in honey samples is frequently used to obtain information for classification according to the geographical origin. In some cases, using the concentration of a few elements, it is possible to obtain good classification models. Such is the case for the geographical classification of honey from Galicia (Spain), where the concentrations of Mn, Li and Cu - determined by atomic absoprption spectroscopy (AAS)- were enough to reach a correct classification. The multivariate chemometric techniques applied were, principal components analysis (PCA), linear discriminant analysis (LDA) and *k*-nearest neighborhod analysis (KNN): honeys from Galicia were satisfactory classified from other regions (Latorre et al., 1999). In other work, authors included different multivariate tools to classify honey from the same region: PCA, cluster analysis (CA), Bayesian analysis (BA), partial least square regression (PLSR) and neural network analysis (NNA); the metal contents were employed as original data, reached similar results to those obtained previously (Latorre et al., 2000). Then, the characterization of Galician honey was performed using electrothermal atomic absorption spectroscopy (ETAAS) analyzing 13 metals (including the concentrations of Ca, Cd, Cr, Cu, Fe, K, Li, Mg, Mn, Na, Ni, Pb and Zn) in 40 honey samples from two different places of Galicia: polluted and unpolluted regions. The chemometric tools were: PCA, CA, LDA, KNN, soft independent modelling of class analogy (SIMCA), multilayer feed-forward artificial neural networks (MLF-ANN), accurate vector quantization classification systems artificial

neural networks (AVQ-ANN), vector quantization-based classification procedure artificial neural networks (VQBCP-ANN) and learning vector quantization artificial neural networks (LVQ-ANN). The results obtained by PCA and CA showed a good classification for both regions. Also, the different supervised procedures had a recognition ability ranging from 70 to 100%, and prediction ability from 71.8 to 100 % for polluted and unpolluted regions, respectively (García et al., 2006).

In the Canary Islands, honey samples were characterized and differentiated from other regions, based on their metal content obtained by ETAAS and using PCA, CA, discriminant analysis (DA) and logistic regression (LogR) as chemometric tools. The Canary Islands samples were characterized by mean of the Na, K, Sr, Mg, Ca and Cu concentrations: authors inferred that Canary honey could be distinguished from non-Canary honey basing on these elements. DA and LogR showed the best performance and could be useful to detect fraud or adulteration of denomination of origin (Hernandez et al., 2005). Other Spanish honey samples with protected designation of origin (PDO) called "Miel de Granada" -a highly quality product that includes 6 monofloral honeys and two multifloral honeys- were characterized by the determination of 5 elements (K, Na, Ca, Mg and Zn). The elemental data were analyzed by CA and ANOVA, obtaining correct classification of honeys according to their botanical and geographical origin. ANOVA showed significant differences among rosemary honeys from different geographical areas despite the botanical factor weight (De Alda-Garcilope et al., 2012).

In Argentina, the classification of honey from La Pampa was carried out based on their elemental composition. The chemometric tools used for the geographical classification were PCA, CA and LDA. The elemental study was carried out by inductively coupled plasma atomic emission spectrometry (ICP-AES) analyzing 6 elements (P, Fe, Al, Ca, Mg and Na) in 32 multifloral honey samples. The results showed the geographical discrimination of samples in 2 groups: capital samples (next to Santa Rosa, the capital city) and non capital samples (more than 50 km from Santa Rosa city). The major difference between both groups was the content of phosphorous: capital samples had a higher concentration of phosphorous than the non capital samples: PCA score plot and dendogram of cluster analysis, showed two well-defined groups. By LDA the training and validation set of samples gave a 100% of correct results (Camiña et al., 2008). Other honey samples from Argentina were clasified, analyzing 14 trace elements (Br, Ce, Co, Cr, Cs, Eu, Fe, La, Rb, Sb, Sc, Sm, Th and Zn) in 120 samples of light-coloured honeys, using instrumental

neutronic activation analysis (INAA). The elemental composition was used in multivariate statistical analysis to discriminate honeys according to geographical origin. Good results were obtained by using the element analysis combined with LDA for discriminating honeys by regions: the elemental composition is not dependent of harvest year. Eight key variables (Ce, Cr, Cs, Fe, La, Sb, Sc and Zn) were identified by stepwise-LDA providing the maximum discrimination between samples according to their provenance (Pellerano et al., 2012). Then, Argentinean honeys from other three important producing region of Argentina (Córdoba, Buenos Aires and Entre Ríos) were analyzed to develop a reliable fingerprint system to asses their geographical procedence based on elemental and isotopic signatures. This study included multielemental and isotopic data of soils, honey and waters from these regions. The multielemental composition was determined by ICP-MS. $\delta^{13}C$ was measured by isotopic ratio mass spectrometry and the $^{87}Sr/^{86}Sr$ ratio was determined using thermal ionization mass spectrometry. The chemometric analysis of the results showed that honey samples were differentiated by classification and reegression trees (CART) and LDA 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 (CCA) and generalized procrustes analysis (GPA) showed 91.5% of agreement between soil, water and honey samples, in addition to clear differences between studied areas (Baroni et al., 2015).

In Italy, the concentrations of K, Ca, Mg, Na, Al, Zn, Fe, Mn, Cu, Cr, Ni, Se, Pb, Cd, and As in 39 honey samples from different botanical (orange-blossom, lemon-blossom, chestnut, eucalyptus, acacia, sulla and wildflower honeys) and geographical (Sicily and Calabria, Italy) origins were determined by ICP-AES and ICP-MS. The most abundant minerals decreased in the following order: K (mean value: 1.57 mg g^{-1}) >Ca (0.2 mg g^{-1}) >Mg (0.121 mg g^{-1}) >Na (0.092 mg g^{-1}). Mineral content showed differences in honey samples from different geographical origin despite the botanical factor weight and therefore, could be used as a tool to assess the traceability of honeys. The discrimination between Sicilian and Calabrian honeys was achieved by PCA, and also the results of Canonical Discriminant Analysis (CDA) indicate that the 100% of total samples were correctly classified (Di Bella et al., 2015).

The anthropogenic influence in the trace element composition of honeys from Latvia was demonstrated in a study realized in 2009/2010. In this work, acidified water solutions of honey samples were analyzed by atomic absorption spectrometry (AAS) and inductively coupled plasma mass spectrometry (ICP-MS). It was possible to quantify 5 major elements (Ca, Fe,

K, Mg, Na) and 15 trace elements (Al, As, Ba, Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb, Rb, Sr, V, Zn). Results showed slight differences between the selected regions taking into account that the regions selection was based on the available geochemical information. Some regional distinctions were detected in the elemental content of honey, mainly concerning heavy metals. However, it was determined that the elemental profile in honey samples was not strongly connected with geochemical background, but was more influenced by the consequences of anthropogenic activities (Vincevica-Gaile et al., 2012).

Fifteen elements (Al, B, Ba, Ca, Cd, Cr, Cu, K, Mg, Mn, Na, Ni, Pb, Sr, and Zn) were determined in 140 honey samples from six regions in Poland by ICP-MS. The honeys consisted in three types: honeydew, buckwheat and rape honey. This study revealed that chemometric analysis appears as useful tool for detection of authenticity in honey samples. Using the profile of mineral content it was highlighted the relationship between the elements distribution and geographical origin. In order to discriminate honey samples the chemometric methods used were CA, PCA, LDA and CART, showing that the content of Al, Mg and Zn can be considered the markers of geographical origin of Polish honeys (Chudzinska & Baralkiewicz, 2011). Then, honeys and bee products produced in Poland from different botanical origins (acacia, buckwheat, apple, chestnut, lime, dandelion, orange, rape, honeydew, multifloral, and heather) as well as syrup-feed honeys, honeys with natural additives such as chokeberry, cinnamon, pollen and propolis, were analyzed by graphite furnace absorption atomic spectroscopy (GFAAS). Fourteen elements (Ca, Mg, K, Na, P, Co, Mn, Fe, Cr, Ni, Zn, Cu, Cd, and Pb) were determined, from which only 9 (Ca, Mg, Na, K, P, Zn, Cu, Fe, and Mn) were considered for multivariate analysis, because the levels of trace elements (Pb, Cd, Cr, Ni, and Co) were too low or below the limits of detection. It was demonstrated by using PCA and CA that the botanical and geographical provenance as well as technological processing, had strong influence on the elemental composition of honeys (Grembecka & Szefer, 2013).

Twelve minerals were determined in 200 samples of the black locust (*Robinia pseudoacacia*) honey from five Croatian regions during two seasons. The average macroelement concentrations were K (205.57-428.05 mg kg^{-1}), Ca (33.53-329.00 mg kg^{-1}) and Na (23.34-218.04 mg kg^{-1}). Melissopalynological and physicochemical analysis of honey confirmed the botanical origin. PCA showed that Al (honeys from Bjelovar-Bilogora), Fe (honeys from Bjelovar-Bilogora and Istria), Cu (honeys from Eastern Croatia), and K (honeys from Istria) were the characteristical elements for the black locust honey of each region (Uršulin-Trstenjak et al., 2015).

In Brazil, honeys from Bahia region were geographically classified based on their mineral and trace content (a total of 8 elements including Na, K, Ca, Mg, Cu, Fe, Mn and Zn) using PCA and CA. The results obtained by CA showed 3 groups: semiarid region, transitional forest zone and Atlantic forest zone. However, by PCA only two groups were obtained: Atlantic forest zone and semiarid forest zone, while for the transition forest zone, honey samples appeared indistinctly in both groups, indicating that CA could be the most reliable method to classify these type of samples (Santos et al., 2008). In other work, 57 samples of honeys were collected from different regions of Brazil. Forty-two elements were determined (Al, Cu, Pb, Zn, Mn, Cd, Tl, Co, Ni, Rb, Ba, Be, Bi, U, V, Fe, Pt, Pd, Te, Hf, Mo, Sn, Sb, P, La, Mg, I, Sm, Tb, Dy, Sd, Th, Pr, Nd, Tm, Yb, Lu, Gd, Ho, Er, Ce and Cr) by inductively coupled plasma mass spectrometry (ICP-MS). Three data analysis techniques were evaluated to discriminate origin of samples: multilayer perceptron network (MLP), support vector machine (SVM) and random forests (RF). The classification was established separating one subset of samples for training and another for testing, using cross-validation method: 10 different cross-validations criteria were applied to each subset of selected samples. This was repeated 20 times for each algorithm for a total of 200 times for each algorithm and for each subset of samples: it was demonstrated that a subset consisting of only 5 chemical elements (Pb, Tl, Pt, Ho and Er) identified on the basis of the application of two feature selection algorithms, were enough to provide good classifications. The best results –in term of accuracy in classification- were obtained when MLP algorithm was used (Batista et al., 2012). In other report, heavy metals (Pb, Cd and Cr) were determined in honeys from Paraná (Brazil) using GFAAS. PCA was applied to characterize honey samples according to their levels of the trace elements which were also related to the geographical origin. The mean concentrations for the elements followed the order Pb > Cr > Cd. It could be verified that honey samples were geographically separated, especially with regard to Pb and Cd contents. Thus, honey can be considered a bioindicator of environmental contamination, suggesting possible contamination in soil, water and air, which can be related to natural or anthropogenic sources present in the studied regions (de Andrade et al., 2014).

A recent report effectuated on samples collected from two localities in the Eastern Rhodopes Mt. (Bulgaria) was unable to find similar levels in macro and microelement compositions of honeys from the same geographical origin. The studied elements were K, Ca, Mg, P, As, Cd, Co, Cr, Cu, Fe, Mn, Na, Ni, Pb, and Zn, determined by ICP-AES. In general, it was observed that different

elements had different concentrations in honey from the same botanical type even collected from the same geographical region, same locality and same beehive but in different vegetation season (Atanassova et al., 2016).

Capillary electrophoresis (CE) electrochemical method in combination with PCA, was proposed for the classification of honey by geographical origin based on cations determination in honey samples. Capillary electrophoresis is a family of electrokinetic separation methods performed in narrow silicon capillaries. Forty honey samples from Brazil were analyzed to test the proposed method. These samples were dissolved in deionized water and filtered before injection. Potassium, Na^+, Ca^{2+}, Mg^{2+} and Mn^{2+} were determined using Ba^{2+} as internal standard. The CE with UV detector for cation analysis performed in real samples was compared statistically with ICP-MS methodology obtaining good results and reliability. The analytical data suggest a significant influence of the geographical origin on the mineral composition (Maria Rizelio et al., 2012).

Amino Acid Profile and Proteins

The amino acid profile can be useful as original data to obtain geographical classification of honey. The free amino acid composition determined by HPLC, combined with PCA and CA methods, were applied for the geographical characterization of honey from 3 regions of Cordoba province in Argentina. CA and PCA models showed a low ability for the geographical differentiation of honey from the North, Centre and South of Cordoba province. Although the origins of the honeys were different, samples with similar surrounding flora, had similar amino acids profile, indicating that these compounds can be strongly affected by the botanical origin (Cometto et al. 2003).

Moreover, the geographical origin of commercial honey from Hawaii (USA) and other countries, was carried out by determining the fingerprints of honey proteins by matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI TOF MS). The mass spectra profile of 16 commercial samples from Hawaii and 22 commercial honey samples from different countries were analyzed by PCA: the scores plot showed the ability of the model to distinguish Hawaiian honeys from the rest ones, recognizing 15/16 samples. The results showed that the MALDI TOF MS combined with PCA can be an alternative method for the identification of honeys from Hawaii (Wang et al., 2009).

Physicochemical Parameters

Physicochemical analysis performed in honey, ususally not provide reliable information to be used in chemometrics, because these parameters present similar ranges for different honeys, independently of the geographical or botanical origin. However, it have been shown that the variations in compositon of honeys characterized by physicochemical parameters (fructose, glucose, sucrose, pH, total flavonoids, total phenolics, hydrogen peroxide concentration, mineral contents) can influence on the antimicrobial activities against drug multiresistant bacteria and pathogenic fungi (Al-Waili et al., 2013; Kuś et al., 2016).

The characterization of Argentinian honeys from two regions of Buenos Aires province was performed by PCA, using color, free acidity, pH and moisture. Three types of honey were studied: monofloral, mixed and polyfloral honeys. PCA scores plot showed the grouping of two regions from Buenos Aires province (East and Centre regions) using as variables moisture, color, pH and free acidity (Fangio et al., 2010). In other report, melisopalynological and physicochemical analysis was performed on 58 honey samples collected from 5 different geographical environments of the Jujuy province (Argentine) through the application of CCA, PCA and LDA. Forty-five per cent of honeys were monofloral; nearly half of them correspond to native species, among which stood out *Salix humboldtiana*, *Baccharis sp.* and *Ziziphus mistol*. CCA characterized significantly honeys from four different origins using all pollen data. By forward stepwise coupled to LDA, 14 significant variables were identified to build a discriminatory model with three significant discriminant functions, reaching a cumulative variance of 94%; then, 86% of the samples were correctly classified. PCA and LDA applied to physicochemical data allowed to distinguish three different groups with a significant function ($p < 0.01$) that explained 90% of the total variability. Honey corresponding to Subandean Hills of Santa Bárbara was the most accurately classified by pollen and physicochemical data (Rios et al., 2014). Other interesting alternative contribution to the geographic classification of Argentinean honey samples, was proposed by using image analysis: a conventional flow-batch system with a simple webcam to capture digital images was employed. In this methodology, analytical information is generated from color histograms obtained from the digital images employing different color models: RGB (red-green-blue), HSB (hue-saturation-brightness) and grayscale. Three chemometric tools were employed for geographic origin classification: projections algorithm-linear discriminant analysis (SPA-LDA), SIMCA and

PLS-DA. The proposed method demostrated to be a good alternative to be used in quality control laboratories for the classification of honey samples according to their geographical origin (Domínguez et al., 2014).

The combination of physicochemical parameters (pH, free, lactonic and total acidity, electrical conductivity, moisture, ash, lactonic/free acidity ratio and colour parameters), as well as phenolic compounds profile (quercetin, myricetin, kaempferol, chrysin, and syringic acid) determined by HPLC, were used to classify 35 thyme honeys from four different regions in Greece (Irakleio, Hania, Kefalonia and Lakonia). Multivariate analysis of variance (MANOVA) and LDA reached out to 91.2% of correct prediction according to geographical origin of samples (Karabagias et al., 2014).

In Brazil, the geographical characterization of honey by using physicochemical analysis and chemometric tools, was performed in 31 wild honey samples of different mesoregions from Paraná State. The results obtained by PCA indicated that pH, electrical conductivity and hydroxymethylfurfural (HMF) were key parameters to identify the geographical origin of wild honeys from South, Centre, Southeast and West mesoregions suggesting that these regions had different climatic and topological conditions, great floral diversity and different honey processing types (Kuchla et al., 2015).

Volatile Compounds

Mass Spectrometry

The volatile profile can provide important information referred to geographical origin, depending of the type and concentration of analyzed compounds. Based on that, honey samples from Corsica were studied to obtain the discrimination of Corsican honeys -with PDO- from those of other European countries, using PCA and ANN. The type and concentration of the volatile compounds present in honey were obtained by head-space solid-phase microextraction (HS-SPME)-based procedure, coupled to comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry (GC×GC–TOF-MS). By PCA, the score plot showed a low ability for the classification of geographical origin: Corsican and non-Corsican samples were not correctly grouped. However, when neural network analysis based on the multilayer perceptrons (ANN-MLP) was applied, good results were obtained, with prediction and classification abilities of 94.5% and 96.5% respectively. Thus, the method distinguished Corsican honeys (PDO region) from honeys of other

European countries, which can be useful for authenticity analysis (Cajka et al., 2009). After that, new pattern recognition tools were applied on the same analytical data to obtain a new models as SIMCA, discriminant partial least squares (D-PLS) and SVM. The results showed that the range of efficiency and specificity of the proposed models were acceptable (60-91.5 and 42.9-90.5 % respectively) while the sensibility range was optimal from 86.7 to 97.8%. The main conclusion of this study is that the volatile profiles of Corsican honeys allowed their discrimination from honeys of different European origins. In general, all models, except SIMCA, showed good efficiency and specificities, with high sensitivity, independently of year of sampling, which did not affect the classification (Stanimirova et al., 2010).

A methyl derivatization method for $\delta^{18}O$ analysis of individual carbohydrates by gas chromatography isotope ratio mass spectrometry (GC/IRMS) was proposed to improve the $\delta^{18}O$ analysis. The method was tested on honey samples from 23 different countries, which contain glucose and fructose as the only two individual carbohydrates in significant amounts. Other compounds such as amino acids or vitamins may influence the $\delta^{18}O$ values of honey bulk material and therefore have an effect on the relationship to $\delta^{18}O$ values of individual carbohydrates. The proposed method showed not only high accuracy and precision for $\delta^{18}O$ analysis of various individual carbohydrates, but also have an excellent performance test for origin and authenticity of honeys if comparing the results with the $\delta^{18}O$ values of water source and climatic data of the region of production (Lehmann et al., 2016).

Electronic Nose

An electronic device -known as electronic nose- was used to determine the geographical origin of honeys samples from Slovenia, by the determination of volatile compounds and aroma profile. The electronic nose device is a detector unit containing an array of 22 different semiconductor sensors, which can detect volatile compounds without identify them specifically. Multivariate analysis was carried out by PCA on 49 honey samples, in order to determine differences among volatile profiles in samples with the same botanical origin, but with different geographical origin. PCA showed that honey samples from close regions were grouped together, but those from geographically distant regions showed differences, independently of the botanical origin, indicating that the method was useful to identify honey from Slovenia (Cacic et al., 2009).

Sugars

Electronic Tongue

In voltammetric measurements, an electrical current is measured between the working electrode and the counter-electrode, when a voltage pulse is applied among reference and working electrodes. A set of pulses can be put together to form a pulse train in order to extract as much information as possible from the solution. When the potential is applied, electro-active compounds that react to that potential can produce oxidation reduction (redox) reactions and therefore, a current will can be measured. In voltammetry of electronic tongue, data are collected over the whole pulse and not only at the end of the pulse, as in traditional electrochemistry. The electronic tongue creates a data matrix which can be analyzed by multivariate methods. The electronic tongue consisted of four working electrodes made of the metals gold, iridium, platinum and rhodium, an Ag/AgCl reference electrode and a stainless steel counter-electrode (Ivarsson et al., 2005).

Voltammetric electronic tongue (VE-tongue) was employed to classify honey samples from different geographical and botanical origins. Furthermore, VE-tongue was used to detect adulterants such as glucose syrup and saccharose syrup in honey. The data obtained were analyzed by three-pattern recognition techniques: PCA, SVM, and hierarchical cluster analysis (HCA). These methods enabled the classification of 18 honeys of different geographical origins in Morocco. Therefore, this simple method based on VE-tongue could be useful for quality control in the honey industry (Bougrini et al. 2016). Other VE-tongue developed at Zhejiang University with 6 metallic working sensors was used to analyze 4 types of honeys from different floral origins (acacia, buckwheat, data, and motherwort) and 4 types of acacia honeys from different geographical origins. Multivariate statistical data analysis techniques such as PCA and LDA were used to classify honey samples, showing good potential for classification. In addition, 3 regression models obtained by PCR, PLSR, and SVMs were applied for prediction category. These regression models exhibited a clear indication of the prediction ability for geographical origin of honey samples (Wei & Wang, 2014).

Mass Spectrometry

Promising results have been obtained by analytical techniques that combine liquid chromatography (LC) and mass spectrometry. An untargeted metabolomics approach was applied for the classification of honey of

various floral and geographical origins using ultra-performance liquid chromatography-quadrupole time of flight mass spectrometry (UPLC-QToF-MS). Evaluation of data by multivariate data analysis allowed the discrimination of New Zealand and Australian honeys from those produced in other countries. It was also possible to differentiate between mono- and polyfloral honeys within one region, as well as between some monofloral honeys of various geographical origins. Characteristic markers which contributing to the discrimination of different honey types, were selected (Jandrić et al., 2015).

Other interesting technique is the hyphenation of liquid chromatography/isotope ratio mass spectrometry (LC/IRMS). In this case, the sugar fractions of honey (fructose, glucose, di- and trisaccharides) were separated by LC and subsequently chemically oxidized to carbon dioxide using an interface specifically designed for this purpose. The carbon dioxide is separated from the eluent with a selective gas-permeable membrane and transferred into the IRMS for $\delta^{13}C$ measurement using helium as a purge and carrier gas. A reference database of authentic honeys was built up in order to set purity criteria and limit values for authentic honeys. This method was useful as screening technique (qualitative method) and was applied on 79 commercial honey samples from various geographical origins. The procedure demonstrated to have advantages over existing methods in terms of analysis time and sensitivity. In addition, it was the first isotopic method developed that allowed the detection of beet sugar addition (Cabañero et al., 2006).

Gas chromatography/isotope ratio mass spectrometry (GC/IRMS) and LC/IRMS techniques were compared to assess enrichment levels for glucose standards. For GC/IRMS measurements the derivatization correction and the conversion of carbohydrates into CO_2 had a considerable effect on the measured $\delta^{13}C$ values. However, the authors did not find significant differences in the accuracy of the two techniques over the full range of natural $\delta^{13}C$ abundances and ^{13}C-labelled glucose. The difference in the performance of GC/IRMS and LC/IRMS diminished when the $\delta^{13}C$ values were measured in natural samples, because the chromatographic performance and background correction became critical factors, particularly for LC/IRMS. Even though the derivatization of carbohydrates for the GC/IRMS method was complete, LC/IRMS demonstrated to be the best technique due the simpler sample preparation and straightforward isotopic calibration (Moerdijk-Poortvliet et al., 2015).

Undefined Compounds

Infrared Spectroscopy

Infrared spectroscopy is an spectroscopic technique which can bring information about honey and can be useful as classificatory data analysis. The geographical origin of honeys from different countries around the world, was carried out on 125 filtered and 167 unfiltered honey samples: Ireland, Mexico, Spain, Hungary, Argentina and the Czech Republic. Samples were analyzed by NIRS and data analysis was performed by PCA, LDA, SIMCA and D-PLS. The second derivative of transflectance spectra were used as original data and the study of false positive was included. For unfiltered samples (from Ireland, Mexico and Spain) using SIMCA, the classification showed a correct prediction ranging from 94.4 to 96%, but with a high range percentage of false positive (from 0-100%). This problem was solved by means of D-PLS, which showed a 100% correct prediction and 0% of false positives. Similar results were obtained with the filtered honey samples (from Argentina, Hungry, Ireland and the Czech Rep.): by SIMCA, the classifications ranged from 91.7 to 100%, but with high percentage of false positives. By D-PLS2, a correct classification from 96 to 100% and 0% of false positives were obtained. The scores plot for PCA and LDA showed good classification of honey samples (3 groups for unfiltered and 4 groups for filtered honey samples). Due to the good fit of models for geographical prediction, the method was useful to classify filtered and unfiltered honey samples from the studied countries (Woodcock et al., 2007).

Fourier-transformed infrared spectroscopy by attenuated total relectance (FTIR-ATR) was used as instrumental data for the classification of honey samples from several countries of Europe and America (Czech Republic, Ireland, Hungry, Argentina and Mexico). Data were preprocessed by derivative spectra methods and analyzed by PLSR, forward discriminant analysis (FDA) and SIMCA. Correct predictions were obtained by PLSR (93.3%) and FDA (94.7%). However, the best classification was achieved using SIMCA (100%), but it showed very high false positive percentages (Hennessy et al., 2008).

In several works, NIRS was applied to study Corsican honeys. In the first place, NIRS spectra in combination to PCA and PLSR were used to determine fingerprints of Corsican honeys. Three hundred seventy three samples were analyzed, of which 219 were Corsican and 154 non-Corsican honeys. Applying PCA, it was found a large amount of overlap between Corsican and non-Corsican honey samples. However, reducing variables and using second

derivates spectra data, D-PLS regression gave a correct classification of samples ranging from 81.2 to 84.0%. Data processing by jack-knife uncertainty testing was used before D-PLS, improving the results from 86.8 to 90.9%. By using the separate calibration and validation steps the correct classified samples ranged from 84.9 to 88.8%, but using separate calibration and validation sample sets after jack-knife uncertainty testing, correct classification ranged from 87.7 to 94.1%. The use of the jack-knife uncertainty test in NIR spectral data combined to D-PLS was an adequate method for the study of provenance of Corsican honey samples (Woodcock et al., 2009). This study was then expanded for honey and edible oil: NIRS was applied to Corsican honeys which were classified using another family of chemometric tools, namely class-modeling techniques: SIMCA, UNEQ and POTFUN. The predictive ability of the model was performed by assessment of 3 parameters: sensibility, specificity and efficiency. For sensibility, the best results found correspond to the UNEQ method (78.4-94.6%) in comparison to the POTFUN (73.0-86.5%) and SIMCA methods (59.5-70.2%). For specificity, the best results were for SIMCA (87.0-100%) in comparison to POTFUN (69.6-87.0%) and UNEQ (69.6-82.6%); but for efficiency the 3 methods were comparable between them (from 77.1-84%). Based on these results, SIMCA was not considered appropriate because it presented high specificity but low sensibility. The specificity of POTFUND and UNEQ were comparable, but UNEQ had the best range of sensibility: for this reason it was the best class-modelling technique for assessment of Corsican honeys (Olivieri et al., 2011). Then, IR spectroscopy was applied again to Corsican honeys. In this case, FTIR-ATR combined with FDA and PLSR were used. IR spectra were pretreated with standard normal variate (SNV) and first- and second-derivative data. In this work 373 honeys were studied, corresponding to honey seasons 2004-2006, from Corsica and other European countries (Ireland, Italy, Austria, Germany and France). The analysis by FDA considered by year harvest, shows that the prediction ability was dependent on pretreatment data and harvest year; the average classification ability ranged from 67 to 86%; the analysis of all harvests shows a prediction result from 72 to 83%. A similar analysis was performed by PLS-DA, by assessment of ability prediction, considering the individual or total harvest. The analysis by harvest showed a prediction ability (average) from 67 to 86%, while the total harvest showed a prediction ability from 81 to 89%. The method can be useful to confirm the provenance of Corsican honeys, but also to recognize that the IR spectrum of these honeys do not represent a fingerprint itslef in comparison with the rest of the countries (Hennessy et al., 2010). Finally, an interesting review

can be consulted on the principal markers used in the discrimination of the geographical and floral origin of honey and the standard spectroscopic techniques used, such as NIR or FTIR. These techniques are of non-invasive nature and widely applied to the assessment of honey origin (Cavaco et al., 2012).

Multiple Organic Compounds

Nuclear Magnetic Resonance Spectroscopy

[1]H Nuclear magnetic resonance (NMR) has been used to determine geographical origin of honey from Argentina, Hungary and Italy. The applied multivariate tools were PCA and hierarchical projection to latent structures discriminant analysis. The results show the correct classification of acacia and multifloral honey by means of a PCA scores plot. Also, the geographical classification was made by a scores plot of D-PLS, which showed the classification of multifloral samples in three groups from Argentina, Italy and Hungary, indicating the efficiency of the D-PLS as classification tool when NMR data were used (Consonni & Cagliani, 2010).

In China, honey samples from 8 botanic origins, three geographical origins, and five harvest years were analyzed by ^{1}H NMR. Thirty-three components in honey samples were detected and identified, and 20 of them were accurately quantified by internal standard method with relaxation time correction. PCA was used to classify honeys from different botanical and geographical origins. The variations of chemical compositions in honeys, including saccharides, amino and carboxylic acids, confirmed their clustering according to their origins in PCA scores plots. NMR data for the different pairwise honey samples analyzed by Orthogonal partial least squares discriminant analysis (OPLS-DA) allowed to geographical discrimination and identify the compositional variations due to long storage time. Hence, NMR spectroscopy coupled with chemometric techniques offered an efficient tool for quality control of honey (Zheng et al. 2015). On the other hand, a worldwide collection of more than 800 authentic honeys were analyzed through ^{1}H NMR spectroscopy, covering most of the economically significant botanical and geographical origins. In this work, an untargeted profiling of substances contained in the honey and their concentrations was performed and compared with respective compound spectra. Honeys showing an 'untypical' ^{1}H-NMR profile -transgression of typical concentration ranges found for authentic honeys or detection of syrup marker compounds- were considered as

non-authentic or adulterated honey samples respectively. Chemical plant nectar markers can be used to check monofloral honey labeling. Spectral patterns and natural variability were established for multifloral honeys. In addition, chemical markers for sugar syrups were identified by comparison with a commercial dataset. Although the results are qualitative, experiments with fortified samples have confirmed the ability of the method to detect sugar addition up to 10% in favorable cases (Spiteri et al., 2015). For the purspose of improve the detection levels for adulterants, NMR profiling has been complemented with other analytical methods like stable isotope analysis and mass spectrometric for the screening of adulteration markers, in order to achieve reasonable detection levels from 5- to 10% of foreign sugars (Lolli et al., 2008).

In Romania, isotope ratio mass spectrometry (IRMS) was combined with site-specific natural isotopic fractionation measured by nuclear magnetic resonance (SNIF-NMR) for the purpose of establish potential origin markers for quality assessment and classification of honeys. Data on $\delta^{18}O$, and $\delta^{2}H$ of honey measured by IRMS together with the stable isotopes ($\delta^{13}C$ and D/H) in ethanol from fermented honeys were reported, demonstrating that the use of $\delta^{13}C$ value as single parameter to differentiate honey floral variety is not feasible. Accordingly, a particular emphasis was put on the isotopes from ethanol extracts which were specific to a given botanical origin of honey, and its potential in characterizing the substances was highlighted for both assignments of origin and detection of adulterants (Dinca et al., 2014).

Multiple Organic and Inorganic Compounds

It is common to use several combined analytical methods to obtain the much information as possible about the honey classification system. For that, in Argentina the regional classification of honey samples from north and south of country was carried out, based on the analysis of 12 physicochemical parameters and 10 metals on 75 honey samples, using step-wise discriminant analysis (SDA) and KNN analysis as chemometric tools. By conventional KNN analysis, using 15 variables (8 physicochemical parameters and 7 metals) the best model produced a 83% of correct classification. However, the use of SDA as variable selection tool previous to KNN, produced a model using only 6 original variables, with a 99% correct geographical classification. This reduction of variables implied that some variables were masked or cancealed by the discriminatory power of others (correlated variables)

avoiding an over-estimation of model (Baroni et al., 2009). Then, the assessment of geographical origin of honeys from the seven different regions of Buenos Aires province (Argentina) were characterized by combination of the results from their antioxidant capacity (DPPH, FRAP), total phenolic content (TPC), mineral composition, colour and ash. Honeys showed significant differences among their antioxidant capacity (DPPH), ash, colour and mineral content. Good antioxidant activity, low amounts of Cu and Zn (<1.0–1.5 and 0.7–1.8 mg kg^{-1}, respectively) and significant Pearson's correlations (r) among the different parameters were found in the samples. LDA allowed the classification of honeys in their original groups with a prediction success of 98%. The results suggest that honeys could be correctly classified by their geographical origin through their TPC, colour, ash and mineral concentrations (Patrignani et al., 2015).

Also, honey samples from two regions of Madrid province were successfully classified by pollen analysis, physico-chemical parameters and volatile compounds, associated with PCA and SDA. The study was carried out on 46 honey samples and was possible to distinguish honey from mountain areas to those of plain ones. However, a big variability was founded because the different honey sugar sources (honeydew or nectar) (Soria et al., 2004).

In Slovenia, 122 honey samples were classified from four different Slovenian regions: Pannonnian, Alpine, Dinaric and Mediterranean regions. The analytical determinations included physicochemical parameters, elemental content determined by total reflection X-ray fluorescence spectrometry (TXRF) and IRMS. The multivariate tools used in this work were PCA and LDA: good results were obtained in both cases, with a correct geographical classification from 87.5 to 100%. The method can be useful for the verification of the geographical origin of Slovenian honey (Kropf et al., 2010).

In Pakistan, researchers performed the study of physicochemical parameters, metal contents an pollen in 15 honey samples from this country. Determination included density, apparent viscosity, moisture, pH, total soluble solids, free acidity, conductivity, HMF and ashes. Also, the content of Li, K, Na, Fe, Bi, Mn, Ni, Co, Cu, Cd and Hg was analyzed by AAS. The samples were divided into foreign bee honey, local branded honey and unbranded local honey. Using PCA, the results show a good discrimination between the three different types of samples, by means of three first principal components (PCs) in the score plot (Nasiruddin Khan et al., 2006).

The composition of three types of honey (mixed forest, rapseed and monofloral-black locust honeys) produced in the vicinity of an industrial town (Košice, Slovak Republic) was compared. In this report metals and metabolites

were studied and data analysis was discussed with available literature. Higher content of trace elements including those considered non-essential in forest honey (1358.6 ng g^{-1} Ni, 85.6 ng g^{-1} Pb, and 52.4 ng g^{-1} Cd) found in rapeseed and black locust honeys, confirmed that botanical origin rather than the distance for eventual source of pollution (steel factory) affects heavy metals deposition. Benzoic acid derivatives were typically more accumulated in forest honey, but cinnamic acid derivatives (as free and/or glycoside-bound fraction) and some flavonoids were present in rapeseed honey. In terms of quantity, p-hydroxybenzoic and p-coumaric acids were the most abundant. Total phenols, proteins, and thiols were abundant in forest honey. Some elements and phenols contributed to discrimination of honeys based on PCA. This work demonstrated that black locust (acacia) honey was the most suitable for daily consume but also the other monofloral honeys from Central Europe had lower amounts of non-essential elements in comparison with other geographical regions (Kováčik et al. 2016).

CONCLUSION

The data collected in this chapter demonstrate that the use of chemometrics is essential to obtain the geographical classification of honey samples, independently of the selected analytical method, which is reasonable considering the variability of composition in honeys. Factors such as botanical origin, wheather or harvest year, could affect the possibility of geographical classification, depending both of the selected chemometric method and the analytical data. This suggests that to ensure a correct classification, it is necessary to understand the complexity of the matrix sample, as well as to choice the most reliable analytical method and chemometric tools. In some cases, the selection of the best analytical method, require high cost equipments, which not always will be able to ensure a successfully level of classification itself. Therefore, geographical classification of honey depends strongly on three key factors, which are summarized in the following figure (Figure 1).

The correct selection of variables (organic and/or inorganic compounds) or type and range of spectral data will be crucial in a correct classification, which is related to the selected analytical methods. Also the chemometric tools will be important depending to the matrix data and variability in the samples: there are not rules involving the use of these tools and will depend of the grade of knowledge about the system. External factors can also be crucial in the

classification, because the desire property is the geographic one, however, there will be other type of properties which can influence strongly on the classification model: harvest year, wheather, botanical origin, apiculture practices, etc. Likewise, the use of supervised methods is strongly recommended to evaluate the errors of models. Based on these factors, a last consideration must be taken into account: the proposed models for classification of honey samples obtained for a particular region or country, not necessary will be useful for another regions or countries, indicating also that more studies are required to extend the classification frontiers to new regions and countries around the world.
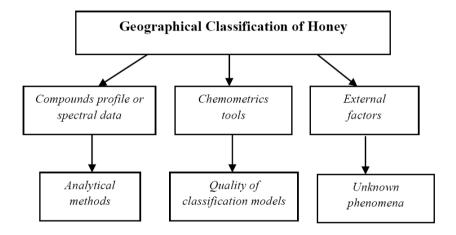

Figure 1. Factors that affect the geographical classification of honey.

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