Geographical and Botanical Classification of Honeys and Apicultural Products by Chemometric Methods. A Review

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Abstract: Evaluations of adulteration, geographical and botanical origin of bee-products are matters of great interest worldwide. These trends are principally related to the international market of food commodities. The chemometric tools appear as new analytical complements which, in recent years, were successfully used to define the geographical and botanical origin of food raw materials or to detect possible adulterations. The classification can be possible using different analysis criteria, such as minor and trace elements, as well as sugars and other items by the chemometric analysis. This review contains a survey of the current multivariate methods published between January 1999 and February 2011, used for the classification and authentication of honey and other bee-products. References are ordered in a way to allow easy reading and further search.

Keywords: Honey, geographical origin, botanical origin, bee products, pattern recognition methods, multivariate calibration, authenticity.

1. INTRODUCTION

Today, confidence in the safety and integrity of the food supply is an important requirement for consumers. The assessment of methods ensuring the authenticity of foodstuffs has thus, become an important issue. Authenticity in particular, is closely related to both food quality and safety issues. Bee products are natural foods and as such, constitute a category of foods heavily affected by adulteration either due to misdescription of the botanical or geographical source or adulteration regarding their composition, such as the addition of illegal products (fructose syrup, water and other substances) usually for commercial purposes.

The geographical and botanical traceability of bee products involves the use of analytical methods that allow for their differentiation based on the chemical composition, thus confirming their authenticity. For this purpose, various parameters such as phenolic compounds, physicochemical properties, minerals and trace elements were determined. As a rule, it is not possible to classify bee products with enough confidence, considering only a single chemical parameter. Modern analytical techniques provide opportunity to collect large amounts of data from various samples. Thus, some multivariate treatments of data are necessary to get relevant results [1]. In essence, these multivariate techniques are powerful tools that allow designing empirical mathematical models that are for instance capable of predicting some values of important properties, which are not directly measurable. The discipline that applies these multivariate techniques to chemical data is called chemometrics.

There are several reviews on this theme, principally on honey authenticity issues [2-3]. Anklam [4] studied the analytical methods for geographical and botanical analysis of honey. In 2005, the use of chemometrics in honey for detecting chemical authenticity and quality parameters was proposed [5]. More recently, in 2009, a complete study about determination of metals in honey was carried out, including the effect of environmental pollution in the concentration of several metals in honey. This revision was carried out by Phol and it included analytical methods to detect metal concentrations in honey [6]. On the other hand, stable radioisotopes were used to determine the geographical origin of protected label, including honey [7-8]. Finally, a complete review about chemometric methods used as classification tools was published [9]. To offer the advances on this subject, this work provides a detail of the articles published during the last decade (from January 1999 to February 2011) that use multivariate methods to prove geographical or botanical source and authenticity of bee products. Since determination of geographical origin is strongly influenced by the differences of the vegetation type between the geographical re-

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gions and thus, to the botanical origin [10], some authors proposed the classification of 2 criteria simultaneously. In addition, in the reviewed publications some trends could be noted, related to the use of methods of analysis in the country of origin of the bee-product. Therefore, the references presented in this work are arranged by country of origin to make the search of results easier. Finally, to facilitate the review's readability, references were presented in a compact way without giving too much detail. Researchers looking for greater detail concerning methods and related issues are advised to consult the cited references.

2. GEOGRAPHICAL ORIGIN OF HONEY

The authentication of geographical origin of honey is an important part of quality control and food safety. International honey prices strongly vary with geographical origin. Generally, in Western Europe and other countries, such as the United States of America and Japan, honey imported from the Far East or South America has a lower price, and is therefore prone to mislabelling because of economic reasons [11]. So, in many countries, foodstuffs including honey are identified by their location through regulatory means. In the European Union (EU), this is regulated by the Protected Geographical Indication (PGI) and the Protected Designations of Origin (PDO) [12].

Research on the determination of the geographical origin or quality brand of honeys is a very active area for the application of chemometric classification procedures. This tendency could be due to the fact that, the determination of single analytical indices on honey samples is not enough to provide to the experimenter, information related to the geographical origin of the product. Following this consideration, it becomes clear that, to authenticate the origin of honey samples, a multivariate analytical approach has to be undertaken. Pattern recognition techniques have been widely applied in food chemistry when an origin-related identification of the products was examined. In general, geographical origin has been addressed by research groups, corresponding with the principal honey producing areas worldwide.

2.1. Argentina

During the last 5 years, Argentina, characterized by its wide variation of weather and great extensions of land became, together with China, one of the most important honey producers worldwide [13]. In general, the problem of the origin of honey from this country was treated, but always limited to small producer regions.

The free amino acid contents determined by High Performance Liquid Chromatography (HPLC) combined with principal component analysis (PCA) and cluster analysis (CA) methods were applied for the geographical characterization of honey from Cordoba province (Central-Argentina). By means of CA and PCA, the results show 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 acid profiles [14].

In another paper, classification of honey samples from the north and southern regions was carried out, based on physicochemical parameters (12 variables) and 10 metals on 75 honey samples, using step-wise discriminant analysis (SDA) and k-nearest neighborhood (KNN) analysis as chemometric tools. By conventional KNN analysis, using 15 variables (8 physicochemical parameters and 7 metals) the best model produced a 83% 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. The authors state that the reduction in the number of variables included in the analysis, with the consistent increase in the percentage of correct assignments, implies that some variables are masked or concealed by the discriminatory power of others (correlated variables). On the other hand, the chemical traceability of honey improves the trust of consumers to certified regional products and also honey provenance [15].

Another important honey production region of Argentina corresponds to La Pampa province. 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. The elemental study was carried out by inductively coupled plasma atomic emission spectrometry (ICP-AES) on 6 elements and 32 multifloral honey samples. The results show the geographical classification of samples in 2 groups: capital samples (next to Santa Rosa, the capital city) and non capital samples. The major difference between both groups was the content of phosphorous: capital samples had a higher concentration of phosphorous than the non capital samples. This aspect can be observed graphically by the PCA scores plot and dendogram of cluster analysis, showing two well-defined groups. By linear discriminant analysis (LDA), the training and validation set of samples yielded 100% correct results. The authors suggest that the method is useful to classify and identify samples of natural honey from areas within the province of La Pampa [16].

Finally, a geographical classification of honeys from two regions of Buenos Aires province (Argentina) was performed, using physicochemical parameters such as color, free acidity, pH and moisture, while PCA was used as multivariate tool. Three types of honey were studied: monofloral, mixed and polyfloral honeys. Although the physicochemical parameters are not often suitable for the classification of honey, the PCA scores plot showed the grouping of two regions from Buenos Aires province (east and centre region groups). The authors showed that the most important variables to differentiate the samples under study, were color and moisture content, thus reducing the dimensionality of the data matrix [17].

2.2. Brazil

Honey samples from the Bahía region (Brazil) were geographically classified based on their mineral and trace content (8 elements), ashes and electric conductivity. The PCA and CA chemometric tools were included. The results obtained by cluster analysis show 3 groups: semiarid region, transitional forest zone and Atlantic forest zone. In the scores plot that resulted from PCA, two groups were obtained: Atlantic forest zone and semiarid forest zone, while for the transition forest zone, honey samples appear indistinctly in

both groups. The authors propose that this characterization is of great importance to detect fraud or adulteration without the need for sophisticated techniques [18].

2.3. France - Corsica

The assessment of geographical origin was applied for fast characterization of honey by analysis of volatile compounds and chemometric methods. In total, 374 samples were collected over two production seasons in Corsica (n = 219) and other European countries (n = 155) with the aim to confirm the authenticity of the honeys labeled as "Corsica" (protected denomination of origin region). The analytical data 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). Twenty six selected volatile compounds were used as variables for PCA and artificial neural networks (ANN) analysis. In PCA, the scores plot shows 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 perceptions (ANN-MLP) was applied, good results were obtained. The best ability for prediction (94.5%) and classification (96.5%) of the ANN-MLP model were obtained when the data from two honey harvests were aggregated in order to improve the model performance compared to separate year harvests. The proposed method aimed at distinguishing Corsican honeys (protected denomination of origin region) from honeys harvested in other European countries, which can be useful for traceability studies [19]. Two years after, new pattern recognition tools were applied on the same analytical data to obtain a new model. In this case, the researchers employed LDA, soft independent modeling of class analogy (SIMCA), partial least squares discriminant analysis (PLS-DA) and support vector machines (SVM) with the recently proposed Pearson VII universal kernel. The results show that the efficiency of the proposed models ranged from 60 to 91.5%, the sensibility ranged from 86.7 to 97.8% and the specificity ranged from 42.9 to 90.5%. The main conclusion of this study is that the volatile profiles of Corsican honeys are specific enough and allow for their discrimination from honeys of different geographical origins. In general, all models, except SIMCA, showed good efficiency, high sensitivity and specificities of data from separate sampling years and of data from both years [20].

Near-infrared spectroscopy (NIRS) was applied several times to study Corsican honeys, too. In the first place, NIRS spectra in combination to PCA and partial least square regression (PLSR) were used to determine the fingerprint Corsican honey. Three hundred seventy three samples were analyzed, of which 219 were Corsican and 154 non-Corsican honeys. Applying PCA, it was found in general, a large amount of overlap between the Corsican and non-Corsican honey samples. However, reducing variables and using second derivate spectra data, PLS-DA regression gave a correct classification of samples ranging from 81.2 to 84.0%. When jackknife uncertainty testing was used before PLS-DA, the results improved 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% and for the PLS models developed using separate calibration and validation sample sets after jack-knife uncertainty testing, correct classification ranged from 87.7 to 94.1%. The application of the jack-knife uncertainty test improved the correct classification of samples, while the combination of PLS-DA of NIR spectral data was an adequate method for the study of provenance of Corsican honey samples [21]. This provenance study was recently expanded for honey and edible oil. In this work, NIRS was applied to Corsican honeys which were classified using another family of chemometric tools, namely class-modeling techniques: SIMCA, Unequal Class Model (UNEQ) and potential function technique (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 [22]. Finally, IR spectrometry was applied to Corsican honeys. In this case, Fourier transformed infrared spectroscopy with attenuated total reflectance (FTIR-ATR) combined with factorial discriminant analysis (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 prediction ability (average) from 67 to 86%, while the total harvest showed prediction ability from 81 to 89%. The method can be useful to confirm the provenance or not of Corsican honeys and to recognize that the IR spectrum of these honeys is not unique in comparison with the rest of the countries [23]. Then, FT-Raman spectroscopy was used to classify honey from Corsica. In this case, PLS-DA and SVM were used as multivariate tools. Two hundred and nineteen Corsican samples and one hundred and fifty-seven non Corsican ones from different botanical origins were analyzed. As exploratory technique, the Fisher criterion was used to select the best wavelength range. In both cases, PLS-DA and SVM, the results were similar: for PLS-DA the prediction ability of correct samples of Corsican honeys went from 86.4 to 87.1% and 82.6 - 92.1% in the case of non Corsican honeys; for SVM the predictions were: 87.3 – 88.3 % for Corsican honeys and 82.9 – 91.6% for non Corsican honeys. On the other hand, the prediction plot for PLS-DA and SVM showed good discrimination ability in both cases: two groups showing non Corsican and Corsican honeys samples. In comparison, FT-Raman and PLS DA yielded better results than FT-

IR PLS-DA. On the basis of results, the new method can be used to determine geographical origin of Corsican honeys [24].

Also, the ^{1}H MNR spectroscopy and multivariate analysis was used to determine Corsican honeys. The multivariate tools included PLS-DA, two step genetic programming (GP) and PLS-GP. A total of 111 samples from Corsica and 253 samples from other places in Europe were analyzed. Using PLS-DA, the scores plot showed quite a good discrimination, with an intermediate zone where both origins overlap. By GP the tree showed a 97.3 % of classification ability to distinguish Corsican and non Corsican honeys. By PLS-GP it was possible to find variables than identify the Corsican honeys: these variables are named as Corsican markers, and showed more sensibility (98.2%) and selectivity (93.0%) than the two-step GP (96.4 and 91.5%) and PLS-DA (72.1 and 81.6%) respectively [25].

All of the studies mentioned in this section [19-25] were a part of the EC FP6 TRACE project [26] sponsored by the European Commission. TRACE aims at improving the health and well-being of European citizens by delivering improved traceability of food products. As a part of this project, all of the samples analysed by the different methods were aliquots of the same samples.

2.4. Greece

The geographical origin of Greek honeys was carried out by the use of absolute pollen grains frequency, which is a melissopalynological analysis, and LDA. One hundred and eighty samples of honey from the same botanical origin (Greek thyme) from different regions of Greece were analyzed. The LDA model had a discriminative power of 90-100%. The discriminant ability of the model depended on the care to be taken when sampling and on accidental contamination. The results showed good prediction ability, being this the only work that used only pollen analysis to obtain geographical classification [27].

Then, the analysis of volatile compounds was applied to the geographical discrimination of honeys from this country. Twenty eight thyme honey samples and 63 volatiles were analyzed by means of solid-phase microextraction coupled to a GC-MS. Four Greek regions were studied and geographically discriminated by LDA: Crete, Leros, Kalumnos, and Kos. A previous PCA model was obtained to select the 45 most important variables that were then used in LDA. The canonical function scores plot showed good discrimination ability for the 4 regions, without overlapping samples. The table of LDA classification showed good predictions, from 71.43% for Kalumnos to 100% to Crete. Also 6 volatile compounds were identified as possible botanical markers for thyme honeys [28].

2.5. Other Countries in America & Europe

The study of geographical origin was carried out on 125 filtered and 167 unfiltered honey samples, from different countries around the world: Ireland, Mexico, Spain, Hungary, Argentina and the Czech Republic. Samples were analyzed by NIRS, spectra were recorded in transflectance mode. Following preliminary examination by PCA, modeling methods applied to the spectral data set were SIMCA and PLS. Various pretreatments were examined. For unfiltered honey, best SIMCA models gave correct classification rates of 95.5, 94.4, and 96% for the Irish, Mexican, and Spanish samples, respectively; PLS2 discriminant analysis produced a 100% correct classification for each of these honey classes. In the case of filtered honey, best SIMCA models produced correct classification rates of 91.7, 100, 100, and 96% for the Argentinean, Czech, Hungarian, and Irish samples, respectively, using the standard normal variate data pretreatment. PLS2-DA produced 96, 100, 100, and 100% correct classifications for the Argentinean, Czech, Hungarian, and Irish honey samples, respectively, using a second-derivative data pretreatment. Overall, while both SIMCA and PLS gave encouraging results, better correct classification rates were found using PLSR. From the view of the authors, due to the good fit of models for geographical prediction, the proposed methods could be useful to classify filtered and unfiltered honey samples from the studied countries [29].

FTIR-ATR was used for the geographical classification of honey samples from Europe and America. One hundred and fifty filtered honey samples from the Czech Rep., Argentina, Ireland, Mexico and Hungry were analyzed. Data processing included first, second and standard derivative spectra. The pretreated data were then analyzed by PLSR, FDA and SIMCA. A 93.3% correct prediction was obtained by PLSR, while by FDA it was 94.7%. The best classifications were achieved using SIMCA (100%); however, models showed very high false positive rates [30].

^{1}H Nuclear magnetic resonance (NMR) was also used to determine geographical origin of honey from Argentina, Hungary and Italy [31]. 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 PLS-DA, which showed the classification of multifloral samples in three groups from Argentina, Italy and Hungary, indicating the efficiency of PLS-DA as classification tool when NMR data were used.

2.6. Pakistan

In a paper published by a research group of Pakistan, physicochemical parameters and metal contents of fifteen honey samples, as well as pollen, were studied. Determination included density, apparent viscosity, moisture, pH, total soluble solids, free acidity, conductivity, hydroxylmethylfurfural (HMF) and ashes. Also minerals and trace metals were determined by AAS (Li, K, Na, Fe, Bi, Mn, Ni, Co, Cu, Cd and Hg). 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 the three first principal components (PCs) in a scores plot [32].

2.7. Slovenia

Electronic nose was used to determine the geographical origin of monofloral honeys from Slovenia, by the determination of volatile compounds and aroma profile. Multivariate analysis was carried out by PCA on 49 honey samples of

black locust (*Robinia pseudoacacia L.*) and 16 honey samples of chestnut (*Castanea sativa Mill.*). The electronic nose device consisted of an automatic sampling apparatus, a detector unit containing the array of 22 different sensors of metal oxide semiconductors and an automatic-random sampling system with controlled temperature. Data obtained by electronic nose analysis were analyzed by PCA in order to determine differences among volatile profiles of samples with the same botanical origin but with different geographical origin. PCA results showed that honey samples from geographically close regions tend to group together, while those from geographically distant regions show differences even though they have the same botanical origin. Authors stated that the volatile profile data obtained by electronic nose and the PCA plots showed that samples had the tendency to group in PCs spaces according to their geographical origin [33].

On the other hand, 122 Slovenian honey samples, which included black locust honey (*R. pseudoacacia L.*), lime honey (*Tilia spp.)* and chestnut honey (*C. sativa Mill.*) 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 the stable carbon-nitrogen isotope ratios using isotope ratio mass spectrometry (IRMS). The multivariate tools used in this work were PCA and LDA. The results show a good geographical classification for every monofloral honey: for black locust honey, Dinaric, Pannonnian and Mediterranean regions were classified; for lime honey, Alpine, Dinaric and Mediterranean and for chestnut honey Alpine, Dinaric and Pannonnian were classified. Good results were obtained again by LDA, with a correct geographical classification from 87.5 to 100%. The method can be useful for the protection and verification of the geographical origin of authentic Slovenian honey [34].

2.8. Spain

In terms of volume, the EU is an important producer of honey, being Spain its leading producer in the last years [13]. Research groups from this country proposed the use of mineral composition of honeys combined with chemometrics to set up authenticity parameters of geographical origin.

From the North-west region of this country (Galicia), a research group proposed to classify geographical origin of honey using chemometric techniques combined with its inorganic composition. The metal profiles of honey provide enough information to enable classification criteria to discriminate samples according to their environmental surrounding. In the first work published by this group, the multivariate chemometric techniques applied were, PCA, LDA and KNN analysis. Using only three metals (Cu, Mn and Li), the geographical origin was correctly achieved [35]. More recently, the authors published a new work to classify honey from the same region, using new multivariate tools as well: PCA, CA, Bayesian analysis (BA), PLSR and neuronal network analysis (NNA) by means of the metal content as original data [36]. Nine metals were determined in 42 honey samples which were divided into two categories: Galician and non-Galician honeys. The elemental analysis produced classification to identify honeys from this region, with similar results to those produced in a previous work [35]. In another work by the same authors, the geographical classification of honey is described based on environmental aspects. This work determined 13 metals in 40 honey samples from two different places of Galicia: urban-industrial polluted and rural unpolluted regions. The analytical determinations were carried out by electrothermal atomic absorption spectroscopy (ETAAS) and the chemometric tools were PCA, CA and different pattern recognition methods, such as LDA, KNN, SIMCA, multilayer feed-forward artificial neural networks (MLF-ANN) and vector quantization classification systems associated with artificial neuronal network: 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). In this work, the scores plot by PCA reflects a good discrimination for both samples, as well as the CA plot. On the other hand, the different supervised pattern recognition procedures had a recognition ability ranging from 70 to 100%, and prediction ability from 71.8 to 100 % for both, urban-industrial polluted and rural unpolluted regions. The method is recommended for the geographical classification of honey from Galicia, based on pollution conditions. Also, different classification rules, which associate the metal content of honeys with their environmental surrounding, were obtained by chemometric pattern recognition procedures, and also the potential use of honey as suitable environmental bioindicator was established [37].

Again, the concentration of metals was used to discriminate honeys from this country. In this case, 24 elements were analysed by inductively coupled plasma optical emission spectroscopy (ICP-OES) in 24 thyme honey samples. The geographical analysis consisted in the distinction of samples from the coast (14) and from the mountains (10) and was performed by CA, PCA and SDA. By CA, the dendogram showed low classification ability, because coast and mountain samples appear together and mountain honey samples were not specifically grouped. A similar situation was shown by the PCA analysis, where the scores plot showed overlapping between coast and mountain honey samples. However, by SDA, and using 10 elements (Al, As, Cr, Cu, K, Li, Mg, P, S and V) 100 % correct classification was obtained. Authors concluded that the mineral composition depends on botanical and geographical origin, as well as on the extent of precipitations [38].

Similarly, honeys from the Canary Islands were characterized by their metal contents, too [39]. In this work, PCA, CA, DA and logistic regression (logR) were applied. From the results, Canary Islands samples can be characterized in terms of their Na, K, Sr, Mg, Ca and Cu contents. Authors inferred that Canary honey could be distinguished from non-Canary honey basing on its metal content. DA and logR showed the best performance to allow for this aim. So, this method could be useful to detect fraud or adulteration of denomination of origin.

On the other hand, honeys from a small region of Central Spain (Madrid province) were successfully classified by pollen analysis, physicochemical parameters and volatile com-

pounds, associated with PCA and stepwise-DA. The study was carried out on 46 artisanal honey samples. The authors concluded that it can be possible to distinguish honey from mountain areas to that of plain ones. The comparative analysis of these results suggested the main cause of their variability was the different honey source (honeydew or nectar) [40].

2.9. USA

The geographical origin of commercial honey from Hawaii (USA) and other countries was carried out based on fingerprinting and barcoding of proteins by using matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI TOF MS). The protein mass spectra of 16 honey samples of known Hawaiian origin were obtained and peak information was extracted to generate protein fingerprints. This information was transformed into a database library of spectral barcodes that were used for differentiation of the geographical origin of honeys based on PCA. The differentiation ability of the database library of barcodes was validated by comparing the results of replicate assays of 5 of the 16 honey samples of known Hawaiian origin obtained directly from the producers. Validation results showed the protein fingerprints of honeys have better comparability with those honeys in the library known to be from the same region than with those of honey samples from other regions. The protein fingerprints were used to distinguish the geographical origins of commercially purchased honey samples with labels indicating that they were produced in different countries and various states of the USA, including Hawaii. The 2D and 3D scores plot obtained from PCA, shows the ability of the model to distinguish Hawaiian honey samples from the rest ones. Finally, the authors conclude that the MALDI TOF MS combined with PCA are an alternative method for identification of honey geographical origin and they suggest that these techniques can be applied for controlling the geographical origin of honeys sold in commerce [41].

3. BOTANICAL ORIGIN OF HONEY

Current legislation about honey origin establishes that [42, 43]: if there is any reference to a particular plant or blossom (this can be pictures or words), then the honey must come wholly or mainly from that blossom or plant - i.e. the honey must be characterised by that blossom or plant. Such descriptions can also be tested by analysis. As expected, the research groups from the countries that produce monofloral honeys were those mostly interested in proposing classification models to authenticate these kinds of honeys.

3.1. Algeria

The botanical classification of Algerian honeys was proposed based on their sugar content. The sugar profile was analyzed by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) for the determination of 11 sugars: fructose, glucose, sucrose, maltose, isomaltose, turanose, erlose, raffinose, melezitose, melibiose and trehalose. The method was performed using a gradient with NaOH solution and ultrapure water. Due to the fact that melibiose was not detected in any sample, the chemometric analysis was performed by DA using 10 sugars. The results show the DA function plot with 4 botanical origin honeys: multifloral, *Eucalyptus*, *Erica arborea* and *Apiaceae*. The graphical results showed a low ability for the classification of all honeys because multifloral honeys overlapped with the rest of honeys. However, *Apiaceae* honeys could be separated from all other honeys by means of their sugar profile [44]. In comparison, the same analytical method was used in 2005 by Nozal and others for Spanish honeys [45]. In this previous work, the same solvents were used on the HPLC method, 14 sugars were determined, but only 6 were used in the chemometric analysis: erlose, nigerose, trehalose, melezitose, isomaltose, and panose, for the discrimination of ling, spike lavender, French lavender, and forest honeys.

3.2. Argentina

Five botanical honey origins were determined: *Medicago sativa* (alfalfa), *Helianthus annuus* (sunflower), *Melilotus albus* (white clover), *Prosopis spp*. (carob) and *Prosopis caldenia* (caldén) by the analysis of organic volatile compounds profile, carried out by gas chromatography and mass spectrometry, combined with chemometric methods: hierarchical CA, SDA, and KNN. From the 35 volatile compounds, the most significant for a good differentiation between floral origins were octanal, benzeneacetaldehyde, 1-octanol, 2-methoxyphenol, nonanal, and 2-H-1-benzopyran-2-one. These variables were selected by analysis of HCA and SDA; then, by KNN analysis a model with good fit of 0% of error was achieved for most honey samples, but *P. caldenia* had 33.33% of calibration error. The proposed method could be an alternative to the melissopalynology to distinguish five different monofloral honeys [46].

3.3. China

Electronic tongue was used to settle the botanical origin of honey, of 13 different kinds of floral origins (including five kinds of acacia honeys) from different places in China. Data analysis was carried out by the use of PCA, CA, and ANN. The electronic tongue device consisted of 7 potentiometric chemical sensors, which allow defining five tastes (sourness, saltiness, sweetness, bitterness, and savory). The monofloral honeys included 25 samples of each botanical origin: acacia, astragali, data, coptis, vitex, motherwort, radix changill, and buckwheat, from different geographical origins. By PCA, the scores plot shows a good classification, with different botanical sub-groups into 3 great groupings defined by the sweetness sensor. Similar results were obtained by CA, which presents a dendrogram able to distinguish 8 monofloral honeys with very good coincidences. The ANN model showed good results: the training set presented from 93.3 to 100% correct samples, while in the test set there was from 75 to 100% prediction ability. On the other hand, a study over 5 geographical origins of acacia honey was carried out; in this case, acacia honeys were geographically classified from 5 counties in China. In the PCA scores plot 5 groups of honey samples can be distinguished, according to their geographical origin. By means of the cluster analysis, the grouping was less effective, but by ANN the training and prediction set showed concordant abilities ranging from 87.5 to 100%. The method showed good ability for both, botanical and geographical determination of honey from this coun-

try and can be useful to control geographical and botanical authenticity [47].

Five floral origins (acacia, astragali, data, vitex and buckwheat) from five geographical origins from Jangxi province (China) were classified based on their rheological properties. The chemometric tools included were PCA, PCR, CA, PCA-PLSR and SVM. For botanical origin, and after optimization of the rheological variables, the PCA 3D scores plot (using the 3 first components) obtained a good classification, with five separate groups. By means of the PCR, a model fit with R^2 = 0.90 was obtained, with an ability prediction of 95%. By cluster analysis, the dendogram also showed a good classification, with only 2 misclassified samples. By PCA-PLSR, the scores plot shows a classification for 3 of 5 monofloral honeys (acacia, astragali, data) with overlapping of vitex and buckwheat, while for the PLS the scores plot shows 2 separate groups (acacia and astragali). The observed-predicted plots for PLS and PCA-PLSR show R^2 values 0.97 and 0.96 for both models respectively, with a good fit. The best results of prediction were obtained by SVM in comparison to PCR, PLS and PCA-PLSR: the R^2 values for different SVM models ranged from 0.96 to 0.97, suggesting a good fit of the model for the botanical prediction. The same multivariate tools and same rheological variables were used for assessment of geographical origin, from different places of the Jangxi province. The results show good prediction ability by PCA, CA, PLS and PCA-PLSR, discriminating five groups according to their different geographical origin. The best results were again obtained by SVM, whose models produced R^2 values from 0.97 to 0.99. This work, managed to determine both, botanical and geographical origins using different chemometric methods, by means of a simple analysis of viscosity properties, for a rapid assessment of the honey samples [48].

3.4. European Countries

A study of monofloral honeys from different countries in Europe was carried out by Raman spectroscopy. Thirteen unifloral type honeys were studied from 51 honey samples: acacia, chestnut, eucalyptus, heather, lime, rape, sunflower, citrus, lavender, rosemary, *Echium plantagineum*, orange and 'flor di sulla'. PCA, LDA and MLP were used as multivariate methods. The geographical origin involved the following countries: Germany, Italy, Denmark, Spain, France, Netherlands, England and Portugal. The LDA scores plot showed good discrimination abilities for acacia, sunflower and rape, but showed overlapping with the rest of botanical honeys. A geographical study performed by LDA showed a general overlapping among all the involved countries. By MLP, 14 samples and 7 monofloral types were studied: the classification table showed that only 1 sample of heather was unclassified, while the rest were correctly classified. The method appears to be useful for botanical non-destructive analysis of monofloral honey from several European countries [49].

3.5. France

The analysis of 18 metals in 86 honey samples with different botanical origins and coming from France and other places was performed by ICP-AES, using correspondence factor analysis and hierarchical cluster analysis as multivariate chemometric tools. The results obtained showed that, due to the variability of the concentration of metals in the different samples, it was not possible to obtain an adequate classification of botanical and geographical origin based on the selected variables [50]. Then, the same research group achieved the classification of seven botanical honeys (fir, cinder heather, chestnut, lavender, acacia, rape and sunflower). The interesting point is that the authors used physicochemical analysis on 469 honey samples for the classification, analyzing the following parameters: moisture, conductivity, diastase activity, pH, free acidity, color, HMF and percentage of fructose, glucose, saccharose, erlose, raffinose, and melezitose. The multivariate analysis was carried out by PCA and DA. By means of conductivity, pH, free acidity and percentage of fructose, glucose, and raffinose, the results showed a good classification of botanical honeys, which is a novelty considering that generally, the physical chemical parameters are not useful for botanical recognition of honey [51].

Other research groups in this country used the sugar composition of honey to differentiate their botanical origin. In the first place, Cordella *et al*. studied the content of thirteen saccharides in honey by anion-exchange chromatography–pulsed amperometric detection (HPAEC–PAD), followed by LDA. The proposed model showed good classification between multifloral and several monofloral honeys, so the method results appear adequate for the detection of botanical authenticity [52]. Later, Cotte et. al. used the content of nineteen saccharides determined by chromatographic analysis (HPAEC-PAD and GC-FID) combined with PCA in seven monofloral honeys: acacia, chestnut, rape, lavender, fir, linden and sunflower in natural samples; and acacia, chestnut, lavender, fir and linden in commercial samples. Only seven variables were needed from the total of sugars,: glucose, fructose, raffinose, trehalose, fructose/glucose, maltose/trehalose and erlose/maltulose. The PCA plot showed a very good classification in both, natural and commercial honey samples. However, the rest of monofloral honeys presented an overlap among them. For this reason, the method can be used to classify monofloral honeys from natural and commercial samples [53].

On the other hand, a conjunction of ANN and LDA was applied for the multivariate analysis of chromatograms. This tool allowed for the characterization of seven floral honeys (robinia, lavender, rosemary, multifloral, chestnut and fir) based on their saccharides content. This method is able to detect monofloral honeys authenticity and avoid botanical adulteration or fraud [54].

3.6. Greece

A validated method for the discrimination and classification of honey samples was performed by gas chromatography–mass spectrometry (GC-MS) fingerprinting of headspace volatile compounds. Combined mass spectra of honey samples originated in different plants and geographical regions of Greece were subjected to orthogonal partial least squares-discriminant analysis (OPLS-DA), and orthogonal partial least squares-hierarchical cluster analysis (OPLS-HCA). Botanical origin included chestnut, citrus, cotton, fir,

heather, pine and thyme. Analyses revealed an excellent separation between honey samples according to their botanical origin with the percentage of misclassification to be as low as 1.3% by applying OPLS-HCA, whereas OPLS-DA showed overlapping only for fir and pine. Analyses revealed an excellent separation between honey samples according to their botanical origin with the percentage of misclassification to be as low as 1.3% by applying OPLS-HCA, whereas OPLS-DA showed overlapping only for fir and pine. The fragments (m/z) responsible for the observed separation were assigned to phenolic, terpenoid and aliphatic compounds, which were present in the headspace of unifloral honeys. On the other hand, a variable classification for citrus and thyme honeys was obtained, according to their geographical location. Results suggested that the developed methodology is, in general, sound and reliable for the botanical classification of honey samples and the study of differences in their chemical composition [55].

3.7. India

A study of four monofloral honeys was carried out in this country. Fifteen samples for every monofloral honey were analyzed*: Litche chinensis* (litchi), *Citrus sinensis* (sweet orange), *Ziziphus mauritiana* (ber) and *Prunus persica* (peach). Analytical tests included: moisture content, pH, free acidity, reducing sugars and sucrose contents, fructose⁄glucose ratio, ash and proline content, invertase and diastase activities, hydroxymethylfurfural and mineral content (sodium, potassium, iron, calcium, zinc, and copper), as well as rheological properties of honey. The chemometric tools included were PCA and LDA. The PCA scores plot shows good ability of discrimination: using 3 PCs in the model, which explained 90% of total information, the model showed 4 groups, concerning each monofloral honey. The most significant variables were proline, potassium and free acidity. By the LDA, 100% of cases were correctly discriminated in the classification and validation steps. This study can be applied to decide the authenticity of monofloral honeys from India [56].

3.8. Israel

NIRS has been used for the chemometric characterization of honey, for the analysis of perseitol, which is a sugar that is found in a specific honey (avocado). PLSR was used as chemometric tool on 109 honey samples. The validation analysis of perseitol was performed by HPLC by detection with differential diffraction. The fit of the model showed a $R^2 = 0.95$, using the second derivative spectra, while for the prediction set, the results showed a $R^2 = 0.87$. A good fit of the model as well as a satisfactory prediction error were achieved, which allow for the use of this technique for the determination of authenticity of avocado honeys based on their perseitol content [57].

3.9. Italy

LDA was used to classify six different floral honeys from this country. The analytical determinations were physicochemical parameters and carbon isotope ratio ($^{13}C/^{12}C$). The results showed the ability of the model for the graphical classification of six types of honey from different botanical origins, in a data set projection onto the space spanned by the first two variables selected by stepwise LDA and by pairs of varieties: wildflower/honeydew, chestnut/eucalyptus, eucalyptus/sulla and sulla/heather. The method was able to characterize honey from every botanical origin, which makes it useful for determining fraud or adulteration of these botanical honey varieties [58].

Honey samples from the same country were evaluated on the basis of the mineral content and physicochemical analysis to determine their botanical origin [59]. In this work, all samples were from the Marche region (Italy), the botanical varieties studied were acacia, multifloral and honeydew. Analysis of mineral content was carried out by atomic absorption spectrometry (AAS) and all data were analyzed by the PCA and LDA models. The useful variables for the chemometric models were the contents of: Ca, Cu, K, Mg, Mn, Na and Fe. The scores plot for PCA discriminated 3 different botanical origins; similar results were obtained by canonical discriminant plot. With those variables, the LDA model had an ability of prediction of 100% for the studied varieties (acacia, multifloral and honeydew). The method was useful to determine botanical origin from the Marche region, but authors inferred that further studies are necessary to characterize botanical origin of honey from other places in Italy.

Diffuse reflectance mid-infrared Fourier transform spectroscopy (DRIFTS) was used in combination with PCA, discriminant analysis (DA) and classification tree analysis (CTA) to control the botanical origin (robinia, chestnut, citrus and polyfloral) of 82 Italian honey samples [60]. PCA did not yield good results, but DA distinguished four botanical groups using the reduced spectra database. The DA model showed a correct classification for the calibration step from 76-100%, while for the validation step it varied from 56-89%. Using the CTA model, the calibration had a correct classification ranging from 95 to 100%, while the validation ranged from 33-93%. Authors concluded that DRIFTS combined with multivariate analysis, constituted a rapid technique to classify honey samples of different botanical origins.

Then, botanical origin classification of honeys from Siena was carried out based on their metal contents. In this paper, honeydew and monofloral honey were distinguished, using 23 elements analyzed in 51 honey samples from the same geographical origin (Siena, Italy). The analytical determinations were made by ICP-OES and inductively coupled plasma mass spectrometry (ICP-MS). PCA was used as multivariate tool. The projection of each sample (scores) in a two-dimensional factorial map showed a clear separation between the honeydew and nectar samples. From these results the authors conclude that the PCA analysis highlighted the relationship between element distribution and honey type. Some geological and geochemical features appear to affect honey chemistry, especially with regard to Ca, Na and Mn. In this work, monofloral honeys could not be classified by botanical origin; however, they could clearly be distinguished from the honeydew samples [61].

NMR was used as an analytical tool to classify monofloral honey samples. The high resolution nuclear magnetic resonance method (HR-NMR) and 2D high resolution nu-

clear magnetic resonance method (2D HR-NMR) were used in combination with PCA and GDA to classify 71 honey samples from 5 botanical origins (robinia, chestnut, citrus, eucalyptus and polyfloral). D_2O and DMSO were also tested as solvents to prove the degree of resolution of the NMR spectra. At first, the authors applied the PCA model to analyze the data matrix. A poor separation between samples was achieved from the scores plot (D_2O and DMSO spectrum) since samples were not grouped according to their botanical origin. The main identified reason was the high number of overlapping singles using monodimensional HR-NMR spectra. Then, by using 2D HR-NMR spectra, a clear classification was obtained by general discriminant analysis with both solvents: in this case, the scores plot showed the five groups matched every botanical origin, which proved that 2D HR-NMR in combination with GDA were useful to distinguish successfully the 5 botanical origins of Italian honeys [62].

^{1}H NMR spectra by pulse sequence, in combination with PCA and PLS-DA were used to distinguish a total of 118 samples of chestnut, acacia, linden, and polyfloral honeys. In this case, chloroform was used as solvent. Compared to [44], the use of pulse sequence, as well as the previous separation of several compounds from sugars using chloroform, may have improved the spectral quality. Also, authors found 8 chemical markers, which helped to obtain better multivariate results by use of ^{1}H NMR. The PCA scores plot was shown, in three separate graphics: chestnut-linden, acacia-chestnut and polyfloral-chestnut groups. In the three cases, the distinction of botanical origin was clear, but obtained separately. However, the incorporation of PLS-DA as multivariate tool, achieved a 3D scores plot (3 first components), with a good classification for all botanical origins in just one plot. Also, by analysing the PLS-DA weight plot, it was possible to determine the effect of the chemical markers over the PLS-DA model. The method can be promising to discriminate unifloral and polyfloral honey samples using a single spectroscopic measurement with reduced sample preparation time and molecular characterization of the components [63].

3.10. Italy and Hungary

Electronic nose in combination with ANN-MLP was used to classify four monofloral honey samples: *Robinia pseudoacacia L., Rhododendron spp. and Citrus spp.* Seventy samples were analyzed, out of which 64 were from Italy and only 6 *Robinia pseudoacacia L* from Hungary. The electronic nose device was conformed by 22 sensors of different semiconductor materials. A preliminary PCA model was obtained to select the most important variables to use in ANN-MLP. The PCA scores plots for PC1 vs PC2 and PC3 vs PC4 showed low discrimination ability: in the first plot only *Rhododendron spp* could be distinguished from the rest, while in the second plot Italian *Robina pseudoacacia L.* was distinguished from the rest. However, the use of ANN-MLP improved significantly the discrimination ability of the method: 100% of all samples (14 Italian *Robinia pseudoacacia L.*, 6 Hungarian *Robinia pseudoacacia L.*, 30 *Rhododendron spp.* and 20 *Citrus spp*) were correctly classified, using only 3 of 22 sensors without the need to isolate volatile compounds [64].

3.11. Morocco

There are several works from this country that describe the characterization of the botanical origin of honeys from Morocco. The analytical determinations were different, but in all cases, multivariate analysis was applied. In the first place, the characterization of monofloral (*Eucalyptus*, *Citrus*, *Lithrus* and *Umbelliferae*) and honeydew honey from the Northwest of Morocco was performed by the analysis of 13 sugars in 98 honey samples by GC-MS and classification by PCA and SDA. The analysed sugars were: fructose, glucose, sucrose, maltose, malutose, xilose, trehalose, kojibiose, gentiobiose, raffinose, isomaltose, melezitose and erlose. By PCA, the classification was not possible. However, better results were obtained by the SDA analysis, in which the classification of honeydew from the rest of monofloral honey was possible, with 100% correct classification. However, the classification of monofloral honey was lower, and ranged from 43 to 75% [65].

After that, authors proposed a new method for the characterization of honeydew and monofloral honeys (*Eucalyptus*, *Citrus*, *Lithrus* and *Umbelliferae*). In this case, they used physicochemical parameters (moisture, pH, acidity, HMF, diastase activity and proline) as original data and PCA and SDA as multivariate analyses. Similar to the previous work, the best results were obtained by SDA, which showed a correct characterization of honeydew and eucalyptus from the other botanical origins, but the rest of monofloral honeys could not be classified. Authors state that that the physicochemical parameters are insufficient to achieve a perfect discrimination of the five unifloral honey classes considered, except for the eucalyptus and honeydew types [66].

Then, the same authors proposed to use mineral content and electrical conductivity as analytical variables to classify 98 samples of honeys with the same botanical origin. The analytical determination was carried out by ICP-AES, analyzing 6 elements: K, Mg, Mn, Cu, Fe and Zn. Multivariate tools included PCA, LDA MLP [67]. Similar results that in [65] and [66] were obtained, including overlapping of botanical origins by PCA; but better results than with SDA and MLP: in these cases, it was possible to classify honeydew (100% correct) and *Eucalyptus* (92% correct) from the rest of the botanical origins, in agreement with the results obtained previously [66].

Next, the authors proposed the botanical classification of the same botanical honeys: *Eucalyptus sp.*, *Citrus sp.*, *Lythrum sp.*, *Umbelliferae* and honeydew. In this work, they combined different analytical variables to obtain a total of 30 parameters measured in 39 honey samples, including physicochemical analysis (moisture, pH, acidity free, acidity lactonic, HMF, diastase activity, electrical conductivity, proline, ashes), mineral content (Zn, Mn, Cu, Fe, Mg and K), sugar profile (fructose, glucose, sucrose, maltose, maltulose, isomaltose, trehalose, gentiobiose, kojibiose, raffinose, erlose and melezitose) and colour. The multivariate tools used in this paper were CA, PCA and SDA [68]. When all variables were used, a good classification of honeys, according to their floral type was not achieved by CA and PCA, except for honeydew from the other monofloral honeys, with similar results to those obtained in [65]. However, this previous classification was useful to find the most important variables,

which were: moisture, free and lactonic acidity, HMF, diastase activity, electrical conductivity, fructose, sucrose, isomaltose, raffinose, melezitose, mineral content (Zn, Fe, Mn and K) and colour. By SDA, the results were improved in comparison to the preliminary CA and PCA plots. The scatter plot for the SDA showed the discrimination of 3 groups: honeydew, *Eucalyptus* and the rest of honeys (*Citrus*, *Lythrum* and *Umbelliferae*). The prediction ability of the model was 100% for all botanical origins, except for *Lytrum* which was 85%. The method demonstrated ability to discriminate *Eucalyptus* and honeydew from other Morocco's monofloral honeys [68]. On the other hand, except for the case of sugar profile [65], the combination of all variables together, suggested that the classification ability of multivariate models is not improved, in comparison to the separate use of physicochemical parameters [66] or mineral content [67].

3.12. Poland

The study of three botanical origins of honey from Poland was performed. Honeydew, buckwheat and rape honey samples were classified based on the analysis of 13 elements. The analytical determination was carried out by using ICP-MS. The chemometric tools included in this work were CA and PCA. The best results were obtained by CA, since the dendogram plot shows 3 groups according their botanical origin. However, PCA scores plots (PC1 vs PC2 and PC1 vs PC4) did not show the 3 groups, only 2 groups could be seen, which indicates that in this case, a good discrimination was not achieved. [69].

3.13. Portugal

Electronic tongue combined with PCA and LDA were used to classify 3 different monofloral honeys *(Lavandula, Erica* and *Echium*) in 50 honey samples. The electronic tongue consisted in a twenty-channel electrode built with different types of polymeric membranes and potentiometric detection. With this device, the PCA scores plot presented 4 groups, which show only a partial separation of the honey samples, since all groups had mixtures of monofloral honeys. However, by means of the LDA discriminant functions, showed in a scores plot, it was possible to distinguish three groups with quite well-defined boundaries, in agreement with the different botanical origins: honey samples were correctly classified according to the following results: 53% for *Lavandula,* 83% for *Erica* and 78% for *Echium*. Honey samples were analyzed by the pollinic analysis and the results showed, in general, an acceptable prediction ability for the study of monofloral samples [70].

3.14. Slovenia

Total reflection X-ray spectrometry was used in combination with chemometric tools for the classification of 7 botanical origins in honey: acacia (*Robinia pseudoacacia*), floral (nectar of different flowers), lime (*Tilia spp*.), chestnut (*Castanea sativa*), spruce (*Picea abies*), fir (*Abies alba*) and forest (mixed honeydew type of honey). Two hundred and sixty four samples from three different harvest years were analyzed. The most significant elements used for chemometric analysis were Cl, K, Mn and Rb. The multivariate tools included in this work were PCA and regularized discriminant analysis (RDA). By using PCA, the scores plots by year showed a low discrimination ability, because only acacia and chestnut honeys were recognized; however the rest of the honey samples were not correctly classified. By RDA, the classification model was improved, with an ability of 90% for the classification and prediction steps. Authors consider that, due to the complexity of honey samples, it was difficult to obtain a better classification, but the method could be used in multivariate statistical analysis of other food products. [71].

3.15. Spain

This country is an important producer of monofloral honeys [13]. Several research groups from this country used different composition variables to determine botanical origin: mineral composition, carbohydrates profile, amino acid profile and physicochemical parameters.

First, the concentration of 15 metals and physicochemical parameters was analyzed in 46 samples of monofloral honeys (ling, heather, rosemary, thyme, honeydew, spike lavender and French lavender) from Soria province. The data analysis was carried out by PCA and DA. Results show that by PCA, heather and ling honey were classified apart from the rest of monofloral varieties, while by means of the DA, it was possible to classify correctly 90% of honey samples, according to their botanical origin [72].

Then, the content of several metals (Zn, P, B, Mn, Mg, Cu, Ca, Sr, Ba, Na and K) was performed by ICP-AES in 40 samples of monofloral honey from Spain: eucalyptus, heather, orange blossom and rosemary. The used multivariate tools were PCA, CA and LDA. The results show a good modelling, as well as a good discrimination ability, for the 3 multivariate methods, using all elements. The methods were able to determine very well the botanical origin of the four monofloral honeys from different places of Spain [73].

From the point of view of the physicochemical parameters and sugar profile, the classification of blossom and honeydew honeys was performed. The analysis included the following parameters: moisture, water activity, electric conductivity, colour, hydroxymethyl furfural, acidity, pH, proline, diastase and invertase. The sugar profile included fructose, glucose, sucrose, maltose, isomaltose, trehalose, turanose and melezitose, which was performed by HPLC and differential refractive index detector. All analyses were performed on 76 honey samples. The chemometric methods included were PCA and SDA. The PCA scores plot for the second and third PCs showed a quite good clustering for blossom and honeydew honeys, with some overlapping at the centre of the graphic. But by SDA, a 95.8 and 100% correct classification was obtained for honeydew and blossom honey, respectively. For this reason, sugars and physicochemical analysis of honey, in combination with SDA can be useful to determine authenticity of blossom honeys from Spain [74].

To obtain the botanical classification of honey from Soria (Spain), the profile of 14 carbohydrates of 77 natural honey samples was performed by HPLC-PAD, using PCA and canonical discriminant analysis (CDA) as multivariate tools.

The botanical honeys were spike lavender, French lavender, forest, thyme, ling and multifloral. For the PCA model, all sugar variables were used. The scores plot was shown under the way of a centroid plot (instead of all samples). The centroid scores plot showed points well resolved, with good discrimination between forest, thyme, French lavender and ling, while spike lavender and multifloral honey appear as quite overlapping. CDA was carried out in a sequential way (4 steps) using six sugars (erlose, nigerose, trehalose, melezitose, isomaltose, and panose) obtaining a range of samples correctly classified from 80 to 100 %, with better prediction results for ling, spike lavender, French lavender and forest honeys [45]. Amino acid analysis has been frequently used to classify botanical origins of honeys from Spain. Thereby, the study of five monofloral honeys (rosemary, orange blossom, eucalyptus, lavender and thyme) was performed using the content of 23 amino acids. The analysis was carried out by HPLC and UV detector with prior isolation and derivatization of amino acids from honey samples. A PCA model was obtained using 8 amino acids (proline, phenylalanine, tyrosine, lysine, arginine, glutamic acid, histidine and valine). A 3D scores plot using the first, second and third PC, showed that the lavender group had the best discrimination, while the rest of honeys showed overlapping. Authors stated that amino acid composition was not absolutely able to distinguish the studied botanical origins of honey samples from Spain [75].

In another work, the analysis of amino acid profile and physicochemical parameters was carried out on 46 honey samples from central Spain. The study involved the discrimination of floral honey from honeydew, using PCA and CA. The physicochemical variables included a complete analysis: pH, conductivity, ash, glucose, fructose, free acidity, lactonic acidity, total acidity, water content, diastase activity, R-glucosidase, α -glucosidase, total phenols, chromatic parameter, net absorbance, amino nitrogen, protein and total nitrogen. The amino acid profile involved the analysis of 23 amino acids: aspartic acid, glutamic acid, asparagine, serine, glutamine, histidine, glycine, threonine, arginine, β-alanine, α-alanine, α-aminobutyric acid, tyrosine, γ-aminobutyric acid, methionine, valine, tryptophan, phenylalanine, isoleucine, leucine, ornithine, lysine and proline. The classification analysis was performed separately for both, physicochemical parameters and amino acid profile: for the first, PCA and CA were used while for the second, only CA was informed. The best results were obtained by means of the physicochemical parameters, either for PCA and CA, due to the fact that it produced two separate well-defined groups, where all samples belonged to their own group. By means of the amino acid profile, CA showed that only a sample of honeydew was misclassified, but the rest were correctly classified: in this case, glutamic acid and tryptophan were the best variables to achieve the best differences between floral honeys from honeydew [76].

3.16. Sudan

Five botanical origins (*Ziziphus, Helianthus, Acacia seyal, Azadirachta* and *Acacia nilotica*) of honeys from Sudan were classified by using their amino acid profile, determined by an amino acid analyzer while data analysis was carried out by PCA, CA and LDA. Seventeen amino acids were analyzed on 15 samples of monofloral honeys. This paper showed the PCA loadings plot, with the relevant original variables, but the scatter plot for samples is not shown. The dendogram obtained by CA showed good classification for *Ziziphus* and *Azadirachta*, but there was overlapping with the rest of the botanical honeys. However, by LDA, 100% correct classifications were obtained for 4 monofloral honeys: *Ziziphus, Helianthus, Acacia seyal, Azadirachta*, while for *Acacia nilotica* the ability of the model was 66.67%. LDA was able to identify honey source based on their amino acid composition with good prediction ability, considering the low number of samples for botanical origin analysis [77].

3.17. Switzerland

The Swiss region is an important market of honey in Europe; it has a high annual per capita consumption of honey (1.0 – 1.8 kg), together with other countries of the EU such as Germany, Austria, Portugal, Hungary and Greece. Swiss researchers proposed the analysis of volatile compounds, using MS-based electronic nose combined with PCA and FDA to classify monofloral honeys. Sampling obtained by use of head space (SHS), solid phase micro extraction (SPME) and inside-needle dynamic extraction (INDEX) was performed. The monofloral honeys included acacia, chestnut, dandelion, lime, fir and rape. The best results were obtained by the SPME sampling mode, in combination with PCA and FDA using 7 ionic masses to obtain a correct classification ranging from 88-100% of monofloral honeys [78].

The characterization of monofloral and polyfloral honeys was carried out using front-face fluorescence spectroscopy (FFS) as preliminary study. The measurements were carried out in diluted honey and the normalized spectral data of emission were analyzed by the PCA and LDA. The botanical honeys were the following: acacia, alpine rose, alpine polyfloral, honeydew, chestnut, rape and polyfloral. By means of the LDA, a good classification of honey samples was obtained, with a recognition ability ranging from 67 to 100%: rape honeys had a low prediction ability (67%) but the rest of honey had a 100% correct classification [10].

After that, other authors from this country carried out a new botanical classification by FFS and multivariate analysis. Seven botanical origins were studied (acacia, alpine rose, chestnut, rape, honeydew, alpine polyfloral and lowland polyfloral) in 62 honey samples. The assays were carried out using excitation and emission normalized spectra data. The used multivariate tools were PCA and FDA. PCA was used as a reducing variables tool, but the best results were obtained by FDA, which showed a very good prediction ability for monofloral honey samples (100%) but low ability for polyfloral honeys (50-66.7%). The results obtained using PCA-FDA were performed by combination of excitation and emission spectra data. The ability of this PCA-FDA combined model demonstrated that coupling different normalized spectra of honey samples, allowed to classify different monofloral honeys in a very efficient way; however, for polyfloral honeys it had a low classification ability [79].

The classification of monofloral honeys, based on Fourier-transformed infrared (FTIR) spectroscopy and surface acoustic z-nose sensor was preformed. Seven monofloral honeys were studied. The multivariate tools involved were

PCA, canonical variate analysis and probabilistic neuronal network. The varieties of honey analyzed were: orange blossom, carrot, wildflower, clover, buckwheat and alfalfa. The accuracy of analysis was 100% and the multivariate models had a fit with a value of $R^2 > 0.98$ for the PCA-canonical variate analysis and probabilistic neuronal network [80].

The botanical origin of 364 honey samples using FT-NIR combined with LDA was determined from the following varieties: acacia (*Robinia pseudoacacia*), alpine rose (*Rhododendron spp*.), sweet chestnut (*Castanea sativa*), rape (*Brassica napusvar. oleifera*), fir honeydew (*Picea spp.* and *Abies spp.*), lime (*Tilia spp.*); dandelion (*Taraxacum s.l.*) and multifloral honeys. A first classification, considering every group separately, had a prediction ranging from 39 to 96% in the calibration step and from 19 to 100% in the validation step by using LDA. But if samples of dandelion, alpine rose, lime, rape, and polyfloral honey samples are combined in groups, the classification is improved. In this case the calibration ability ranged from 81 to 96% and the validation one from 79 to 88%. By using two groups of honeys (monofloral and non-monofloral honey) the LDA model improved from 87 to 96% in the calibration step and 84 - 93% in the validation step. Also, the classification of honey from blossom and honeydew was studied. In this case, the ability to classify ranged from 92 to 94 % to determine both types of honey. The results demonstrate that NIR spectroscopy is a rapid and nondestructive tool for the determination of the botanical origin of blossom-polyfloral and blossom-honeydew honeys [81].

Finally, Ruoff and others [82] used Fourier transformed mid-infrared spectroscopy with attenuated total reflectance (FT-MIR-ATR) combined with PCA and LDA, to discriminate 11 unifloral honeys (acacia, alpine rose, chestnut, dandelion, heather, lime, rape, fir honeydew, metcalfa honeydew, oak honeydew) and polyfloral honeys. The results showed a good prediction ability using the jackknife criterion with a correct prediction of 69% for polyfloral honey and 91-100% for monofloral honeys. The authors stated the differences between physicochemical properties of monofloral and polyfloral honeys were small. Also, only a few compounds could be correlated to a particular variety. In this context, the chemometric approach based on a spectroscopic "fingerprint" seems more promising than the use of certain chemical markers. From the results, the chemometric method showed superior performance for classifying monofloral honeys. In addition, it was noted that the IR spectra of the honeys studied were strongly dependent on their botanical origin rather than their geographical origin.

3.18. Switzerland and Germany

One thousand seventy five honey samples from Germany and 131 honey samples from Switzerland were analyzed to be classified according to their botanical origin, including rape, lime tree, false acacia, heather, cornflower, clover, sunflower and honeydew honey. The analysis included physicochemical parameters, sensory analysis and FTIR spectra. The classificatory analysis was carried out using only PCA calibration and validation. A first PCA analysis showed low classification ability, ranging from 20 for sunflower to 100% for false acacia. In the case of blends, the correct predictions ranged from 0 for heather blend to 80% for false acacia blend. By combination of physicochemical parameters and FTIR spectra, the correct classifications for pure honeys ranged from 50% for cornflower to 100% for heather, but minor prediction abilities for blend honeys were observed. Authors state that the prediction ability is improved by incorporating physicochemical and sensory analysis to the FTIR spectra. The use of other multivariate tools could also improve the results [83].

3.19. Switzerland, Italy & Germany

Again, the front-face fluorescence spectroscopy (FFS) was used as analytical tool for the determination of the botanical and geographical origin of honey, by chemometric (LDA) data analysis. The proposed method studied 374 honey samples from Switzerland, Italy and Germany. The botanical origin of samples was: acacia (*Robinia pseudoacacia*), alpine rose (*Rhododendron spp*.), sweet chestnut (*Castanea sativa*), rape (Brassica napus var. oleifera), fir honeydew (*Abies and Picea spp*.), oak honeydew (*Quercus spp*.), honeydew from Metcalfa pruinosa, heather (*Calluna Vulgaris*), lime (*Tilia spp*.), dandelion (*Taraxacum s.l.*) and polyfloral honeys. Authors used three different options for fluorescence spectrum: two wavelengths of excitation (at 270 and 310 nm) and one of emission (at 420 nm). The best results for botanical classification were achieved using a combined method of excitation (270 nm) and emission (420 nm). All monofloral honeys were correctly classified (more than 97%), but the dregree of accuracy of the polyfloral honey classification was quite lower (55%). Using only the emission at 420 nm, the results were something lower (more than 87% for monofloral honeys and 50% for polyfloral honey). By means of a two-step LDA, first grouped by botanical origin, and then as monofloral and polyfloral honey, the classification was improved, giving 100% correct classification for monofloral and 75% for polyfloral honeys. On the other hand, a study of geographical origin was performed. By means of discriminant scores plots, two clusters were obtained for fir honeydew honey (from Switzerland and Germany), and three clusters for lime honey from Italy, Switzerland and Germany. For acacia, lime, dandelion, and honeydew (spruce and fir), LDA showed correct classification ranging from 63-65% to distinguish honey from Switzerland and Germany. The method can be useful to evaluate botanical or geographical fraud of unifloral and polyfloral honey samples from these countries [84].

3.20. Uruguay

In this country, a study of the botanical origin of honey was carried out. In this work, honeys were classified according to six different botanical origins: pasture, *Eucalyptus spp*., *Citrus spp*., *Baccharis spp*., multifloral and others. The analytical variables were physicochemical parameters: moisture, pH, electrical conductivity, hydroxymethyl-2-furaldehyde and colour. Multivariate tools included PCA and LDA. With these variables, the ability of the model to provide a correct classification is low: the scores plot of the two first PCA represents the 86% of original information, but the work could not discriminate the different botanical honeys. Something similar occurs with the model obtained by LDA, whose errors ranged from 17-80% according to the botanical

origins. Authors stated that this could be explained by the low number of samples analyzed (30 samples) and that further studies are necessary to improve the accuracy of the multivariate models [85].

4. ADULTERATION OF HONEY

The analysis of substances used in honey for adulteration is an important application of multivariate tools to detect possible frauds. The addition of invert or other sugars in honey is a common case of adulteration. In general, the majority of analyses was performed by mid- and near- infrared spectroscopy.

The study of adulteration of honey with cane and beet sugars was performed using Fourier transform Raman spectrometry (FT-Raman) and multivariate data analyses by LDA and canonical analysis to control the quality of studied samples. Also, PLS and PCR analysis were successfully used for the quantitative analysis of both, cane and beet sugars. The results showed a correct discrimination between samples of natural honey and honey with added sugar [86].

On the other hand, the combination of PLS, LDA and FT-IR was useful to determine sugars, which are frequently used as adulterants in honey. The adulterants analyzed were glucose, fructose, sucrose, cane invert and beet invert. Three monofloral honeys were studied: orange blossom, buckwheat and clover. For simple sugars, LDA showed an ability prediction of 100% with 2 PC for adulterated samples, while for complex sugars (cane invert and beet invert) 100 % was achieved using 4 PC. Authors stated that the method was successful in discriminating from the original honey samples and can be used for a rapid detection of adulteration in 3 varieties of monofloral honeys [87].

The mid-infrared spectroscopy, using FTIR-ATR in combination with chemometric analysis, proved to be a good analytical tool to control possible sources of adulteration of honey, especially with sugars. In this case, the multivariate tools were PLSR and KNN analysis. By processing spectrum (normal, first derivative and second derivative spectrum of honey samples), PLS-DA had a discrimination ability of 84.9, 94.4 and 100% for adulterations of 7, 14 and 21% respectively, using second derivative spectra, while by quantitative PLS regression the prediction ability for the same concentrations was 82.2, 95.8 and 100%. Similar results were obtained by KNN, with 80.8, 94 and 100% correct samples for the same percentages of adulteration, if samples are grouped as honey and non-honeys; however the ability prediction was lower if samples are grouped according to their adulterant content. PLS and KNN were able to detect and quantify, adulterations of sugar above 7%, so the method can be useful for a rapid screening of sugars in honey for quality control [88].

The use of syrups for bee feeding affects the sugar composition of honey. The detection of industrial sugar syrups using anion-exchange chromatography–pulsed amperometric detection (HPAEC–PAD) and chemometric analysis was proposed. The botanical origins were the following: fir, chestnut, thyme-rose and adulterated honeys. Authors used chromatographic data combined with PLS and LDA. By LDA, the canonical analysis plot showed good discrimination ability for the 4 types of honeys, but the best results were obtained by the observed-predicted PLS plot, which showed a great ability to detect adulterants from 10%. The results indicated that it was possible to detect the use of syrups for bee feeding (instead of the natural nectar used by bees), which produces changes in the final composition of honey. On the other hand, the method was useful to detect the adulteration of honey with sugar syrups from 10 to 40 %, with an ability to predict close to 100% [89].

Again, honeys were studied by FTIR-ATR to find falsification by syrup in Ireland. The multivariate analysis was performed by the PCA, SIMCA model and KNN. The obtained spectra were transformed and second-derivative mid-infrared spectra were used to multivariate analysis data for 580 honey samples. The study of adulteration included commercial syrup: fructose/glucose syrup (50:50 and 45:55), partial invert cane syrup (32:32:36 fructose/glucose/sucrose), high fructose corn syrup, dextrose syrup and inverted beet syrup. The scores plot showed that PCA was able to classify the authentic samples from the other adulterated honeys. Also, using the SIMCA model with 6 PCs, the classificatory model showed a predictive ability of 96.2% to recognize genuine honeys from the others. Besides, the SIMCA model was able to predict adulterated honey samples with partial invert cane syrup (51.2 %), inverted beet syrup (97.5%) and cane syrup (95.8%). By the KNN model, authentic honey was correctly predicted with an ability of 88.8%, while adulterated honeys by fructose-glucose syrup and inverted beet syrup were correctly predicted with 75.5 and 74% accuracy, respectively. On the basis of the results obtained, the authors stated that the method was unlikely to be commercially useful and they indicate the difficulty in discriminating between these sample types by mid-infrared ATR spectra. [90].

Fourier transformed near infrared spectrometry (FT-NIRS) combined with PLS-DA chemometric tool were used to detect adulteration of 71 commercial honey samples from China. Samples were previously analyzed by the stable carbon isotope ratio technique to fix the adulterated samples: 44 adulterated and 27 pure honey samples. Five different PLS-DA models were obtained using different NIR spectral ranges. The calibration produced 91.49-94.87% correct classification, while in the validation step the result showed 86.96-93.75% correct prediction. Finally, the proposed method was applied to the samples, the results were: 100% correct classification rate for unadulterated honey and 95% of correctness for adulterated honey was gained. Authors suggest the combination of FT-NIRS and PLS-DA is a rapid and low-cost method to control commercial honey adulterations [91, 92].

FTIR-ATR was used again to quantify three different adulterants, as well as the geographical origin of honeys from Mexico. The adulterants studied were corn syrup, high fructose corn syrup and inverted sugar. Samples were collected in Chiapas, Oaxaca, Estado de México and Morelos states. PLSR was used to model the signal from each possible adulterant and pure honeys. Good linear fits were observed on predicted plots ($R^2 = 0.99$) for corn syrup, inverted sugar and high fructose corn syrup. Also, all samples were classified correctly according to their geographical procedence, using 3D-PLS scores plots. From these results,

authors agree that the use of FTIR-ATR combined with PLSR allow both, for the quantification of the content of several adulterants, as well as for the classification of honey samples from four different Mexico states [93].

Falsifications of honey with jaggery syrup were studied by the NIRS transflectance method and data analysis by PCA and PLSR. PCA was used to reduce the original variables and to detect the most important ones, while the adulterant was quantified by the PLS technique. The linear fit of the model was quite low ($R^2 = 0.81$), while the real samples had a standard error of 4.55%. However, this non-destructive method could be useful to discover adulterations with jaggery syrup in honeys [94].

Another study of honey adulteration with sugar syrup was carried out using one-dimensional (1D) and two-dimensional (2D) NMR coupled with LDA: 63 genuine honeys and 63 adulterated honeys were studied. The spectra used for the multivariate analysis were DMSO-d_6 ^{1}H NMR and DMSO-d_6 ^{1}H-^{13}C HMBC. The scores plot of canonical functions 1 and 2 for DMSO-d_6 ^{1}H NMR, showed a good discrimination for all samples (authentic, 10, 20 and 40% of adulterant), but the scores plot for DMSO-d_6 ^{1}H-^{13}C HMBC showed an overlapping of samples with 10 and 20% of adulterant. However, in both cases the authentic samples were well resolved. The classification and prediction for DMSO-d_6 ^{1}H NMR had an ability of correct prediction ranging from 90.5 to 100% for adulterated samples and 100% for authentic samples, but DMSO-d_6 ^{1}H-^{13}C HMBC had a lower prediction ability: from 76.2 to 100 % for adulterated samples and 100% for the true ones. Authors consider the obtained results were effective enough for honey adulteration detection with sugar syrup [95].

5. CHEMOMETRIC CLASSIFICATION OF OTHER BEEHIVE PRODUCTS

The use of chemometric tools has included the study of cuticular waxes from bees to determine properties and ecology of beehives. The multivariate tool used included the LDA, while capillary gas chromatography was used as analytical method. The substances analyzed were alkanes, alkenes, alkadienes, branch alkanes, esters, unsaturated esters, hydroxyalkil esters, organic acids and alcohols. The results showed that the discriminant function and chromatographic data allowed to classify successfully the age, sex and caste of bees of *Apis mellifera carnica*, using alkanes, alkenes, alkadienes, branch alkanes and esters as original variables [96]. Later, the same authors published another work on the discrimination of cuticular waxes in *Apis mellifera*, using GC-.MS and LDA. The results showed that it is possible to discriminate castes of bee workers: food storers, foraged, queen attenders and drones [97].

Propolis from Brazil was studied on the basis of the fingerprints obtained by electrospray ionization mass spectrometry (ESI-MS) and chemometric analysis by PCA. The propolis samples were obtained from 4 regions in Brazil: north, south, northeast and southeast, while the apicultural origin was diverse (11 different species of stingless bees). The PCA model showed the behaviour of samples according to the chemical composition of propolis, but this is not in agreement with its geographical origin. Authors, based on the classification and ESI-MS spectrum, concluded that the bee species used different plants from Brazil to produce propolis, as *S. terebenthifolius*, *S. bipunctata*, *Baccharis dracunculifoia*, and *Arauraria tree* [98].

The study of 49 propolis samples from different places around the world was performed by determination of their fingerprinting by easy ambient sonic-spray ionization mass spectrometry (EASI-MS). The mass spectra were analyzed by PCA. The PCA 3D scores plot showed a geographical classification based on 5 groups: group 1: Spain, Portugal and Iran; group 2: Korea, Australia, India, USA, Canada, Chile and South Brazil; group 3: Brazil and India; group 4: Brazil, Venezuela and Chile; and group 5: Korea. Every group was characterized by a distinct mass peak, which depends on the plant where it is, on the resin and on their geographical origin. EASI-MS permits the grouping of samples according to their botanical and geographical origin, identifying the plant origin of propolis, comparing extracts of its resins with known plant samples [99].

Finally, Argentinean propolis were classified based on their elemental content analyzed by neutron activation analysis (NAA). The chemometric tools were SDA and KNN. Eight elements (Br, Co, Cr, Fe, Rb, Sb, Sm and Zn) were selected for both chemometric methods. A certified reference material (CRM) was used to evaluate the analytical accuracy of data. The propolis samples were geographically classified according their provenance: La Pampa, Buenos Aires and San Luis provinces. The results shown by the SDA projection plot had a good grouping of propolis samples, according to the 3 geographical provinces, with a prediction ability from 93.8-100% for calibration and 90.2-96.9% for cross validation. By the KNN method, for the calibration and cross-validation set, the prediction ability ranged from 93.8-100% in both cases. The elemental analysis, in combination with chemometric tools can be useful to ensure the provenance of propolis to consumers [100].

6. CONCLUSIONS

From this review we can infer that, along the last decade, the interest in the authentication of honeys and bee products was in continuous growth. It is possible to group the chemical tracers in three principal groups: mineral contents, organic contents (such as sugars or amino acids) and spectroscopy data. Moreover, it was shown that most of the research groups used PCA and CA as first exploratory data analyses, but these tools were not sufficiently satisfactory to classify samples according to some criteria. Among the discriminant techniques, LDA continues occupying an important place between the most widely used tools. However, on the last years, the use of SVM and hybrid techniques, such as PLS-DA evidenced a growing tendency. The use of multivariate methods, not only for the botanical and geographical classification purposes, but also for adulteration of honey and classification of other apicultural products, will continue to rise because these tools are today necessary as a complement of chemical analyses.

In general, the best performance was achieved by the use of PLS-DA and NNA, followed by SDA and LDA. However, the performance of every multivariate tool will depend

on every particular case, on the number of samples, similarity of composition, selected analytical variables, and so on.

From the point of view of the number of studied cases, in general, the main contributions were performed by the European countries, because they have interest in the analysis based on geographical and botanical origins of honey- with a noticeable emphasis in the case of Corsican honeys' studies. Spain and Morocco, among others, can also be included in those countries interested in honey classification.

Regarding analytical procedures, they included a wide use of instrumentation. In general, the IR spectroscopy was one of the most widely used, but also others, such as Raman spectroscopy, NMR, chromatography technique, MS, AES and the incorporation of electronic nose, only to refer to some of them. The use of more selective and sensitive instrumentation tends to the search of specific markers for each type of honey.

In general, the studied species included elemental (or metal) analysis, amino acids or sugar profiles, classical physicochemical parameters and several volatile compounds. In this sense, the use of these species has not changed much along time, but its detection could be improved through the use of new analytical methods.

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LIST OF ABBREVIATIONS

AAS	=	Atomic Absorption Spectrometry
ANN	=	Artificial Neural Networks
ANN-MLP	=	Artificial Neural Network Based On The Multilayer Perceptions
AVQ-ANN	=	Vector Quantization Classification Systems Artificial Neural Networks
BA	=	Bayesian Analysis
CA	=	Cluster Analysis
CTA	=	Classification Tree Analysis
DA	=	Discriminant Analysis
D-OPLS	=	Discriminant Orthogonal Partial Least Squares Analysis
DRIFTS	=	Diffuse Reflectance Mid-Infrared Fourier Transform Spectroscopy
EASI-MS	=	Easy Ambient Sonic-Spray Ionization Mass Spectrometry
ESI-MS	=	Electrospray Ionization Mass Spectrometry
ETAAS	=	Electrothermal Atomic Absorption Spectroscopy
EU	=	European Union
FDA	=	Factorial Discriminant Analysis
FFS	=	Front-Face Fluorescence Spectroscopy
FTIR	=	Fourier Transform Infrared Spectroscopy
FTIR-ATR	=	Fourier Transform Mid Infrared Spectroscopy By Attenuated Total Reflectance
FT-NIRS	=	Fourier transformed near infrared spectrometry
FT-Raman	=	Fourier Transform Raman Spectrometry
GC×GC–TOF-MS	=	Two;Dimensional Gas Chromatography–Time Of Flight Mass Spectrometry
GC-MS	=	Gas Chromatography Mass Spectrometry
GDA	=	General Discriminant Analysis
HMF	=	Hydroxymethylfurfural
HPAEC-PAD	=	High Performance Anion-Exchange Chromatography–Pulsed Amperometric Detection
HS-SPME	=	Head-Space Solid-Phase Microextraction
ICP-AES	=	Inductively Coupled Plasma Atomic Emission Spectroscopy
ICP-MS	=	Inductively Coupled Plasma Mass Spectrometry
INDEX	=	Inside-Needle Dynamic Extraction
IRMS	=	Isotope Ratio Mass Spectrometry
KNN	=	k-Nearest Neighborhood
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
MLF-ANN	=	Multilayer Feed-Forward Artificial Neural Networks
MLP	=	Multilayer Perceptron
NNA	=	Neutron Activation Analysis
NIRS	=	Near-Infrared Spectroscopic
NMR	=	Nuclear Magnetic Resonance
PCA	=	Principal Components Analysis
PCR	=	Principal Component Regression
PDO	=	Protected Designations of Origin
PGI	=	Protected Geographical Indication
PLS	=	Partial Least Square

PLS-DA	=	Partial Least Squares Discriminant Analysis
PLSR	=	Partial Least Square Regression
POTFUN	=	Potential Function Techniques
RDA	=	Regularized Discriminant Analysis
SDA	=	Sequential Discriminant Analysis
SHS	=	Sampling Head Space
SIMCA	=	Soft Independent Modelling of Class Analogy
SPME	=	Solid Phase Micro Extraction
SVM	=	Support Vector Machines
TXRF	=	Total Reflection X-Ray Fluorescence Spectrometry
UNEQ	=	Unequal Class Model
VQBCP-ANN	=	Vector Quantization Based Classification Procedure Artificial Neural Networks

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