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Classification of Italian honeys by mid-infrared diffuse reflectance spectroscopy (DRIFTS)

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Abstract

Diffuse reflectance mid-infrared Fourier transform spectroscopy (DRIFTS) and multivariate statistical analysis methods were used for the identification and classification of honey from different floral sources. The 82 honey samples (robinia, chestnut, citrus, polyfloral) were scanned by DRIFTS in the region 4000–600 cm^{-1} and also transformed in 1st and 2nd derivatives. Spectral data were analyzed by principal component analysis, general discriminant analysis and classification tree analysis. Classification accuracy near 100% was obtained by discriminant and classification tree analyses. Classification models were successfully validated with one-third leave out method and a classification of about 100% were achieved.

Keywords: DRIFTS; Honey; Botanical origin; Multivariate statistical analysis; Classification

1. Introduction

The Italian annual nectar and honey dew honey production was estimated at about 13.000 tons for the year 2004 (Osservatorio Nazionale della Produzione e Mercato del Miele [National Observatory on Honey Production and Commercialization], 2005). The large variety of melliferous sources also enables Italy to produce characteristic unifloral nectar honeys and a high number of polyfloral nectar honeys. Among unifloral honeys, robinia (*Robinia pseudoacacia* L.), citrus (*Citrus* spp.) and chestnut (*Castanea sativa* L.) honeys are very relevant for their production levels, distribution and consumer preference, besides, many other unifloral honeys are produced in more limited amount. Each product is unique on the basis of the nature, amount and combination of the various components that give to honeys a unique and individual organoleptic character. The control and characterization of quality and botanical origin of unifloral honeys is of great importance and interest in apiculture. Today the most important techniques to determine or certify the unifloral origin of honeys are the palynological analysis besides the evaluation of organoleptic characteristics. To avoid errors these control must be conducted by a group of very expert testers and if the classification of honeys is attempted only on the basis of the above mentioned parameter, it can sometimes be imprecise or ambiguous.

Various techniques were used in the past to determine the floral source of honey i.e. determination of saccharides, phenolic compounds, amino acids, proteins, physicochemical parameter or aroma compounds, (Abu-Tarboush, Al-Kahtani, & El-Sarrage, 1993; Andrade, Ferreres, Gil, & TomasBarberan, 1997; Anklam, 1998; Azeredo, Azeredo, Souza de, & Dutra, 2003; Conte, Gracco, & Piasenzotto, 2003; Hermosin, Chicon, & Cabezudo, 2003; Latorre et al., 1999; Nozal et al., 2005; Radovic et al., 2001; Serrano, Villajero, Espejo, & Jodral, 2004), however, the experimental method to determine this could be improved.

Unlike near-infrared reflectance spectroscopy (NIR) which has been extensively used to characterize food,

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agriculture products and honey and to quantify different components of certain foods (Afseth, Segtnan, Marquardt, & Wold, 2005; Corbella & Cozzolino, 2005; Davies, Radovic, Fearn, & Anklam, 2002; Garcia-Alvarez, Ceresuela, Huidobro, Hermida, & Rodriguez-Otero, 2002; Garcia-Alvarez, Huidobro, Hermida, & Rodriguez-Otero, 2000; Kim, Singh, & Kays, 2006; Quy, Ding, Tang, & Xu, 1999) the use of mid-infrared (MIR) techniques for the same purpose has been more limited. This is particularly true when it comes to performing diffuse reflectance mid-infrared Fourier transform spectroscopy (DRIFTS). DRIFTS was used in the past years to determine the degree of esterification in pectic substances (Gnanasambandan & Proctor, 2000; Monsoor, Kalapathy, & Proctor, 2001). More recently DRIFTS was used to characterize rice (Gangidi, Proctor, & Meullenet, 2002; Ozgul-Yucel & Proctor, 2004) and in a study on beans (Maurer, Ozen, Mauer, & Nielsen, 2004)

The mid-infrared region potentially has advantages over the NIR for the analysis of food because NIR is limited in its usefulness for spectra interpretation, due to the many overlapping bands and also because only bands related to OH, NH or CH are present. On the other side, MIR region is very rich of informations related to the molecular structures of compounds present in the samples, therefore Fourier transform mid-infrared spectroscopy can be used as an optical sensing technique to obtain biochemical fingerprints of samples rapidly with minor risks to produce artifacts if compared to other techniques and offers a valid way for qualitative characterization. Although considerable work is still needed to determine completely the effect of the different factors on the analytic results recorded for qualitative or quantitative purpose. Recently Sivakesava and Irudayaraj (2001) and Kelly, Downey, and Fouratier (2004) have applied FTIR spectroscopy to detect inverted beet sugar adulteration in honey; Irudayaraj, Xu, and Tewari (2003); Tewari and Irudayaraj (2004) have applied micro-attenuated total reflectance to quantify saccharides in multiple floral honeys and to classify different honey types (Tewari & Irudayaraj, 2005).

The objective of this work was to determine the feasibility of classification of Italian honeys based on mid-infrared spectra obtained by DRIFTS and multivariate statistical analysis of spectroscopic results, this technique, at our knowledge, is never been applied previously on honey.

2. Experimental

2.1. Material

(a) Ultrapure water, obtained by a MilliQ Plus 185 system from Millipore (Milford, MA. USA).
(b) Potassium bromide SPECTRANAL® (Fluka) (Buchs, Switzerland). Before use potassium bromide was completely dehydrated at 110° in oven.

2.2. Sampling

Eighty-two honey samples consisting of 22 robinia (*Robinia pseudoacacia* L.) honeys, 12 citrus species honeys, 19 chestnut (*Castanea sativa* L.) honeys and 29 polyfloral honeys were collected by CRA-INA (Consiglio per la Ricerca e la Sperimentazione in Agricoltura-Istituto Nazionale di Apicoltura; [Council for Research in Agriculture-Italian National Institute for Apiculture, Bologna, Italy]) during the years 2003–2005. To assure and certify the botanical origin of honeys CRA-INA performed preventively the palynological and organoleptic analysis of selected samples.

2.3. Samples preparation

One gram, exactly weighted, of each sample was dissolved in 100 ml of ultrapure demineralized water, after the complete dissolution, 4 ml of the solution were exactly transferred on glass plates, added of 900 mg of dehydrated potassium bromide (spectroscopy grade) and immediately frozen at −50 °C. After freezing, sample was freeze-dried, using a Edwards Modulyo freez dryer, until the complete removal of water. At the end of this process sample appeared as a very hygrofilous, fine white powder and was maintained under vacuum in a desiccator over phosphoric anhydride until analysis. This process was performed to avoid or minimize errors due to water absorbing peaks in DRIFTS analysis.

2.4. Spectra acquisition and transformation

DRIFTS measurements were carried out using a Perkin–Elmer series 160 Fourier Transform Spectrometer with a diffuse reflectance sampling accessory equipped with a static sample cup (1.5 cm in diameter). Anhydrous potassium bromide spectroscopy grade was used for the background spectrum, the scansions were taken for each sample from 4000 to 600 cm^{-1} at resolution of 2 cm^{-1} and 64 scansions were co-added before Fourier transform. Samples were analyzed in a randomized order and three replications of FTIR analysis for each sample were performed on three different sub-samples. The three obtained spectra were averaged and the average spectra was stored then the 1st and the 2nd derivative of averaged spectra were calculated. After the calculations all original and derivatized spectra were normalized and the spectral variables corresponding to the regions 4000–3648 cm^{-1}, 2400–1502 cm^{-1} and 750–600 cm^{-1} were deleted reducing the dataset to 998 variables. All calculation were performed using Spectrum version 2.00 Perkin–Elmer software. The original and derivative spectra were transferred to statistical software, organized and stored in three datasets of 998 variables and 82 samples each.

2.5. Statistical analysis

In order to achieve a reliable differentiation between different honey types, pattern recognition and supervised

pattern recognition procedures were applied to the datasets. The most important use of these chemometric methods is to represent the *n*-dimensional dataset in a smaller number of dimensions, usually two or three. This allows the observations of groupings of cases, which can define the structure of the dataset; therefore, principal component analysis (PCA), general discriminant analysis (GDA) and classification trees analysis (CTA) were used in this work in the attempt to classify honeys according to their spectroscopic profile. PCA allows to express a large portion of total variance of data with a smaller number of variables, which can be used to represent graphically the population of samples and to identify the most significant of original factors. GDA is used to determine whether a given classification of cases into a number of groups is an appropriate one. It can be used, for instance, to test whether a particular clustering of cases obtained from a unsupervised technique is a likely one. Also, this analysis can be use in the attempt to classify unknown samples. CTA are used to predict membership of cases or objects in the classes of a categorical dependent variable from their measurements on one or more predictor variables. The goal of classification trees is to predict or explain responses on a categorical dependent variable, and as such, this technique has much in common with the techniques used in the more traditional methods such as discriminant analysis. Starting with all observations in a single group and a set of independent variables, the observations are split into two groups on the basis of the independent variable or linear combination of variables that is judged to be the most important in reducing the total deviance in the categorical dependent variable. The two resulting groups are split further by the same or an alternative independent variable or linear combination that continues to reduce the total variation within the dependent variable. The partitioning is repeated until further splitting will not reduce the variation of the dependent variable. The flexibility of classification trees make them a very attractive analysis option, but when the typically more stringent theoretical and distributional assumptions of more traditional methods are met, the traditional methods may be preferable. Indeed, as an exploratory technique, or as a technique of last resort when traditional methods fail, classification trees are, in the opinion of many researchers, unsurpassed. In the past years CTA has been successfully used in different areas of healthcare such as case mix groups (Smith et al., 1992), hospital bed capacities (Harper & Shahani, 2002), cancer survival groups (Garbe et al., 1995), intensive care (Ridley et al., 1998) and maternity care (Harper & Winslett, 2004). Three different building tree methods were applied to datasets: (a) Discriminant-based Univariate Splits (DUS); (b) Discriminant-based Linear Combination Splits (DLCS), for both these approaches the divisions are calculated using quadratic discriminant analysis as in QUEST (Quick, Unbiased, Efficient Statistical Trees) (Loh & Shih, 1997); (c) Classification & Regression Tree-style Exhaustive Search for Univariate splits (C&RT) (Breiman, Friedman, Olshen, & Stone, 1984). For all of these three building methods, the FACT-style direct stopping was used as stopping rule (Loh & Vanichestakul, 1988).

To perform GDA and CTA a stronger reduction of variables respect complete datasets was necessary, therefore two different methods to select the variables were applied. The first method consisted in the selection, as statistical descriptors, of wave numbers corresponding to the principal signals in original and derivatized spectra, in this way three new datasets were obtained with 28 factors for original spectra, 22 for 1st derivative and 24 for the 2nd derivative respectively, this approach was applied in GDA and CTA; for GDA also the second method, consisting in the selection of 79 (number of samples – freedom degrees of considered system) wave numbers with the highest factorial loadings in PCA, was used. To estimate the prediction capacity of the proposed method in a first step of CDA and CTA, all samples were considered as calibration set, in a second step a one-third cross-validation method was applied.

To verify the effect of the presence of polyfloral honeys, the most performing techniques were repeated also on dataset without polyfloral honey samples. All statistical calculations was performed using Statistica 6.1 for Windows (StatSoft® Italia, Vigonza, Italy).

3. Results

3.1. Characterization of honey by DRIFTS

Fig. 1 shows the overlay normalized spectra of the four honey types, as evident all spectra are very similar, and present the typical band of sugars. The spectral region between 750 and 1500 cm^{-1} corresponds to the absorption region of principal monosaccharides and disaccharides of honey (fructose, glucose, sucrose and maltose). In the

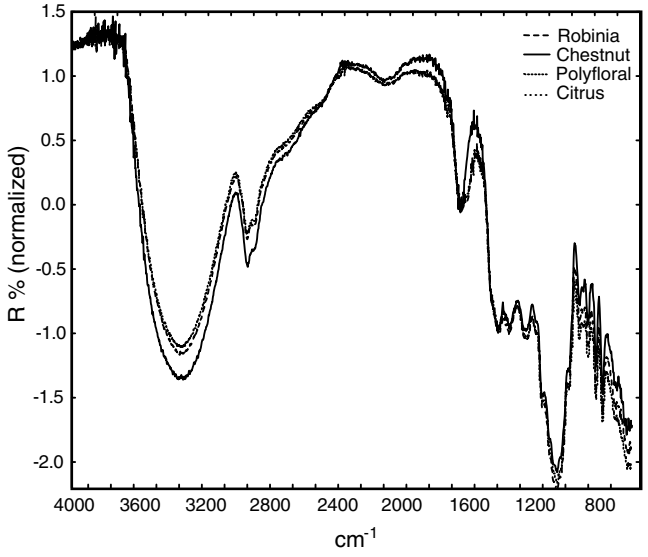

Fig. 1. DRIFTS spectra from four different floral sources honeys.

region 750–900 cm^{-1} the signals are related to the anomeric configuration of saccharides. The bands in the 900–1150 cm^{-1} are related to C–O and C–C stretching modes and those around 1200–1480 cm^{-1} are due to the bending modes of O–C–H, C–C–H and C–O–H. The bands at 2800–3000 cm^{-1} are related to the stretching mode of C–H groups and O–H of carboxylic groups. Bands around 3600 and 1600 cm^{-1} are related to O–H stretching mode and to residual water.

3.2. Results of statistical analysis

Principal component analysis. In Fig. 2, the principal component analysis results related to the dataset containing original spectra is reported, as evident this statistical method is only marginally able to group samples in function of the botanical origin. The first two components explain the 89.31% (PC1 80.23 + PC2 9.08) of the total variance. The application of PCA to the derivative datasets do not permit to improve the quality of the results, in fact the analysis of dataset containing the 1st derivative spectra the first two components explain only the 58.88% (PC1 32.41 + PC2 26.47) of the total variance, percentage that decrease to 41.09 (PC1 24.06 + PC2 17,03) in the case of the dataset of 2nd derivative spectra.

General discriminant analysis. In the case of the reduced datasets obtained by the selection of the most important signals, only the dataset containing original spectra shows interesting results (Fig. 3) in fact the model is able to group the 82 samples in four evident cluster. Performing analysis on datasets of 1st and 2nd derivatives the results become worst. Considering the datasets obtained by statistical selection of 79 most significant factors by PCA the quality of results seems to improve as evident from data reported in Table 1.

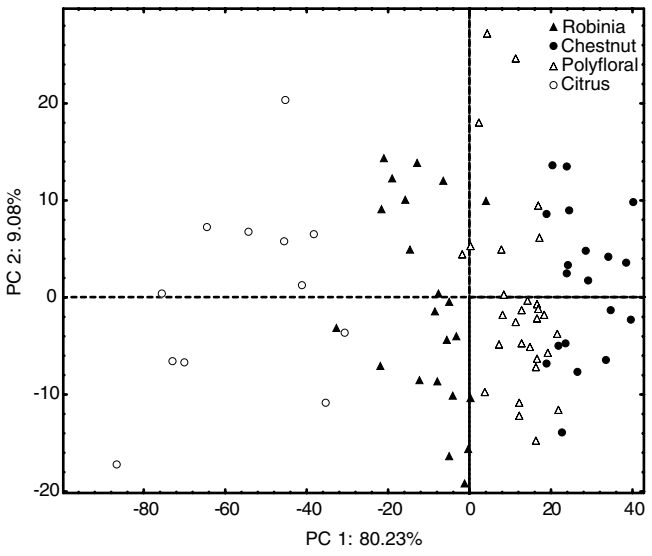

Fig. 2. DRIFTS-based honey classification using PCA on original spectra dataset.

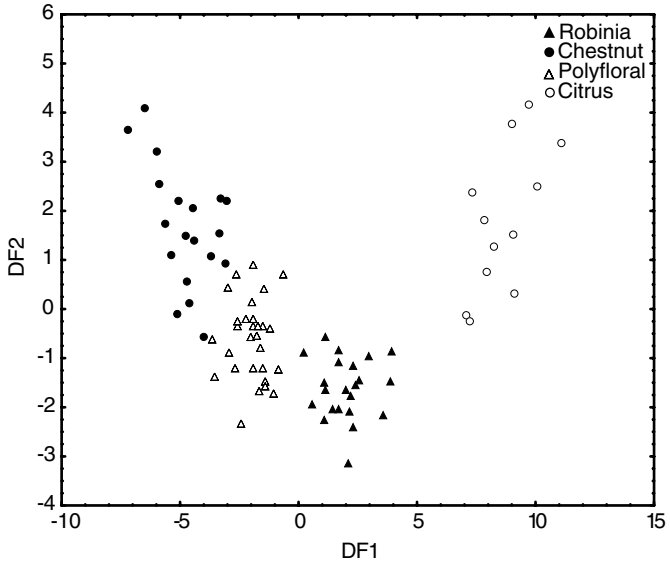

Fig. 3. DRIFTS-based honey classification using GDA on original spectra reduced dataset.

On all datasets a cross-validation test was performed using the one-third leave out method. The results obtained for calibration and validation sets are reported in Table 1. The best results was obtained from dataset of original spectra with selection of variables by PCA.

Classification trees. The obtained results are summarized in Table 2. As evident the best results were obtained for original spectra dataset, in particular if the performance in one-third leave out validation was considered, in fact the capacity of classification decreases notably in 1st and 2nd derivative datasets. As regard the classification capacity, among the tree building methods the most performing results DUS. In general, when the results of CTA were judged, the number of terminal nodes must be considered and a tree with a small number of terminal nodes must be preferred if the same capacity of classification is reached by different approach, therefore considering the classification results and the number of terminal nodes DLCS and C&RT appear to be a good compromise between classification capacity and the complexity of the tree. In Fig. 4 the classification tree obtained from original spectra by C&RT method is reported.

Considering datasets without the polyfloral honeys samples, the results seems to improve notably. As regard PCA analysis the clustering of samples is quite more evident and the first two components explain the 89.57% (PC1 82.23 + PC2 7.34) of the total variance. The same results are obtained by CDA on dataset containing the most important signal in these cases also the classification capacity increases to 98%, the same trend is evident also in CTA and confirms that in the considered spectroscopic technique the presence of polyfloral honey is the most important factor to consider in the analysis of results.

In conclusion DRIFTS coupled with appropriate multivariate statistical analysis seems to be a efficient and rapid

Table 1
Calibration and validation results using GDA on reduced and PCA-reduced datasets

	Variables (v) selection mode	Correct classifications (%)		
		All samples calibration ($n = 82$)	One-third validation	
			Calibration ($n = 55$)	Validation ($n = 27$)
Original spectra	Principal signals ($v = 28$)	100	100	85
	PCA ($v = 79$)	100	100	89
1st derivative	Principal signals ($v = 22$)	76	76	56
	PCA ($v = 79$)	100	100	60
2nd derivative	Principal signals ($v = 24$)	83	89	59
	PCA ($v = 79$)	100	100	75

v = no of variables in reduced datasets.

Table 2
Calibration and validation results using CTA with different building tree methods on reduced datasets

	Building tree methods	All samples calibration ($n = 82$)		One-third validation ($n = 55 + 27$)		
				Calibration set		Validation set
		Terminal nodes	Correct classifications (%)	Terminal nodes	Correct classifications (%)	Correct classifications (%)
Original spectra ($v = 28$)	DUS[a]	6	98	10	100	93
	DLCS[b]	4	100	4	100	85
	C&RT[c]	5	100	5	100	89
1st derivative ($v = 22$)	DUS[a]	35	88	26	96	48
	DLCS[b]	11	99	7	96	15
	C&RT[c]	23	95	17	98	56
2nd derivative ($v = 24$)	DUS[a]	37	95	26	95	37
	DLCS[b]	8	100	6	100	33
	C&RT[c]	22	98	16	100	37

v = no of variables in reduced datasets.
[a] Discriminant-based univariate splits.
[b] Discriminant-based linear combination splits.
[c] C&RT-style exhaustive search for univariate splits.

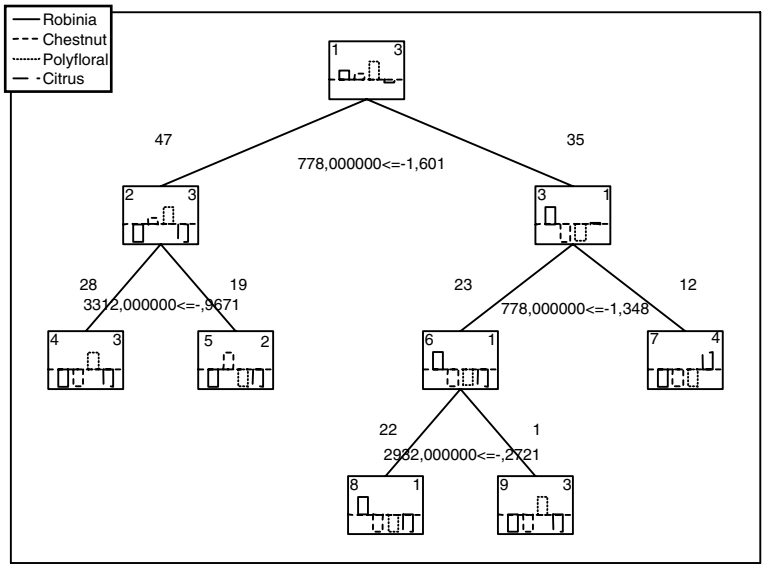

Fig. 4. DRIFTS-based honey classification using CTA (C&RT-style method) on original spectra reduced dataset.

technique for the classification of honey samples of different botanical origin, this is particularly evident if only unifloral samples are introduce in the system. Nevertheless DRIFTS appears as a sufficient efficient technique also in the presence of polyfloral honeys.

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