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Analytical Methods

Near infrared spectroscopy applied to the rapid prediction of the floral origin and mineral content of honeys

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ABSTRACT

Consumers demand to know the floral origins of honeys. Therefore, the use of simple and reliable techniques for differentiating among honeys by their origins is necessary. Multivariate statistical techniques and near infrared spectroscopy applied to palynological and mineral characteristics make it possible to differentiate among the types of honey collected from Northwestern Spain. Prediction models using a modified partial least squares regression for the main pollen types (*Castanea*, *Eucalyptus*, *Rubus* and *Erica*) in honeys and their mineral composition (potassium, calcium, magnesium and phosphorus) were established. Good multiple correlation coefficients (higher than 0.700) and acceptable standard errors of cross-validation were obtained. The ratio performance deviation exhibited a good prediction capacity for *Rubus* pollen and for *Castanea* pollen, whereas for minerals, for *Eucalyptus* pollen and for *Erica* pollen the ratio performance deviation was excellent. Near infrared spectroscopy was established as a rapid and effective tool to obtain equations of prediction that contribute to the honey typification.

1. Introduction

Honey is the natural sweet substance produced by bees from the nectar of plants, from secretions of living parts of plants or from excretions of plant sucking insects. The bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave the honey in the honey comb to ripen and to mature. As a natural, unprocessed and easily digested food, this product can be regarded as an important part of the human diet (Arvanitoyannis & Krystallis, 2006). Increasing honey consumption can be attributed to the consumer interest in natural foods with health benefits.

Honey originates from different plants whose partial contributions profile the characteristics of the final product. There is a great scientific and commercial interest worldwide concerning the characterisation of unifloral honeys; however, this characterisation is extremely complex (Dobre, Georgescu, Alexe, Escuredo, & Seijo, 2012; Seijo, Escuredo, & Fernández-González, 2011; Woodcock, Downey, & O'Donnell, 2009). Knowledge of the botanical origins of the honeys is important to avoid commercial frauds. However, in the European Union, there are no normative documents relating to the quality requirements of the different honey types.

Melissopalynology has been principally used to determine the source, content and relative concentrations of pollen grains in honey (Anklam, 1998). Pollen identification requires specialised and trained professional personnel with an extensive knowledge of pollen morphology. In addition, the interpretation of pollen spectra must consider the diverse floral biology of plants. Some plants, such as *Castanea sativa* or *Eucalyptus*, produce many pollen grains and appear overrepresented in the sediment of the blossom honeys, whereas other species, such as *Robinia pseudoacacia*, *Tilia*, *Citrus* or *Rosmarinus*, produce fewer pollen grains and, therefore, are underrepresented pollen types (Louveaux, Maurizio, & Vorwohl, 1978; Persano-Oddo, Piazza, Sabatini, & Accorti, 1995; Von der Ohe, Persano-Oddo, Piana, Morlot, & Martin, 2004).

The honey production of an area primarily depends on the vegetation and on the climate because the flowering and nectar production season can be different for the same species in different areas (Herrero, Valencia-Barrera, San Martín, & Pando, 2002). In Galician (Northwestern Spain), there are two clearly differentiated areas for honey production, the coast and the inland area. Coastal lands were intensively forested with *Eucalyptus globulus*, whereas inland is predominated by deciduous forests with *C. sativa* and *Quercus*. The progressive decline in agricultural activity has led to large tracts of previously arable land becoming overgrown with shrubs, of which *Rubus* is the predominant taxa (Escuredo, Seijo, & Fernández-González, 2011). Additionally, Ericaceae (*Erica*

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australis, *E. arborea*, *E. umbellata* and *E. cinerea* or *Calluna vulgaris*) and Rosaceae (mainly *Rubus* sp.) are abundant in this territory (Seijo et al., 2011).

Some chemical compounds have been evaluated in honey due to their relations with botanical and geographical origins. Among these chemical compounds, minerals are particularly important because the mineral content is dependent on the plant's absorption from the soil and from the environment (Alda-Garcilope, Gallego-Picó, Bravo-Yagüe, Garcinuño-Martínez, & Fernández-Hernando, 2012; Batista et al., 2012; González-Miret, Terrab, Herranz, Fernández-Recamales, & Heredia, 2005; Nanda, Sarkar, Sharma, & Bawa, 2003). The mineral composition would seem to be a good basis for a classification system primarily because minerals are stable and can be associated with the soil where melliferous flora grows (Batista et al., 2012; Fernández-Torres et al., 2005).

Great attention and effort have been focused on replacing pollen analysis by other identification parameters using a combination of analytical techniques and statistical tools (Anklam, 1998). Recently, spectroscopic techniques, such as near-infrared (NIR) spectroscopy, were used for the quantification of food compositional parameters; however, these techniques can also be used for the determination of complex quality properties, such as texture and sensory attributes (González-Martín, Hernández-Hierro, Barros-Ferreiro, Cordón, & García-Villanova, 2007; González-Martín et al., 2011; Lin, Zhao, Sun, Chen, & Zhou, 2011; Woodcock, Downey, & O'Donnell, 2009). Near infrared (NIR) spectroscopy is characterised as a rapid method with low cost, and the processing of the sample before analysis is minimal (Blanco & Villarroya, 2002; Chen et al., 2012; González-Martín et al., 2007, 2011; Qiu, Ding, Tang, & Xu, 1999). NIR spectroscopy can be applied to honey analysis for major components and for physical parameters (Davies, Radovic, Fearn, & Anklam, 2002; Downey, Fouratier, & Kelly, 2003; Qiu et al., 1999; Ruoff et al., 2007), as well as for prediction of the geographical origins of honeys (Chen et al., 2012; Woodcock, Downey, Kelly, & O'Donnell, 2007).

Considering the difficulties and the large period required for the analysis of pollen, as well as the environmental and the economic costs of chemical analyses, the NIR technology was used. Therefore, the aim of the present work was to use NIR spectroscopy as a quick and easy method to obtain mathematical equations for the prediction of the principal pollen types and minerals in honeys collected from Northwestern Spain. Finally, using multivariate statistical techniques, the honeys were discriminated according to their botanical origins using the mineral profile and the principal pollens presented in the sediments of honeys.

2. Materials and methods

2.1. Honey samples

Sixty honey ($N = 60$) samples were directly collected by beekeepers in different areas (inland and coast) from Galician (Northwestern Spain). The samples were transferred into glass bottles and were stored at 4 °C in dark conditions until used.

For the NIR analysis, 45 honey samples were randomly selected for the calibration set, whereas the remaining 15 samples formed the validation set.

2.2. Melissopalynological analysis

Samples were characterised using melissopalynological analysis to determine the principal pollen types and their botanical sources. The pollen analysis was based on the method established by Louveaux et al. (1978). For this analysis, 10 g of honey was dissolved in bidistilled water and centrifuged at 3373*g* for 10 min. Then, the obtained sediment was re-dissolved and centrifuged. The pollen spectrum of each honey sample was determined using light microscopy (Olympus Bx 50) by counting between at least 800 pollen grains in two 100 μl aliquots. The results were expressed in percentages.

2.3. Mineral analysis

The mineral composition was determined using an Atomic Absorption Spectrophotometer (Varian Spectra A-220 Fast Sequential; Agilent Technologies, Santa Clara, CA, USA). The identified minerals were potassium, calcium, magnesium and phosphorus. The samples were heated and sonicated to facilitate their homogenisation. Aliquots of 0.5 g honey were transferred into Teflon-coated vessels and digested in a microwave (CEM MARSX press model) oven after the addition of 5 ml of HNO_3–H_2O_2 (9:2) mixture (Caroli, Forte, Iamiceli, & Galoppi, 1999).

2.4. NIR spectroscopy and pretreatment

Honey samples were stirred to dissolve the crystals and were heated at 40 °C to obtain the homogenised honey before NIR analysis (Escuredo, Seijo, Salvador, & González-Martín, 2013). In total, 0.5 g of honey was deposited on a cam-lock cup (capsules for liquid-pasty samples, IH-0345-1 model, Foss NIR system) of a 0.1 mm optical path for their analysis, measuring the reflectance in the IR zone close to 1100–2498 nm. For each sample, 32 scans were recorded, with a spectral resolution of 2 nm. To minimise the sampling error, spectral readings were taken in triplicate for each sample. Subsequently, the mean of these replicates in the statistical treatment was used.

2.5. Statistical analysis

2.5.1. Spearman's rank correlation and canonical biplot analysis

Spearman's rank correlations were applied for evaluate the relations between the principal pollen types and minerals quantified. The coefficients varied between −1 and +1, representing the simple linear relation between variables. The correlation was considered significant at $P < 0.05$. Previously, an analysis of variance (ANOVA) was used to compare the quantified variables in the samples of honey according to their floral origins. The data were log-transformed to improve the normality and homogeneity of variance before analysis, and the Bonferroni post hoc test was applied.

Additionally, a canonical biplot was performed with the palynological information and mineral data of the honey samples. This method is a multivariate analysis method similar to MANOVA (Multivariate Variance Analysis), which permits simultaneous plots of different groups to be compared (Amaro, Vicente-Villardón, & Galindo-Villardón, 2004). The biplot method analyses multivariate data through techniques for the simultaneous representation of the *n* rows and *p* columns of a data matrix in reduced dimensions. Classical biplot methods are graphical representations of principal component analyses (PCA) that are used to obtain linear combinations that successively maximise the total. The results are usually plotted as dispersion diagrams of the data matrix and vectors for the different variables. This graphical representation allows the evaluation of approximations between groups of honey samples according their floral origins and mineral compositions.

These statistical analyses were performed using SPSS 19.0 Statistics software for Windows and STATGRAPHICS® Centurion XVI software.

2.5.2. Principal component analysis

Principal component analysis (PCA) was performed using NIR spectral data from samples of the calibration set using WinISI II