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Determination of the botanical origin of honey by sensor fusion of impedance e-tongue and optical spectroscopy

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ABSTRACT

The aim of this study was to discriminate four commercial brands of Portuguese honeys according to their botanical origin by sensor fusion of impedance electronic tongue (e-tongue) and optical spectroscopy (UV–Vis–NIR) assisted by Principal Component Analysis (PCA) and Cluster Analysis (CA). We have also introduced a new technique for variable selection through one-dimensional clustering which proved very useful for data fusion. The results were referenced against standard sample identification by classical melissopalynology analysis. Individual analysis of each technique showed that the e-tongue clearly outperformed the optical techniques. The electronic and optical spectra were fitted to analytical models and the model coefficients were used as new variables for PCA and CA. This approach has improved honey classification by the e-tongue but not by the optical methods. Data from the three techniques was then considered simultaneously. Simple concatenation of all matrices did not improve the classification results. Multi-way PCA (MPCA) proved to be a good option for data fusion yielding 100% classification success. Finally, a variable selection method based on one-dimensional clustering was used to define two new approaches to sensor fusion, and both yielded sample clusters even better defined than using MPCA. In this work we demonstrate for the first time the feasibility of sensor fusion of electronic and optical spectroscopy data and propose a new variable selection method that improved significantly the classification of the samples through multivariate statistical analysis.

1. Introduction

The determination of the geographical and botanical honey origin is of the outmost importance not only because of specific legislation (Codex Alimentarius Commission, 2001; European Union, 2001) but also because of market demands. Additionally, unifloral honey types have higher market value due to their limited production and availability.

Melissopalynological analysis, i.e., the identification and quantification of pollen grains in the honey sediment, is the reference method when determining the botanical origin of honey. However, this method involves a laborious counting procedure requiring specialized knowledge and expertise in the interpretation of results making the identification of the honeys botanical origin rather difficult and very time-consuming (Bianchi et al., 2005; Kaškonienė and Venskutonis, 2010).

Hence, there is a need for detection methods that are non-invasive, faster, more manageable and adequate for wide-scale sample screening. In the past decade, electronic devices such as the electronic-tongue (e-tongue) or the electronic-nose (e-nose) have seen increased use, and coupled with chemometrics have been shown to be useful tools for the discrimination of honeys botanical origin [for a review see Cavaco et al. (2012)]. E-tongue systems are emerging as fast and easy to-handle measurement techniques with very promising applications in food process control and quality evaluation including the determination of honeys floral origin (Dias et al., 2008; Hruskar et al., 2008; Wei et al., 2009; Escriche et al., 2012).

Visible–Near infrared (Vis–NIR) and ultraviolet–visible (UV–Vis) spectroscopies are also widely used for honey screening. They are not particularly sensitive techniques, but they can be very useful in probing bulk material with little or no sample preparation as required for real-time measurements. Vis–NIR spectroscopy can be used in all stages of food processing, from raw material analysis

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to finished product verification (Nicolai et al., 2007), while UV–Vis spectroscopy is useful to detect a wide number of antioxidants, such as phenolic compounds, responsible for the honey biological properties (Antolovich et al., 2000; Estevinho et al., 2008) and considered to be good markers for the authenticity and botanical origin of honey (Tomás-Barberán et al., 2001).

The combined use of two or more techniques should improve sample classification; however, the main challenge is how to combine data coming from each technique. There are few examples of sensor data fusion in food science. Ozer et al. (1995) used sensors for the determination of several fruit quality parameters and fused the data to improve the efficiency of automated fruit sorting. Li et al. (2007) used the fusion of volatile profiles obtained from e-nose and zNose™ to improve the detection of damaged apples. The fusion of electronic taste and aroma sensing was applied successfully to the determination of the floral origin of six varieties of honey (Hallis et al., 2010). A combination of e-nose and e-tongue measurements was used to determine sugar-adulterated honey among 18 samples (Zakaria et al., 2011). In all cases the enormous amount of data generated by these systems must be processed using appropriate multivariate analysis techniques (Cavaco et al., 2012).

The aim of this study was to group Portuguese honey of different botanical origin using a non-invasive e-tongue and spectroscopy (Vis–NIR and UV–Vis) sensor fusion approach combined with chemometrics. This study presents two innovative approaches, namely the sensor fusion of electrical and optical measurements, and also a new data fusion technique based on a criterion for individual variable selection, which shows great potential for the classification of honey samples.

2. Materials and methods

2.1. Honey samples

Thirteen commercial Portuguese honey nectar samples of the same production lot were purchased locally. The samples represented four unifloral types, four producers, four harvest years and three geographic origins as stated on the product label. The four unifloral honey types were *Citrus* spp. (orange blossom, OB), *Helianthus annuus* (sunflower, SF), *Lavandula stoechas* (French lavender, FL) and *Arbutus unedo* (strawberry-tree, AU). The 13 samples were distributed as follows: 3 OB, 4 AU, 3 FL and 3 SF. All samples were kept in their original package and left at room temperature until further analysis. All determinations here reported were conducted in duplicate or triplicate, the value used for data analysis was the corresponding mean value.

2.2. Melissopalynological analysis

All honey samples were tested by melissopalynology to confirm the botanical origin stated on the product label (Lieux, 1980; Louveaux et al., 1978; Von Der Ohe et al., 2004). A random sample (20 g) was dissolved in 40 mL of distilled water at 40 °C, stirred and then centrifuged for 15 min at 4640*g* (Kubata, model KN-70, Japan). The supernatant was removed and a mixture (10 mL) of sulphuric acid and acetic anhydride (1:9) was added to the remaining sediment in each tube. The reaction mixture was placed in a 70 °C water-bath for 10 min (acetolysis). After 10 min centrifugation at 4176*g*, the supernatant was removed and a drop of liquefied Kaiser's glycerol gelatin (Merck, Darmstadt, Germany) was added to the pollen grains sediment. Finally, the entire mixture was spread on a glass slide over an area of about 20 × 20 mm. For each sample/triplicate, at least 800 pollen grains were counted. The pollen grains were grouped by pollinic types according to Valdés et al. (1987) and the corresponding data was expressed as percentages.

2.3. General sample preparation for e-tongue and optical analysis

Prior to instrumental analysis, honey samples were incubated in a water bath at 50 °C overnight to dissolve any crystalline material, manually stirred to ensure homogeneity, and adjusted with distilled water to a standard total solids content (70 °Brix). This is essentially a standardization procedure to level all the samples to the same soluble solids content and water content, allowing for consistent comparisons. Secondary dilutions were further performed to optimize the response of each instrument. In each case the dilution factor was the same for all the samples, and thus the premises of standardization were not affected. After the dilutions the honey samples (∼15 mL) were placed in Falcon tubes and allowed to equilibrate at room temperature (23 ± 2 °C) for 30 min before being used.

2.4. E-tongue measurements

The e-tongue consisted of four working electrodes [10 × 5 mm^2 plates of aluminum (Al), gold (Au), platinum (Pt) and indium thin oxide (ITO)]. These were embedded in a composite material and placed around a gold reference electrode. The electrodes were connected to an Agilent 4284A Precision LCR Meter (Santa Clara, CA, USA). The e-tongue was immersed in a 30 mL aqueous solution prepared by diluting a 20 g honey sample in 20 mL deionized water. Each electrode was set to a 50 mV voltage and a 20–2000 Hz frequency scan. The data collected at room temperature (23 ± 2 °C) included capacitance (C, nF), conductance (G, μS) and the ratio $G/\log(\omega)$ where ω is the angular frequency. Prior to each measurement, the sensor was conditioned in the honey solution until stable readings at the 20 Hz scanning frequency were observed. The sensors were rinsed with deionized water after every measurement. The e-tongue analysis generated a total of 252 (=3 × 4 × 21) experimental values per sample, i.e., three measurements (C, G, and $G/\log(\omega)$) with four electrodes (Al, Pt, Au and ITO) and 21 frequencies.

Our e-tongue performs impedance measurements, not the more usual voltammetric or potentiometric measurements. It delivers effective values for capacitance and conductance, accounting for the bulk honey and surface effects at interface honey/electrode. Also, the LCR meter is a two port instrument and our setup did not include a counter-electrode.

2.5. UV–Vis spectroscopy

An aliquot (5 μL) of honey sample diluted in deionized water (100 mL) was placed in a 1 cm path-length quartz cell (UV–Visible Spectrophotometer, Cary 50 Bio, Varian, Australia) using distilled water as a blank. In the 200–400 nm wavelength range, a total of 201 data points with an average separation of about 1 nm were collected in triplicate for each honey type.

2.6. Vis–NIR Spectroscopy

Honey samples were placed in 1 mL plastic 1-cm cuvettes. Spectra were recorded as absorbance (USB4000-Vis–NIR Spectrometer, Ocean Optics, USA). The first and last points showed a pronounced instrumental oscillation and were removed from the analysis limiting the data to the 403.9–1032.2 nm range yielding a total of 3347 data points. All Vis/NIRS measurements were conducted in triplicates against a blank of distilled water.