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Physicochemical characterization of *Lavandula* spp. honey with FT-Raman spectroscopy

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ABSTRACT

This study aimed to evaluate the potential of FT-Raman spectroscopy in the prediction of the chemical composition of *Lavandula* spp. monofloral honey. Partial Least Squares (PLS) regression models were performed for the quantitative estimation and the results were correlated with those obtained using reference methods.

Good calibration models were obtained for electrical conductivity, ash, total acidity, pH, reducing sugars, hydroxymethylfurfural (HMF), proline, diastase index, apparent sucrose, total flavonoids content and total phenol content. On the other hand, the model was less accurate for pH determination. The calibration models had high r^2 (ranging between 92.8% and 99.9%), high residual prediction deviation - RPD (ranging between 4.2 and 26.8) and low root mean square errors.

These results confirm the hypothesis that FT-Raman is a useful technique for the quality control and chemical properties' evaluation of *Lavandula* spp honey. Its application may allow improving the efficiency, speed and cost of the current laboratory analysis.

1. Introduction

Honey is a natural food product produced by honey-bees that possesses a high amount of available sugars [1] and is a rich source of amino acids, vitamins, minerals and other biologically active compounds [2]. Honey carbohydrates are composed up of about 70% monosaccharides (mainly glucose and fructose), 10–15% disaccharides and a minor concentration of trisaccharides [1]. The chemical composition of this beehive product depends on the botanical and geographical origin [3], which may be evaluated through several methodologies.

Usually, a sample is classified as *Lavandula* spp. monofloral honey (common name: Lavender honey) when the percentage of pollen grains from *Lavandula* spp. is higher than 15% [4,5]. Even so, this monofloral honey may present a large variation in pollen spectrum resulting from the large variability in the ecosystems surrounding the apiaries. *Lavandula* spp. honey is characterized by a pleasant floral aroma, sweet taste and a light amber colour. Its chemical and sensory characteristics make it a much-appreciated honey with a high commercial value in Portugal and in the international market, which make it essential to ensure an efficient and specific quality control for this product.

Vibrational molecular spectroscopy techniques are very useful for food and beverages' analysis due to their flexibility, efficiency and low cost [6]. Particularly, the use of FT-Raman is advantageous due to the small volume of sample required, the high data reproducibility and speed of analysis. Furthermore, in comparison to other spectroscopy techniques like FTIR or NIR, Raman has the advantage that spectral information avoids the interference related to the water molecule.

Spectroscopic techniques like FTIR, NIR or Raman spectroscopy have been used in the identification as well as quantification of the chemical composition of different products in food, pharmaceutical and other industries. Particularly, FT-Raman methodology is based on the scattering of light from near infrared radiation due to the vibrational energy of the molecules in the sample. FT-Raman has been used in food analysis, namely: quantitative analysis of vitamin A [7]; sugars in honey [8]; determination of erucic acid content in canola oil [9]; detection of vitamins B_2 and B_{12} in cereals [10]; classification of different vegetable oils and identifying adulteration on virgin olive [11]; assessment of the quality of Southern Italian honey Types [12]; qualitative analysis of food fraud [10]; controlling protected designation of

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origin of wine [13].

Regarding honey analysis, Kizil et al. [14] evaluated the chemical changes induced by gamma irradiation on the fructose content of honey. Batsoulis et al. [15] applied and modified a standard HPLC-based method and used FT-Raman spectroscopy to evaluate fructose and glucose percentage. Also, Corvucci et al. [16] demonstrated that Raman spectral data, in combination with PCA models, could be a good tool to identify the botanical origins. In addition, more recently, Tahir et al. [17] applied FT-Raman for the prediction of phenolic compounds (catechin, syringic, vanillic, and chlorogenic acids) measured by HPLC–DAD, antioxidant activity and ferrous chelating capacity measured by Spectrophotometry in honey.

As such, this study aimed to assess the potential of FT-Raman spectroscopy to be an accurate tool for the fast analysis of monofloral *lavandula* honey's quality.

2. Material and methods

2.1. Samples

One hundred (n = 100) Apis mellifera's honey samples were harvested by beekeepers from apiaries located in different regions of Portugal: Alentejo (n = 8), Almancil (n = 10), Bragança (n = 12), Castelo Branco (n = 7), Chaves (n = 10), Lisboa (n = 6), Lousã (n = 6), Marialva (n = 4), Mirandela (n = 15), Torres Vedras (n = 3), Vila Flor (n = 12) and Vimioso (n = 7). Samples were delivered at the laboratory and kept in the dark at 5 °C until further analysis, which occurred in no more than one month after the extraction from the hives; none of the samples had signs of fermentation or spoilage.

In order to ensure that all samples could be classified as *Lavandula* spp. monofloral honey, palynological analysis was performed. Those samples that did not meet the requirements to be considered as monofloral *Lavandula* spp. honey were rejected.

The qualitative pollen analysis was performed using the acetolysis method, as recommended by Louveaux et al. [18] and Von der Ohe et al. [5]. The examination of the pollen slides was carried out with a Leitz Diaplan microscope (Leitz Messtechnik GmbH, Wetzlar, Germany) at 400 × and 1000 ×. A minimum of 1000 pollen grains were counted per sample. The recognition of the pollen grains was performed using the reference collection of the School of Agriculture of the Polytechnic Institute of Bragança as well as different pollen morphology guides and palynology atlas. The following terms were used for pollen frequency classes: predominant pollen (P, more than 45% of pollen grains counted), secondary pollen (S, 16–45%), important minor pollen (IM, 3–15%) and minor pollen (M, 1–3%).

2.2. Physicochemical analysis

The physicochemical parameters of Lavender honey samples assessed in a first phase were: ash content (%); electrical conductivity (mS/cm); 5-hydroxymethylfurfural content (HMF) (mg/kg); free acidity (meq/kg), diastase activity (Schade units/g); reducing sugars (%); apparent sucrose (%); pH and proline (mg/kg). The determinations were carried out in agreement with the Official Methods of Analysis of Association of Official Analytical Chemists (AOAC, 1990) [19], Harmonised methods of the International Honey Commission [20] and the Codex Alimentarius [21].

The protein content (mg/kg) was determined according to the method described by Nogueira et al. [22].

Regarding the total phenolic content of the honey samples, it was estimated following the Folin–Ciocalteau method while the total flavonoid were evaluated using the methodology proposed by Elamine et al. [23]. Three replicate analyses (n = 3) were made using each sample. Results are expressed as mean value ± standard deviation.

2.3. Raman data acquisition and processing

The Raman spectra of the honey samples were acquired using a FT-Raman spectrometer (BRUKER, MultiRAM) equipped with a 180° high-throughput collecting lens, a ultra-high sensitivity Liquid Nitrogen-cooled Ge Diode detector, an integrated 1064 nm (9392.5 cm^{-1}), diode pumped, Nd:YAG laser with a maximum output power of 500 mW, for a working spectral range of 3500–70 cm^{-1} Stokes Shift. The instrumental parameters used for spectra acquisition were: spectral resolution: 4 cm^{-1}; scanner velocity: 5 kHz; number of sample scans: 100.

The system was operated using the OPUS software provided by the manufacturer. In order to minimize disturbances in the measurement conditions, an automatic motorized XY sample stage was used, accommodating well-plates with 96 sample positions, thus eliminating the need to constantly open the spectrometer for changing samples. Two measurements were performed for each sample. Mean spectra were used in all subsequent calculations.

The spectra were collected at a constant room temperature of 20 °C.

PLS regression was done based on the spectral decomposition using OPUS 7.5.18 BRUKER software according the methodology used in Anjos et al. [24].

The spectral data were regressed against the measured parameters, using the pre-processing methods for PLS-R analysis: multiplicative scatter correction (MSC); minimum-maximum normalization (MinMax); vector normalization (VecNor); straight line subtraction (SLS); constant offset elimination (ConOff); first derivative (1stDer); second derivative (2ndDer); first derivation with multiplicative scattering correction (1stDer + MSC); first derivation with vector normalization (1stDer + VecNor); first derivation with straight line subtraction (1stDer + SLS).

The total number of samples was randomly split into two groups (Set 1 corresponding to 70% of samples; and Set 2 containing the remaining 30% of the samples). This separation into two groups was performed automatically by the software OPUS (v 7.5 Build 7, 5, 18 (20140810), Bruker Optik GmbH, Ettlingen, Alemanha), in order to ensure the representability of the samples.

The first group of 70 samples were used for internal validation (cross-validation) and a second one, the remaining 30 samples, for test (validation set). Wavelength selection was done iteratively by comparing and combining wavenumber ranges, and automatically by defining significant wavenumber ranges with the help of the Martens uncertainty test. In a first step the infrared dataset was regressed against the calibration components, and by means of full cross-validation with one sample omitted a significant number of PLS components was obtained.

The results of the cross-validation were tested for a maximum rank of 10, higher values of coefficient of determination (r^2) and ratios of performance to deviation (RPD) and lower root mean square error of cross validation (RMSE) as the test set validation [25].

3. Results and discussion

Monofloral status generally refers to the presence of a single pollen type in quantities greater than 45% of the total pollen content in the pollen spectrum analysis. However, for honey samples containing under-represented pollen grains, like *Thymus vulgaris*, *Rosmarinus officinalis*, *Citrus* spp., *Lavandula* spp. and *Arbutus unedo*, the botanical classification must be achieved with a lower pollen frequency percentage - usually ranging between 10% and 20% [2]. The results of honey's pollen profile analysis allow determining its floral origin and confirmed that all samples could be classified as *Lavandula* spp. monofloral honey.

As mentioned before the Lavandula spp. monofloral honey needs to have a percentage of pollen grains from *Lavandula* spp. higher than 15% [4,5]. In this study the percentage of *Lavandula* spp. pollen grains of each honey sample ranged from 16% to 83%, evidencing that all samples analysed could be commercialized as *Lavandula* spp.