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Chemical and physical parameters of Portuguese honey: classification of Citrus, Erica, Lavandula and Eucalyptus honeys by multivariate analysis and FTIR-ATR spectroscopy

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Abstract

In this study eight samples of monofloral honey from Portugal, selected from four different botanical origins: *Eucalyptus* ssp., *Erica* spp., *Lavandula* spp. and *Citrus* spp, were studied. For each monofloral honey two samples from different producers were analysed. Several analyses were carried out: moisture content, water activity (aw), colour, ash, pH, free acidity, electrical conductivity and total phenol content.

All the data were statistically tested using variance analysis and principal component analysis (PCA) with the aim of classifying the honeys and identifying the most significant parameters in the classification. Measurements with FTIR-ATR spectroscopy were performed in order to correlate the previously chemical parameter and the obtained spectra.

The one-way analysis of variance, considering the factor botanical origin shows that, in general, this factor is highly significant. In particular it can be stated ($p<0.001$) that this occurs in almost all variables, that explained between 81% and 98% of the total variance.

From the statistical study by PCA of the variables analyzed, two factors explain 86.3% of the total variance between honey types. We can find a good correlation between ash, pH, free acidity, electrical conductivity, colour and total phenol content parameters that was explained by the factor 1 (66.6%). The moisture content and aw are explained by the second factor (19.8%) and are not correlated with the previously factor. In fact the water activity is a variable rarely described as discriminant for the different honey types.

PCA analysis shows a good separation between three groups: 1 - *Eucalyptus* Honey; 2 - *Erica* honey; 3 - L*avandula* and *Citrus* honeys. Usually the *Lavandula* species and *Citrus* species growth in similar habitats, consequently present a great percentage of both kind of pollens. In fact with the Scheffé test only the ash content and electrical conductivity are significantly different for these monofloral honeys.

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Keywords: Honey, Botanical origin, Physico-chemical analysis, FTIR – ATR spectra

Introduction

Forest loss and fragmentation are widely recognised as the two most important factors responsible for the degradation of the environment in Portugal. The continuing forest loss is a telling measure of the imbalance between human needs and wants and nature's capacity.
Beekeeping offers a great potential for development. Also, beekeeping is advocated to improve human welfare by alleviating poverty through increased household income; it is a source of food and nutritional security, raw materials for various industries, medicine, improved biodiversity conservation and enhancing environmental resilience.
Many authors (Mateo and Bosch-Reig, 1997; Persano-Oddo et al, 1995) have suggested the use of physicochemical criteria (pH, sugar content, electrical conductivity, proline, enzymatic activity, water content) analyses for the characterization of unifloral honeys. So, it is important to test the same methodologies in the characterization of monofloral honeys and at the same time find other faster methodologies.

Material and methods

Different commercial honeys from different floral sources were used in this study, namely: *Eucalyptus*; *Erica*; *Lavandula* and *Citrus*. For each monofloral honey two samples from different producers were analysed.
All honey samples were tested by the usual available physico-chemical tests: moisture content, aw, colour, ash, pH, free acidity, electrical conductivity. All determinations were made using methods adopted by the International Honey Commission and Portuguese standards (Bogdanov, 2002, NP-1307, 1976).
Total phenol content was determined by a modification of the Folin–Ciocalteu method and the results expressed as mg gallic acid equivalents (GAE)/100 g of honey.
The different parameters were analysed by a multivariate approach and Variance analysis. ATR - FTIR spectra were acquired with a Bruker FT-IR spectrometer (Alpha) using a diamond single reflection attenuated total reflectance (ATR) device and a zero filling of 2. Duplicate spectra per sample were obtained with 32 scans per spectrum at a spectral resolution of 4 cm^{-1} in the wavenumber range from 4000 to 400 cm^{-1}.
Principal component analyses (PCA) and partial least squares regression (PLS-R) modeling were performed using OPUS Quant 2 (Bruker Optics, Ettlingen, Germany).

Results and discussion

On Table 1 is reported the mean and standard deviation of different parameter evaluated in the honey samples. It seems that the honey with the same botanical origin, even from different regions, has similar total phenol content. Moreover, because the different honey samples from the some botanical origin present other kinds of pollen, we can observe a higher variability. This leads us to conclude that the floral origin is an important determinant in the antioxidant content. Similar results are reported by other authors (Beretta et al, 2005; Anjos et al, 2009).

Table 1. Physico-chemical parameters of honey samples (mean and standard deviation)

Samples	Moisture content (%)	Electrical conductivity (µS/cm)	Water Activity Aw	Ash (%)	pH	Free acidity (meq/Kg)	Total phenol content (mg GAE/100g)
Ua	19.3±0.1	673.0±8.0	0.61±0.02	0.32±0.01	3.9±0.1	28.96±0.45	61.99±0.40
Ub	18.3±0.1	883.3±5.0	0.58±0.02	0.42±0.01	4.1±0.0	40.93±0.46	83.40±1.11
Ra	17.8±0.2	263.3±4.0	0.57±0.01	0.07±0.01	3.1±0.0	31.88±0.18	25.47±0.42
Rb	17.4±0.1	186.6±1.6	0.56±0.02	0.01±0.00	3.1±0.0	22.14±0.19	39.84±0.16
La	18.7±0.1	198.9±1.2	0.60±0.01	0.06±0.01	3.3±0.0	20.23±0.16	15.40±1.09
Lb	18.4±0.0	216.0±2.0	0.58±0.01	0.05±0.01	3.2±0.0	28.88±0.98	23.77±0.91
Ea	17.0±0.1	431.7±4.6	0.56±0.01	0.16±0.02	3.6±0.0	23.75±0.89	22.81±0.91
Eb	16.8±0.1	483.0±5.6	0.55±0.01	0.16±0.01	3.5±0.0	23.18±0.22	24.11±3.23

All the other parameters are according to the reference values for honeys, but we can find some differences reported for different monofloral honeys. For the electrical conductivity, well correlated with the ash content, higher values for the *Erica* honey and lower values for the *Lavandula* and *Citrus* honeys were found. The colour parameters are well correlated with the phenol content and are clear that the darker honeys present higher phenol content.

The correlations described by PCA analysis show a good separation in the different physico-chemical parameters measured in the honey samples but also a good separation by different samples with different botanical origin. PC1 includes most of the information (up to 67% of the total variance) due to a higher correlation between the colour, ash, pH, free acidity, electrical conductivity and phenol content. PC2 explains 20% of the total variance, with the contribution of moisture content and Aw.

The score plot in the space of the two new variables PC1 and PC2 show clearly that the honey with a great amount of *Erica* pollen and *Eucalyptus* pollen present different physico-chemical behaviour. *Lavandula* honey are well separated from the previously samples too, but for the sample “a” the properties are very similar to the citrus honey. That behaviour could be explained because *Lavandula* and *Citrus* species grow in similar habitats, and their pollens are produced almost at the same time In fact, with the Scheffé test, only the ash content and electrical conductivity are significantly different for these monofloral honeys.

Figure 1 show the average ATR-FTIR spectrum of the all honeys in this study. The spectrum is dominated by two water bands at 3284 cm^{-1} (OH stretch) and 1641 cm^{-1} (OH deformation) and from about 1500 to 750 cm-1 the contribution from mono- and disaccharides of the honey. The most important ones being the band with a maximum at 1025 cm^{-1} from the C-O and C-C stretch and COH deformation.

The PCA of the raw spectra of all honeys in the range of 1800 to 742 cm-1 allowed the separation of the honeys in three groups according to the honey composition. The first group includes *Erica* honey, the second group includes the *Eucalyptus* from South of Portugal (Lagos) and a third group that included the *Citrus*, the *Lavandula* and the *Eucalyptus* honeys. The third group had in common the same geographic origin.

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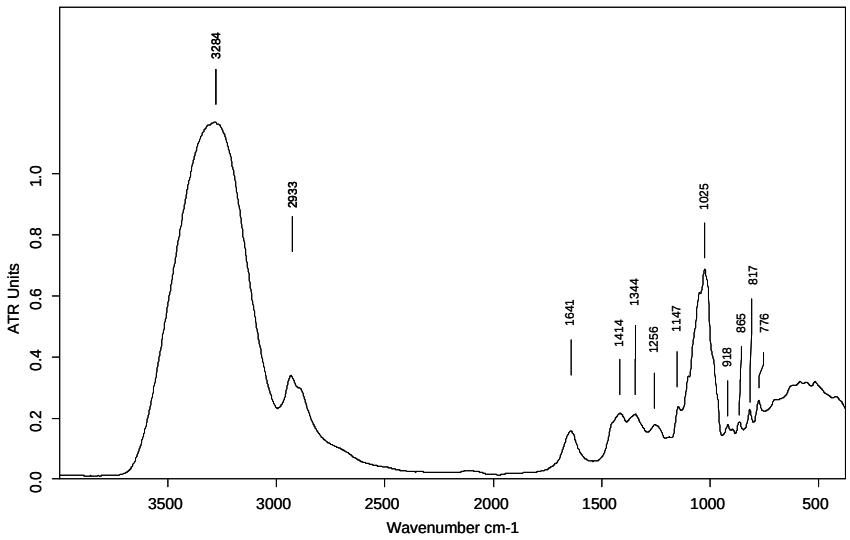

Figure 1. ATR-FTIR spectrum of the average spectra from all honeys acquired from 4000 to 450 cm^{-1}.

Promising models were obtained by PLS-R modelling with high coefficients of determination of the cross-validation for electrical conductivity (R^2= 0.88; RPD=2.9); ash content (R^2= 0.91; RPD=3.3); colour-L parameter (R^2= 0.97; RPD=6); colour-A parameter (R^2= 0.95; RPD=4.8); and phenols (R^2= 0.87; RPD=2.8). However, the small number of samples did not allow a proper validation of the models.

Conclusion

In the honey samples analysed all the parameters are in acordance with the standard values (*Codex Alimentarius*, NP-1307). Honeys with diferent botanical origin have diferent physico-chemical caracteristics namely in the phenol content, color and electrical conductivity.
PCA for physico-chemical parameters and raw spectra is a good methodology to separate honeys with different botanical origin. Moreover, in a preliminary approach, it seems that the models obtained by PLS-R modelling could be a good and faster methodology to predict the physico-chemical parameters in honey samples

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