Research Article

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Evaluation of FT-Raman and FTIR-ATR spectroscopy for the quality evaluation of *Lavandula* spp. Honey

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Abstract: Monofloral *Lavandula* spp. honey is very appreciated by consumers due to its characteristic and pleasant aroma and flavor. Given the economic importance of this type of honey, it is important to develop a rapid and non-expensive methodology that allows certifying its quality. In this context, this study aimed to compare the applicability and accuracy of FTIR-ATR and FT-Raman techniques for the quality evaluation of *Lavandula* spp. honey. Calibration models, with PLS regression models, were obtained for both methodologies concerning the following parameters: total acidity, reducing sugars, hidroximetil-furfural (HMF), electrical conductivity, ash, proline content, diastase activity, apparent sucrose, total flavonoids, and total phenolic contents. The calibration models had high regression coefficients, \( r^2 \) (FTIR-ATR: 0.965–0.996; FT-Raman: 0.983–0.999), high ratios of performance to deviation, RPD (FTIR-ATR: 5.4–15.7; FT-Raman: 7.6–53.7), and low root mean square errors (RMSEs; FTIR-ATR: 0.005–3.0; FT-Raman: 0.004–1.02). These results corroborate the potentiality of FTIR-ATR and FT-Raman for quality evaluation and evaluation of the chemical properties of *Lavandula* spp. honey even though FT-Raman technique provided more accurate models.

Keywords: vibrational spectroscopy, lavender honey, chemical composition, quality evaluation

1 Introduction

Honey is a natural sweet food product produced by honeybees that is mainly composed of sugars, and it is also a rich source of amino acids, vitamins, minerals, and other biologically active compounds (da Silva et al. 2016). Even though the general composition of honey is similar, the particular geographical and botanical origin is a key factors influencing the final quality, the specific chemical composition, and the bioactive properties of each honey type (Ohe et al. 2004; Anjos et al. 2015a).

In the European beekeeping context, monofloral honey from *Lavandula* spp. is a very appreciated high-quality type of honey owing to its peculiar and pleasant aroma and taste, presenting a characteristic sweet flavor with sour notes (Escriche et al. 2017). Usually, honey samples are classified as *Lavandula* spp. monofloral honey if the content of pollen grains from *Lavandula* spp. is above 15% (Ohe et al. 2004). However, monofloral honey may present considerable differences on the pollen spectrum resulting from the large variability in the ecosystems around the beehive.

Given the importance of this particular type of honey, some studies focused on the physicochemical and sensory properties of *Lavandula* spp. honey (Gomes et al. 2011; Castro-Vázquez et al. 2014; Estevinho et al. 2016). For consumer and industrial purposes, the physicochemical quality of honey is regulated by the European Union Council Directive 2001/110 (EU 2001), which provides limits regarding many parameters, such as moisture content, reducing and nonreducing sugars, free acidity, electrical conductivity, ash content, diastase activity, and 5-hydroxymethylfurfural (5-HMF) content.
Techniques like FTIR-ATR, NIR, and FT-Raman spectroscopy have been used in many different fields, such as food chemical characterization and food authenticity (Anjos et al. 2016, 2020; Cebi et al. 2017; Mandrile et al. 2017; Qin et al. 2017; Kasprzyk et al. 2018). Indeed, some studies were performed with the aim of applying FTIR-ATR technique to predict the properties of bee products, such as sugar profile (Gallardo-Velázquez et al. 2009; Anjos et al. 2015b; Tahir et al. 2017), content of phenolic compounds, and antioxidant activity (Tahir et al. 2017) or to identify adulterations (Gallardo-Velázquez et al. 2009; Gok et al. 2015). Other authors also evaluated the efficacy of this technique for monitoring protein degradation during the storage of royal jelly (Tarantilis et al. 2012). Some authors determined the sugar content of honey samples with good accuracy using FT-Raman (Batsoulis et al. 2005; Özbalci et al. 2013), while Mignani et al. (2016) used this technique for the quality assessment of Italian honey. Anjos et al. (2018) performed a calibration model for some honey analytical parameters using FT-Raman. Also, both methodologies have been reported to be effective for the quantification of the phenolic compounds and antioxidant activity of Sudanese honey (Tahir et al. 2017).

In this context, the aim of this study is to compare the ability of FTIR-ATR and FT-Raman spectroscopic techniques for the quality evaluation of *Lavandula* spp. honey samples, using partial least squares regression (PLS-R).

2 Material and methods

2.1 Honey samples characterization

Monofloral *Lavandula* spp. honey samples (*n* = 90) from different regions of Portugal were assessed in this study. Upon receipt into the laboratory, none of the samples had signs of any visible contamination, such as fermentation, spoilage, or field residues. Samples were kept in the dark at 5°C until further analysis, which occurred in no more than 1 month after.

Honey is classified as monofloral of a specific botanical origin when a certain percentage of pollen grains of that botanical species are present. This percentage is, for most of the botanical types, above 45% of the total pollen content. However, for honey samples containing under-represented pollen types, similar to the case of *Lavandula* spp. honey, monofloral classification is achieved whenever the percentage of pollen grains belonging to the botanical family is above 15% (Estevinho et al. 2013). In this study, the monoflorality of *Lavandula* spp. honey was verified by performing the palynological analysis following the acetolysis method (Louveaux et al. 1978; Ohe et al. 2004). The examination of the pollen slides was carried out with a Leitz Diaplan microscope (Leitz Messtechnik GmbH, Wetzlar, Germany) at 400× and 1,000×, and a minimum of 1,000 pollen grains were counted per sample.

The physicochemical characterization of honey samples was performed in agreement with the Official Methods (AOAC 1990; IFS 2001; IHC 2009) by analyzing different parameters such as moisture content (%), ash content (%), electrical conductivity (mS/cm), 5-HMF (mg/kg), total acidity (meq/kg), diastase activity (Schade units/g), reducing sugars (%), apparent sucrose (%), pH, proline (mg/kg), total phenolic content (mg/100 g), and total flavonoids content (mg/100 g). The protein content (mg/kg) of honey samples was determined according to the method described by Nogueira et al. (2012).

The determination of the total phenolic content of the honey samples was carried out using the Folin–Ciocalteau method, while total flavonoids were quantified using the methodology proposed by Elamine et al. (2018).

All analyses were performed using three independent replicates, and results are expressed as mean value ± standard deviation.

2.2 FTIR-ATR and FT-Raman data acquisition and processing

All spectra, obtained with FTIR-ATR (ALPHA, Bruker Optik GmbH, Ettlingen, Germany) or FT-Raman (Bruker Optik GmbH, Ettlingen, Germany), were acquired at a constant room temperature of 20°C. The FTIR-ATR honey spectra were acquired in Bruker FTIR spectrometer (Alpha) with a resolution of 4 cm⁻¹ in the wavelength region 4,000–400 cm⁻¹, using a diamond single reflection attenuated total reflectance (ATR). All spectra were obtained with 32 scans, and the background measurement was made using air.

The FT-Raman spectra of the honey samples were acquired using a FT-Raman spectrometer (BRUKER, MultiRAM) with a spectral resolution of 4 cm⁻¹, scanner velocity of 5 kHz, and 100 scans per sample. The FT-Raman was equipped with a 180° high-throughput collecting lens, a ultra-high sensitivity liquid nitrogen-cooled Ge Diode detector, an integrated 1,064 nm (9392.5 cm⁻¹),
diode pumped, and Nd:YAG laser with a maximum output power of 500 mW, for a working spectral range of 3,500–70 cm\(^{-1}\) Stokes shift.

The FTIR-ATR and FT-Raman systems were operated using the OPUS software provided by the manufacturer, and two measurements of the same samples were performed. Mean spectra were used in all subsequent calculations.

PLS regression was done based on the spectral decomposition using OPUS 7.5.18 BRUKER software, where 63 samples were used for the calibration set and remaining 27 for the validation set, which were randomly selected. The spectral data were regressed against the measured physicochemical parameters to obtain a significant number of PLS-R components. Before PLS-R analysis, the spectra were preprocessed as described in the study by Anjos et al. (2017, 2016) by MSC – multiplicative scatter correction; MinMax – minimum maximum normalization; VecNor – vector normalization; SLS – straight line subtraction; ConOff – constant offset elimination; 1st Der – first derivative; 2nd Der – second derivative; 1st Der + MSC – first derivation with multiplicative scattering correction; 1st Der + VecNor – first derivation with vector normalization; and 1st Der + SLS – first derivation with straight line subtraction.

The model that fitted best with the physicochemical properties was made according to the higher values of coefficient of determination \(r^2\), higher values of ratios of performance to deviation (RPD), and lower root mean square error (RMSE). The construction of this model is important for the industry to provide a quick access to the chemical parameters that allow decision-making for quality control (join or reject honeys batches).

**Ethical approval:** The conducted research is not related to either human or animal use.

3 Results and discussion

3.1 Honey samples characterization

A honey sample is classified as *Lavandula* spp. monofloral when the percentage of pollen grains from this species is higher than 15% (Ohe et al. 2004; Gomes et al. 2011). Before further analysis, it was confirmed that the 90 samples were correctly classified as such. Figure 1 depicts the results obtained for the pollen profile analysis of all samples, expressed as mean values and corresponding standard deviations, as well as minimum and maximum values. The results obtained for the percentage of pollen from *Lavandula* spp. confirm that all

![Figure 1: Pollen percentages in monofloral *Lavandula* spp. honey samples (minimum, maximum, mean, and standard deviation).](image-url)
samples had more than 15%, with variable concentrations up to 59%, being on average 32.7 ± 10.2%.

Even though the samples are classified as monofloral, they contain pollen grains from other botanical families. The percentages of other botanical pollen types found on *Lavandula* spp. honey were quite variable (Figure 1), with very small amounts in some species, such as *Eucalyptus* spp. (4.1 ± 0.9%), *Apium* spp. (6.7 ± 4.0%), or *Thymus* spp. (6.9 ± 4.5%), while higher amounts in others species, such as for example *Echium* spp. (23.46 ± 10.8%) or *Chamaespartium* spp. (23.11 ± 0.3%).

The variability of the physicochemical parameters is shown in Figure 2, where it is possible to confirm that all legal parameters were fulfilled, namely, acidity <40 mg/kg, diastase activity >8 Schade units/g, electrical conductivity <0.8 mS/cm, reducing sugar >60%; total acidity <50 meq/kg, and apparent sucrose <10% (EU 2001). Moisture variance is not shown not plotted in Figure 2, that all of them had moisture percentages less than 20%; in agreement with the limits established by the applicable legal parameters were fulfilled, namely, acidity <40 mg/kg, diastase activity >8 Schade units/g, electrical conductivity <0.8 mS/cm, reducing sugar >60%; total acidity <50 meq/kg, and apparent sucrose <10% (EU 2001).

The pH value of *Lavandula* spp. honey ranged from 2.8 to 4.5 (Figure 2), which are similar to those reported previously for Portuguese *Lavandula* spp. honey (Gomes et al. 2011; Estevinho et al. 2016).

Although proteins are minor components in honey, they are representative of the pollen source and have been reported as important chemical markers for the floral classification of honey and for detecting product’s adulteration (Won et al. 2008). The values obtained in this study ranged between 0.21 and 0.52% and are similar to those reported in the previous study (Estevinho et al. 2016).

According to the European Directive (EU 2001), almost all honey must have values for apparent sucrose content less than 5%. However, due to their floral origin, *Lavandula* spp. may have values of apparent sucrose up to 10%. In this study, all samples are in accordance with the European standards, and the higher value obtained for apparent sucrose was 7.21%.

Usually honey samples have important bioactive properties due to, among other factors, their total phenols and flavonoids contents. The total phenols and flavonoids contents of the analyzed honey samples are similar to those reported by other authors (Gomes et al. 2011; Estevinho et al. 2016) and varied from 88 to 229 mg/kg and from 5.7 to 15.8 mg/kg (Figure 2) respectively.

### 3.2 FTIR-ATR and FT-Raman comparison

FTIR-ATR and FT-Raman spectra of *Lavandula* spp. honey are shown in Figure 3. The spectral information obtained from FT-Raman is similar to that reported by previous studies, which determined fructose and glucose in honey (Batsoulis et al. 2005; Anjos et al. 2015a), assessed the accordance with legislation of honey (Mignani et al. 2016; Kasprzyk et al. 2018), predicted the content of phenolic compounds and antioxidant activity in honey (Tahir et al. 2017), and used other analytical parameters (Anjos et al. 2018).

The two spectroscopic techniques (FTIR-ATR and FT-Raman) measure the interaction of energy with the molecular bonds in a sample but differ in some fundamental ways. FTIR-ATR spectroscopy depends on a change in the dipole moment (measures absolute frequencies at which a sample absorbs radiation), while FT-Raman spectroscopy depends on a change in polarizability of a molecule (measures relative frequencies at which a sample scatters radiation). Honey spectra obtained using both methodologies show most of the spectral peaks in the 400–1,500 cm$^{-1}$ region for FTIR-ATR and 190–1,500 cm$^{-1}$ region for FT-Raman (Figure 3).

FTIR-ATR spectroscopy is sensitive to functional group vibrations and polar bonds, especially −OH stretching in water (large peak at 3,276 cm$^{-1}$); however, this peak does not appear in FT-Raman as explained earlier. Some peaks were also observed at 1,631 cm$^{-1}$, which was attributed to the −OH from the water.

The peaks observed around 2,900 cm$^{-1}$ may be assigned to the C–H stretching (Figure 3). However, the several calibration tests performed before the final essays suggest that this peak does not contribute with discriminant information for the calibration process.

The regions with more information for the calibration models were those between 1,500 and 400 cm$^{-1}$ for FTIR-ATR and between 1,500 and 190 cm$^{-1}$ for FT-Raman, and for that reason, they are shown in more detail in Figure 4.

According to some authors (Kizil et al. 2002; Paradkar and Irudayaraj 2002), the peaks observed in FT-Raman spectra (Figure 4) at 1,461 cm$^{-1}$ are associated with vibration of COO− group; at 1,367 cm$^{-1}$ with bending vibration of CH$_2$ group; and at 1,265 cm$^{-1}$ with bending of C–H and O–H bonds and vibration of C−O−H, C–C−H and O–C–H groups. The stretching vibration of C−O and
C–O–C and vibration of C–N of protein and amino acids appeared at 1,123 cm$^{-1}$ (Kizil et al. 2002). The peak at 1,072 cm$^{-1}$ was associated with the carbohydrates bending vibration of C–H and C–O–H as well as with a small contribution of the proteins and amino acids vibration of C–N bonds (Kizil et al. 2002). According to Zhu et al.
(2010), the peaks at 916 and 979 cm$^{-1}$ are associated with the vibration of C–H and C–O–H and also due to vibration in the two anomers of fructose and glucose, while peaks present at 825 cm$^{-1}$ may be assigned to the vibration of C–H and CH$_2$ (Mathlouthi et al. 1980; Kizil et al. 2002). Peaks obtained around 707 cm$^{-1}$ have been reported to correspond to the stretching of C–O and C–C–O, O–C–O bending and the peaks at 776 were assigned to the C–C stretching and C–H vibrations present in glucose (Kizil et al. 2002). The next bands at 625 and 520 cm$^{-1}$ were attributable to ring deformations and C–C–O and C–C–C deformation and that from 190 to 500 cm$^{-1}$ were observed skeletal vibrational modes, namely C–C–O and C–C–C, C–O and C–C (Tahir et al. 2017).

Figure 3: Average FTIR-ATR and FT-Raman spectrum of the Lavandula spp. honey for all spectral region.

Figure 4: Average FTIR-ATR and FT-Raman spectrum of the Lavandula spp. honey from 1,500 to 190 cm$^{-1}$. 
For the FTIR-ATR spectra, the most sensitive absorption region of the honey’s major components is situated between 750 and 1,500 cm\(^{-1}\) (Figure 4). The results showed that the most important peaks were observed at 1,017, 1,098, 816, 825, and 916 cm\(^{-1}\). According to some authors (Tewari et al. 2003; Bureau et al. 2009), the more important spectral regions and their causes are as follows: 1,411 cm\(^{-1}\) is a combination of O–H bending of the C–OH group and C–H bending of the alkenes; 1,321 cm\(^{-1}\) is due to O–H bending of the C–OH group; 1,110 cm\(^{-1}\) corresponds to the stretching of the C–O band of the C–O–C linkage; 1,043 and 1,264 cm\(^{-1}\) correspond to the C–O stretch in the C–OH group as well as the C–C stretch in the carbohydrate structure; and 916 cm\(^{-1}\) corresponds to the C–H bending of the carbohydrates.

### 3.3 PLS models

To identify the best methodology to develop calibration models for the quality evaluation of *Lavandula* spp. monofloral honey, PLS multivariate analysis was undertaken with spectral data. The preprocesses described in Section 2 were performed for all samples aiming to establish the best calibration models for each parameter and each technique (FTIR-ATR and FT-Raman).

Tables 1 and 2 summarize the results obtained for the optimal calibrations established for each parameter measured by FTIR-ATR and FT-Raman, respectively. Values of \(r^2\) for selected models ranged between 0.965 for pH and 0.996 for total acidity of *Lavandula* spp. honey for FTIR-ATR and between 0.983 for electrical conductivity and 0.999 for of total acidity, HMF, diastase index, and total phenol content for FT-Raman. Concerning the results obtained for calibration, almost all values of \(r^2\) are higher for the models performed with the spectral information collected in FT-Raman. Only for electrical conductivity, the value is slightly superior for the calibration in FTIR-ATR. For cross-validation, the values of \(r^2\) were lower than those obtained for calibration, nevertheless, being better in FT-Raman than in FTIR-ATR. The only case for which a low value of \(r^2\) was obtained was for cross-validation of pH data in FTIR-ATR.

Regarding the values of residual prediction deviation (RPD), higher values were obtained for FT-Raman (Table 2), and particularly for total acidity, 5-HMF, and total phenolic content.

All the other accuracy measurements (bias and root mean square error) are low, thus demonstrating the potential of both techniques. The potentiality of these techniques has already been demonstrated for *Lavandula* honey with FT-Raman (Anjos et al. 2018) and for other kinds of honey with both techniques (Anjos et al. 2015b; Batsoulis et al. 2005; Mignani et al. 2016; Tahir et al. 2017; Kasprzyk et al. 2018). The innovation in this study is the comparison of both techniques, which has not been achieved for honey and the number of analytical parameters that are evaluated, since the previous study.

### Table 1: Results for calibration and cross-validation for honey samples \((n = 90; 63 \text{ for calibration set and } 27 \text{ for validation set})\) for FTIR-ATR

| Parameters | Spectral range \((\text{cm}^{-1})\) | Preprocessing | Rank | Calibration set | Validation set | Bias |
|------------|---------------------------------|--------------|------|-----------------|----------------|------|
|            |                                 |              |      | \(r^2\)         | \(RMSE_c\)     | \(RPD\) | \(r^2\) | \(RMSE_v\) | \(RPD\) |
| pH         | 1,725–1,346 + 1,220–1,093 + 967–461 | VecNor      | 10   | 0.965 | 0.066 | 5.4  | 0.666 | 0.195 | 1.7  | 0.0122 |
| TA         | 1,326–1,219 + 1,075–824 + 575–449 | None        | 10   | 0.996 | 0.393 | 15.7 | 0.947 | 1.31  | 4.4  | -0.0009 |
| EC         | 1,599–1,220 + 840–714             | 2nd Der     | 9    | 0.985 | 0.007 | 8.1  | 0.924 | 0.016 | 3.6  | 0.0010 |
| A          | 1,346–1,093 + 840–714 + 587–460  | 2nd Der     | 10   | 0.977 | 0.005 | 6.6  | 0.873 | 0.011 | 2.8  | -0.0006 |
| HMF        | 1,452–1,325 + 1,200–1,075 + 700–575 | MSC        | 10   | 0.991 | 0.120 | 10.3 | 0.814 | 0.500 | 2.3  | 0.0023 |
| P          | 1,472–1,436 + 1,093–967 + 716–460 | 1st Der + MSC | 10  | 0.988 | 3.0   | 8.9  | 0.919 | 6.9   | 3.5  | -0.2500 |
| Di         | 1,472–1,346 + 840–587             | MSC         | 10   | 0.994 | 0.227 | 12.4 | 0.925 | 0.722 | 3.7  | -0.0269 |
| RS         | 1,725–1,599 + 1,472–1,346 + 1,220–714 | MSC      | 9    | 0.994 | 0.296 | 12.6 | 0.927 | 0.933 | 3.7  | 0.0365 |
| PC         | 1,346–1,220 + 1,093–967 + 840–714 | SLS        | 9    | 0.967 | 0.016 | 5.5  | 0.859 | 0.031 | 2.7  | -0.0017 |
| AS         | 1,599–1,472 + 1,346–1,220 + 967–714 | 1st Der + SLS | 10  | 0.969 | 0.188 | 5.7  | 0.926 | 0.115 | 3.7  | -0.0110 |
| TFC        | 1,725–1,600 + 1,346–1,220 + 714–587 | ConOff     | 10   | 0.989 | 0.267 | 9.6  | 0.865 | 0.876 | 2.7  | -0.0968 |
| TPC        | 1,725–1,600 + 1,472–1,346 + 1,220–460 | 1st Der + SLS | 10  | 0.995 | 2.71  | 14.3 | 0.956 | 7.45  | 4.8  | 0.3590 |

\(r^2\): coefficient of determination; \(RMSE\): root mean square error; \(RPD\): ratio of performance to deviation; \(MSC\): multiplicative scatter correction; \(VecNor\): vector normalization; \(SLS\): straight line subtraction; \(ConOff\): constant offset elimination; \(1st\ Der\): first derivative; \(2nd\ Der\): second derivative.

\(\text{AS}\): apparent sucrose; \(\text{TFC}\): total flavonoids content; \(\text{TFC}\): total phenolic content; \(\text{PC}\): protein content; \(\text{PC}\): protein content; \(\text{TFC}\): total flavonoids content; \(\text{TFC}\): total phenolic content;
addressed sugar profile in different honey samples and only for Raman spectroscopy (Anjos et al. 2018).

4 Conclusions

This study allowed establishing calibrated optimal models with preprocessed spectra for the estimation of physicochemical parameters of in Lavandula spp. monofloral honey by means of FTIR-ATR and FT-Raman spectra, with high values of correlation coefficient and low values of bias and rot mean square error, in general. Nevertheless, the fitting was better for the vase of FT-Raman when compared with FTIR-ATR. Hence, the results hereby reported suggest that FTIR-ATR and FT-Raman spectroscopy have good potential for the rapid and nondestructive assessment of quality evaluation parameters of Lavandula spp. monofloral honeys. In addition, these techniques can also prove useful for the assessment of some nutritionally relevant parameters such as phenolics and flavonoids contents.

Although the potential of these techniques for the aforementioned purpose was validated considering the methodology and number of samples used in the study, further research will be needed to obtain a robust calibration model that will allow implementation in routine quality control laboratory procedures. Finally, even though both methodologies seem suitable for Lavandula spp. honey analysis, it is possible to infer that a more powerful accuracy will be obtained with FT-Raman.

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Ofélia Anjos and Helena Pereira: software; Ofélia Anjos and Leticia M. Estevinho: writing – original draft; Raquel P. F. Guiné, Helena Pereira, and Ofélia Anjos: writing – review and editing.

Conflict of interests: The authors declare no conflict of interest.

Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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