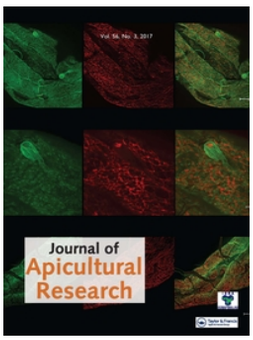

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ORIGINAL RESEARCH ARTICLE

Application of FTIR-ATR spectroscopy on the bee pollen characterization

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Bee pollen contains almost all nutrients required by the human organism as well as diverse health-promoting substances. However, its composition and nutritional value greatly depend on the botanical origin. As such, it is important to develop a rapid and non-expensive methodology that allows studying its characteristics, making labelling more objective and easier. The FTIR-ATR technique was used to predict some nutritional parameters in 126 bee pollen samples. FTIR-ATR spectrum obtained in the region between 4000 and 400 cm^{-1} with PLS Regression models were used to correlate spectral information with the data obtained using reference methods. In this first approach with pollen samples, good correlation models with appropriate accuracy were obtained for the evaluated parameters with r^2 varying from 74.8 to 97% and residual prediction deviation between 2.0 and 5.8. These results suggest that FTIR-ATR may be a useful technique for assessing bee pollen's composition.

Aplicación de la espectroscopía FTIR-ATR a la caracterización del polen de abeja

El polen de abeja contiene casi todos los nutrientes requeridos por el organismo humano, así como diversas sustancias promotoras de la salud. Sin embargo, su composición y valor nutricional dependen en gran medida del origen botánico. Como tal, es importante desarrollar una metodología rápida y económica que permita estudiar sus características, haciendo el etiquetado más objetivo y más fácil. La técnica FTIR-ATR se utilizó para predecir algunos parámetros nutricionales en 126 muestras de polen de abeja. Se utilizó el espectro FTIR-ATR obtenido en la región entre 4000 y 400 cm-1 con modelos de regresión PLS para correlacionar información espectral con los datos obtenidos usando métodos de referencia. En este primer abordaje con muestras de polen, se obtuvieron buenos modelos de correlación con una precisión adecuada para los parámetros evaluados con r2 variando de 74,8% a 97% y una desviación de predicción residual entre 2,0 y 5,8. Estos resultados sugieren que el FTIR-ATR puede ser una técnica útil para evaluar la composición del polen de abeja.

Keywords: bee pollen; chemical composition; Fourier Transform Infrared Spectroscopy; attenuated total reflectance

Introduction

The composition and chemistry of bee pollen have not yet been standardized. In fact, this beehive food product has a very variable chemical composition, with the major compounds being proteins, carbohydrates, sugar, lipids, fibers, vitamins and minerals (Campos, Olena, and Anjos (2016); Estevinho, Rodrigues, Pereira, & Feás, 2012). Also, it has been reported to contain all the essential amino acids needed for the human organism.

The composition of this natural food product not only depends on the plant source (percentages of pollen grains from different botanical families), climatic conditions, soil type and beekeeper activities, but also on the harvest method and processing conditions (Morais, Moreira, Feás, & Estevinho, 2011).

Praised for its good nutritional profile and promising medical applications, bee pollen has been consumed throughout the world since ancient time, receiving increased attention in current years owing to the tendency as a natural diet supplement and a food ingredient. Indeed, several studies ascertain the pharmacological properties of this product including antioxidant, antimicrobial, antiviral, anti-inflammatory, antimutagenic, hepatoprotective and antiallergenic, that have been linked in great extent to the content in phenolic compounds (Campos et al., 2008; Feás, Vázquez-Tato, Estevinho, Seijas, & Iglesias, 2012; Pascoal, Rodrigues, Teixeira, Feás, & Estevinho, 2014).

Considering the dependence on the plant source, the pollen mixtures usually available on the market pre-

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The contributions of each author are: Teresa Dias and Letícia M Estevinho performed the laboratorial analysis of bee pollen samples; António J.A. Santos and Ofélia Anjos performed the spectral information acquired in FTIR-ATR and models development; Letícia M. Estevinho and Ofélia Anjos conceived and designed the experiments and wrote the paper. IPB, IPCB and CBP contributed reagents, materials and equipment tools.

sent high variability in their composition what hampers their labelling. Despite this, the correct labelling is very helpful in guiding consumers toward healthier food choices. For this kind of product (with high composition variability) it is very important have a fast, easy and cheap methodology that allows the different parameters of the nutritional composition required on the label to be analysed.

Attenuated total reflectance (ATR) is a Fourier Transform infrared (FTIR) sampling tool frequently used because it allows qualitative and quantitative analysis of samples that do not require sample preparation.

FTIR-ATR techniques have been applied in several different crud materials (Anjos, Campos, Ruiz, & Antunes, 2015; Anjos, Santos, Estevinho, & Caldeira, 2016; Droussi, D'orazio, Provenzano, Hafidi, & Ouatmane, 2009; Kaya-Celiker, Mallikarjunan, Schmale, & Christie, 2014). In fact, in honey it has been described as useful for the automated and highly sensitive botanical origin estimation (Gok, Severcan, Goormaghtigh, Kandemir, & Severcan, 2015), in the development of calibration models for sugar content estimation (Anjos et al., 2015) and for assessing the presence of adulterants (Gallardo-Velázquez, Osorio-Revilla, Loa, & Rivera-Espinoza, 2009). However, as far as we know, only preliminary studies have been carried out using bee pollen, most of which by our work group. These mainly focus the potentiality of FTIR-ATR spectroscopy for assessing the flavonoid/phenolic acid profile when compared to the results of HPLC/DAD (Anjos, Antunes, Rocio, & Campos, 2012).

For pollen analysis Bağcıoğlu, Zimmermann, and Kohler (2015) conclude that the vibrational spectroscopy (FTIR and RAMAN) is enabled to a biochemical characterization of pollen and detection of phylogenetic variation. These authors' analysed pollen from *Cedrus* and some *Pinaceae* and reported spectral differences connected to specific chemical constituents, such as lipids, carbohydrates, carotenoids and sporopollenins.

In this context, the aim of this study was to evaluate the ability of FTIR spectroscopic technique with ATR in combination with partial least squares (PLS) regression (PLS-R) to quantify moisture, pH, water activity, ash, carbohydrates, lipids, proteins, reducing sugars and fibre content, total phenol content, flavonoids content and antioxidant capacity, important parameters for nutritional labelling of this product.

Materials and methods

Sample characterization

The pollen samples (n = 126) were collected from local beekeepers from diverse locations in the North of Portugal: Miranda, Malhadas, Vimioso, Angueira, Bemposta, Mogadouro and Brunhozinho. Bee pollen was dried by the beekeepers and delivered to the lab, where it was stored in the dark at room temperature (±15 °C) until further analysis.

For all samples, the percentage of pollen grains belonging to each botanical family was determined based on the observation of 300–400 pollen grains (mean value ± standard deviation; 335 ± 29%), according to the procedure previously described in detail by Morais et al. (2011).

Frequency classes were determined as dominant pollen (>45% of a specific pollen type), secondary pollen (15–45%), important minor pollen (3–15%) and minor pollen (<3%).

Chemical analysis

Several analytical parameters were determined in the bee pollen samples, namely: moisture content, ph, water activity, ash, carbohydrates, reducing sugars, lipids, proteins, fiber content, total phenols and flavonoids and antioxidant activity.

Moisture of the pollen samples was determined according to the AOAC procedures (AOAC, 1995). Water activity (aw) was measured using a Rotronic Hygroskop DP model.

pH was measured in the aqueous phase, obtained after mixing 5 g of pollen in 20 mL of distilled water, using a digital pH Meter (pH 526 Multical, WTW, Weilheim, Germany).

Ash content was determined by gravimetry after ignition at 600 ± 15 °C, as previously reported (Carpes, Begnini, Alencar, & Masson, 2007).

The quantification of reducing sugar was performed spectrophotometrically at 540 nm using a spectrophotometer (UV–vis spectrometry Unicam Hekios, UK) according to Silva et al. (2004). Glucose was used as standard.

For the determination of the total lipid content, two grams of pollen were macerated in a mortar with anhydrous Na_2SO_4 (Merck (Darmstadt, Germany). Then, it was extracted with n-hexane (Merck (Darmstadt, Germany) for about 4 h in the Soxhlet apparatus (Carpes et al., 2007).

Protein content was determined by evaluating the nitrogen content in each sample. Nitrogen content was determined using the Kjeldahl method (230-Hjeltec Analyzer, Foss Tecator, Höganäs, Sweden). The crude protein (CP) content was calculated from the total nitrogen using the conversion factor 6.25 (N × 6.25).

The crude fibre percentage was determined by the method recommended by AOAC, 1995).

Total carbohydrate contents (%) were obtained by difference: 100 − (total proteins + moisture + lipids + ash), as described by Estevinho et al. (2012).

The total phenolic content of the extracts was determined using the Folin–Ciocalteu method, as described by Moreira, Dias, Pereira, and Estevinho (2008) and expressed as mg of Galic Acid (Sigma (St. Louis, MO, USA) equivalents per g of bee pollen (GAEs).

Regarding flavonoid contents, it was used the aluminium chloride method (Feás et al., 2012). Quercetin (Sigma (St. Louis, MO, USA) was used in the determination of the standard curve and results were expressed as mg of quercetin equivalents (QE) per g of pollen (QEs).

The antioxidant capacity has been reported to vary according to the type of oxidants and methodology applied in its determination. As such, here it was assessed by calculating the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity (RSA) and by the trolox equivalent antioxidant capacity (ABTS).

The evaluation of the free radical blocking effect of DPPH was performed according to the methodology described by Moreira et al. (2008), with some modifications. The sample extract solution of each BP sample was prepared by weighing 10 mg of BP dried extract to a vial and diluting with 1 mL of methanol (Merck (Darmstadt, Germany). The sample solution was prepared at concentrations ranging from 0.25 to 4.0 mg/mL. 0.3 mL of each concentration and 2.7 mL of methanol solution containing DPPH radicals (6×10^{-5} mol/L) (Sigma (St. Louis, MO, USA) was placed in the test tube. The mixture was shaken vigorously and placed at rest in the dark for 60 min, developing a yellowish colour. The reduction of DPPH radical was determined by measuring the absorbance at 517 nm in a spectrophotometer (Varian UV-Visible Spectrophotometer, Cary 50 Scan). The RSA was calculated b y the following equation:

$$\text{RSA}\,(\%) = [(\text{ADPPH} - \text{AS})/\text{ADPPH}] \times 100 \quad (1)$$

where ADPPH is the absorbance of DPPH solution and AS is the absorbance of the solution when the sample extract has been added at a particular level.

The concentration of the extract that induced a 50% inhibition (EC_{50}) was calculated from the graphic of the percentage effect of eliminating radicals as a function of the concentration of the sample extract solution.

Concerning the trolox equivalent antioxidant capacity assay, it was followed the method described by Re et al. (1999). The extent of inhibition of the sample was calculated using the same formula and compared with a standard curve made from the corresponding readings of Trolox (0.4–0.04 mM) (Sigma (St. Louis, MO, USA). Results were expressed in μmol of equivalent trolox per gram of sample. All analysis was done in duplicate.

The repeatability (*rp*) of each method was calculated according to Luís, Mota, Anjos, and Caldeira (2011) using the analysis of the obtained results of pollen.

The equation used for the calculation of repeatability (*rp*) was:

$$rp = t.\sqrt{2}.\left(\frac{1}{2q}\sum Wi^2\right)^{1/2}$$

where t = value of *t*-Student distribution; q = the number of samples analysed in duplicate; Wi = the absolute differences between duplicates.

FTIR-ATR data acquisition and data processing

Before spectral acquisition approximately 10 g of representative samples were taken and very well mixed and grinded in a mortar according Figure 1.

The pollen spectra were acquired in Bruker FT-IR spectrometer (Alpha) with a resolution of 4 cm^{-1} in the wavelength region 4000–400 cm^{-1}, using a diamond single reflection ATR. All spectra were obtained with 32 scans per and the experiments were carried out at room temperature. Each ten-sample, a background measurement was made using air.

PLS regression was done based on the spectral decomposition using OPUS 7.5.18 BRUKER software. This regression is based on the spectral decomposition in which the original variables are replaced by new variables, which are linear combinations of the original ones.

The spectral data were regressed against the measured parameters in order to obtain a significant number of PLS-R components that was found by automated optimization. The tested pre-processed spectra for PLS-R analysis were (Anjos et al., 2015): 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 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 (RMSECV).

Results and discussion

A total of 126 pollen samples mixture were analysed in order to identify the frequency of each pollen class: predominant pollen, secondary pollen, important minor pollen and minor pollen.

Regarding the floral origin of the analysed samples (Figure 2), *Cistus* spp. was the predominant genus in a higher number of samples. Other important genus that were representative as predominant pollen are *Prunus* spp., *Echium* spp, and *Cytisus* spp. For the secondary pollen some genus are important in the samples namely *Castanea* spp., *Eucalyptus* spp., *Rubus* spp., *Cytisus* spp., *Erica* spp., *Genista* spp. and *Lavandula* spp. These results are in accordance with the predominant species visited by the bees to produce hive's products (Estevinho et al., 2012) in the studied region. These different species that were identified in the samples in different proportions influence the nutritional value and chemical composition as reposted in Table 1. The lower variability is observed for carbohydrate percentage (Coefficient of variation of 4.44%) and the higher for fibre content in percentage

Figure 1. Scheme of samples preparation for analysis in FTIR-ATR equipment.

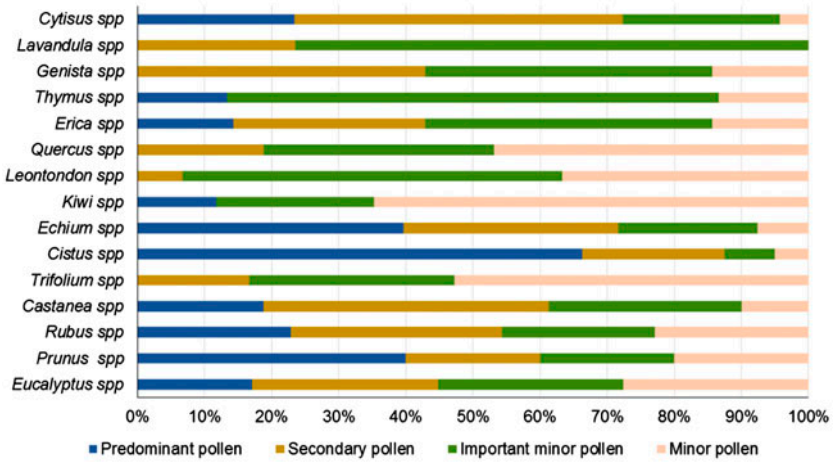

Figure 2. Frequency of each plant genus as predominant pollen, secondary pollen, important minor pollen and minor pollen in the 126 pollen samples mixture analyzed.

Table 1. Statistics of the 126 sample of bee pollen analyses and repeatability values (*rp*) of each parameter determined in pollen.

Parameter	*rp* (%)	Mean ± σ	Min − max	Coefficient of variation
Moisture (%)	0.75	4.98 ± 0.70	3.36 − 6.10	14.05
pH	0.60	4.79 ± 0.53	3.66 − 5.76	11.14
Water activity	0.09	0.34 ± 0.07	0.25 − 0.53	19.19
Ash (%)	0.61	2.73 ± 0.53	1.87 − 3.75	19.40
Carbohydrates (%)	6.01	66.93 ± 2.97	60.37 − 75.19	4.44
Lipids (%)	0.93	5.02 ± 0.72	3.23 − 6.76	14.27
Proteins (%)	6.01	20.57 ± 3.32	12.76 − 27.69	16.12
Reducing sugars (%)	8.12	34.94 ± 3.94	25.56 − 43.42	11.52
Fiber content (%)	0.55	3.40 ± 1.04	1.76 − 5.45	30.48
Total phenolic compounds (mg GAE/g pollen)	2.70	23.9 ± 4.16	16.1 − 35.5	17.4
Total flavonoids (mg QE/g pollen)	0.58	4.1 ± 0.96	2.2 − 5.9	23.2
EC_{50} (mg/g)	0.39	3.5 ± 1.05	2.0 − 6.4	39.1
ABTS (μmol TEAC/g)	12.3	244.9 ± 24.40	203.8 − 298.6	10.0

(Coefficient of variation of 30.48%). The repeatability (*rp*) of the method calculated with the replicas obtained applying repeatable conditions namely same operator, same equipment and same laboratory were plotted in Table 1.

Figure 3 represents the average pollen spectrum with the identification of the more representative bands.

To obtain calibration models for each analysed parameter in pollen samples PLS multivariate analysis was done with spectral data and analytical data. Almost the entire infrared spectral region (4000–400 cm^{-1}) was used for spectral acquisition since the PCA analyses of the spectra did not identify any redundant spectra for rejection.

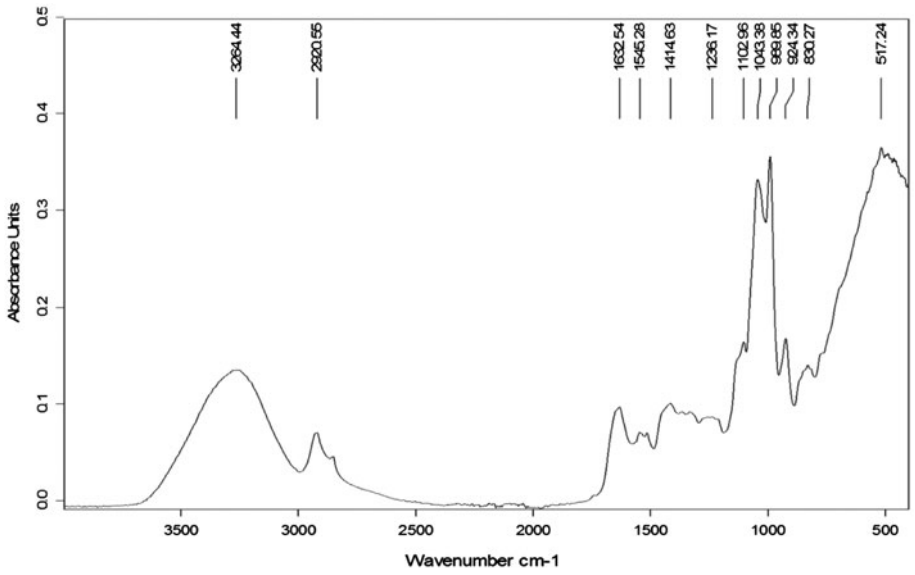

Figure 3. Average ATR spectrum of pollen samples.

The absorption band between 3600 and 3100 cm^{-1} was not considered for all the calibration model because there was a considerable interference of water samples.

The determination of calibration models for all measured parameters in pollen mixtures were developed based on the highest r^2, lowest standard error of calibration and cross-validation, lower number of factors used in the calculation and higher residual prediction deviation.

To select different pre-processing techniques the lowest root mean squared error of prediction (RMSEP) values for a quantitative PLS model is usually chosen. Given that it is possible for the same crude material to use different pre-processing depending on the different chemical functional groups studied in different spectral regions found for each parameter. The model selected depending on the lowest error founded in other to increase the accuracy of the model.

Table 2 represents the values obtained for the calibration and cross-validation of the different analytical determination.

All pollen samples spectra have bands at 2920 cm^{-1} that could be associated to the C–H stretching of carbohydrates (Anjos et al., 2015; Gallardo-Velázquez et al., 2009) in aliphatic structures, such as fatty acids and other long-chain structures like carboxylic acids (Elhajjouji et al., 2007; Movasaghi, Rehman, & ur Rehman, 2008). In fact, for the calibration model obtained for carbohydrates there wereonly bands on the region between 3007 and 2746 cm^{-1}. However, that region was also important in the calibration models of reducing sugar, lipids content, ash content and pH.

The moisture content of the analysed bee pollen samples ranged from 3.4 to 6.1%. The band from 3400 to 3000 cm^{-1} is usually associated to the OH groups and water content in the samples. The band at 1632 cm^{-1} region also contains strong O–H stretching/bending vibrations coming from the water content in the samples.

Two tests were performed for water content measurement with FTIR-ATR, firstly considering with this region in the model development and after without this region. For this parameter good models were not found. The values for r^2 ranged between 73.8 and 58.5 and for RPD between 2.0 and 1.6, so the author decided not to present them.

An important spectral region for the phenolic compounds identification are the bands in the 1700–1500 cm^{-1} regions characteristic of aromatic C–C vibrations, C–O stretching vibrations of amide groups, quinines and conjugated ketones (Leouifoudi et al., 2014; Subari, Mohamad Saleh, Md Shakaff, & Zakaria, 2012). The characteristic bands of the aromatic molecules, C=C stretching appear about the 1545 cm^{-1} (Tarantilis, Troianou, Pappas, Kotseridis, & Polissiou, 2008).

It should be noticed, however, that an absorbance band appeared at 1632 cm^{-1}, which may be assigned to the carboxyl group in asymmetric stretching which has also been previously described in honey samples (Anjos et al., 2015). In spite of that, this interval is also important for the identification of phenolic compounds, alcohols and carboxylic groups and also the hydrogen vibration of amide N–H groups (Susanto, Feng, & Ulbricht, 2009).

According to Elhajjouji et al. (2007) the bands at 1545 cm^{-1} could be assigned to the C–C stretching in aromatic structures, N–H deformation and C–N stretching. Also, according to the literature, the absorption band at 1414 cm^{-1} corresponds to antisymmetric in-plane bending of $–CH_3$ (Tarantilis et al., 2008).

All these regions are important for the diverse parameters under assessment. Indeed, the region

Table 2. Results for calibration and cross-validation for pollen samples (N = 126).

Parameter	Spectral range (cm^{-1})	Pre-processing	Rk		r^2	RMSEE	RMSECV	RPD	Bias
pH	3007 − 2826 + 1769 – 1190 + 932−669	1stDer + MSC	9	C	92.1	0.157		3.6	
				CV	85.8		0.200	2.7	−0.0074
Ash	3007 – 2826 + 1450 – 669	1stDer + MSC	9	C	90.5	0.170		3.2	
				CV	77.6		0.250	2.1	−0.0137
Aw	1709 – 1448 + 932 – 411	1stDer + VN	10	C	95.8	0.0141		4.9	
				CV	90.7		0.0201	3.3	−0.0004
Carbohydrates (%)	3007 − 2746	MSC	7	C	74.8	1.49		2.0	
				CV	62.4		1.76	1.6	0.0606
Lipid content (%)	3007 – 2826 + 1709 – 1448 + 1190 – 411	1stDer + MSC	9	C	87.7	0.259		2.9	
				CV	75.4		0.354	2.0	−0.0075
Protein content (%)	1769 − 669	2ndDer	8	C	88.1	1.16		2.9	
				CV	77.7		1.54	2.1	0.0740
Reducing sugar (%)	3007 – 2746 + 1709 – 1448	ConOff	9	C	81.9	1.74		2.4	
				CV	72.0		2.08	1.9	0.0212
Fiber	3007 – 2826 + 1709 − 1448 + 669−411	MinMax	8	C	97.0	0.188		5.8	
				CV	94.4		0.243	4.3	0.0085
Total flavonoids	2826 − 1448 + 669 − 411	VecNor	10	C	87.2	0.358		2.8	
				CV	69.1		0.534	1.8	−0.0261
EC_{50}	3007 – 2746 + 2027 – 1190 + 669 − 411	VecNor	10	C	89.2	0.357		3.0	
				CV	72.5		0.543	1.9	0.0357
ABTS	1448 − 411	1stDer	7	C	84.5	9.85		2.5	
				CV	64.7		14.2	1.7	1.27

Notes: C – calibration; CV – cross validation; Rk – Rank; N – Number of samples; r^2 – coefficient of determination; RMSEE – root mean square error of estimation; RPD – residual prediction deviation; RMSECV – root mean square error of cross-validation; Bias – mean value of deviation, also called systematic error; 2ndDer – seconded derivative; VecNor – vector normalization; 1stDer – first derivative; MSC – multiplicative scatter correction; MinMax – minimum maximum normalization; ConOff – constant offset elimination.

between 1700 and 1500 cm^{-1} was used for all of the calibration model except for the carbohydrate percentage. The calibration model obtained for the carbohydrate percentage is the worse model obtained, with only a r^2 of 74.8 and a RPD of 2. This results could be explained by the fact that the percentages of total carbohydrates were obtained by the difference of the total proteins, moisture, lipids and ash, and the sum of the errors of each method increases the error in this determination. Additionally, for this parameter the higher value of repeatability was found and for a good calibration model a lower value of repeatability was needed.

Total carbohydrate contents (%) were, as described by Estevinho et al. (2012).

The bands between 1500 and 800 cm^{-1} correspond to the phenolic compounds' characteristic signal and are due to the C–C and C–O vibration of both phenolic and flavonoids compounds (Subari et al., 2012).

The components of the amide-I band are indicative of the presence of protein chain sections with various secondary structures and their relative intensities, were observed in the 1695–1600 cm^{-1} region of the FTIR spectra (Barth, 2007). In this paper this region was also used for protein's content calibration and the region close to 800 cm^{-1} was used in the calibration for pH, ash, aw and lipids content.

According to Karoui et al. (2006) the RPD, that measures the ratio between the standard deviation of the reference data of the validation set and the standard error of prediction of a cross-validation or of the test set validation, must be larger than 2 for a good calibration.

In this study the RPD for the calibration of the FTIR-ATR PLSR model for different measured parameters varied from 2.0 to 5.8, respectively for carbohydrates and fibre contents (Table 2).

The best model found on this work with FTIR-ATR is for fibre quantification with a RPD of 5.8 (r^2 = 97.0). It may be considered that the models obtained for the other parameters are also satisfactory. Nonetheless, regarding carbohydrates and reducing sugar it appears to be better to apply other standard methodologies (Anjos et al., 2015), since the repeatability values obtained for standard method were relatively low. However, the methods used in this work are the official methods used in the nutritional analysis of the food products.

As for total phenolic compounds, total flavonoids, EC_{50} and ABTS, the r^2 obtained in calibration ranged between 84.5 to 92.6 and RPD between 2.5 to 3.7 (Table 2). On the other hand, these models showed lower values of r^2 in cross validation. Regarding ABTS values, the root mean square errors were very high as well as the bias value, suggesting that the model is not significant. Additionally, the repeatability was very high for this parameter, which suggests that the standard methods have higher variability being consequently difficult to calibrate with this technique. The higher variability and low repeatability also results from the samples themselves, since these are mixtures of different pollen grains with diverse origin and chemical compositions.

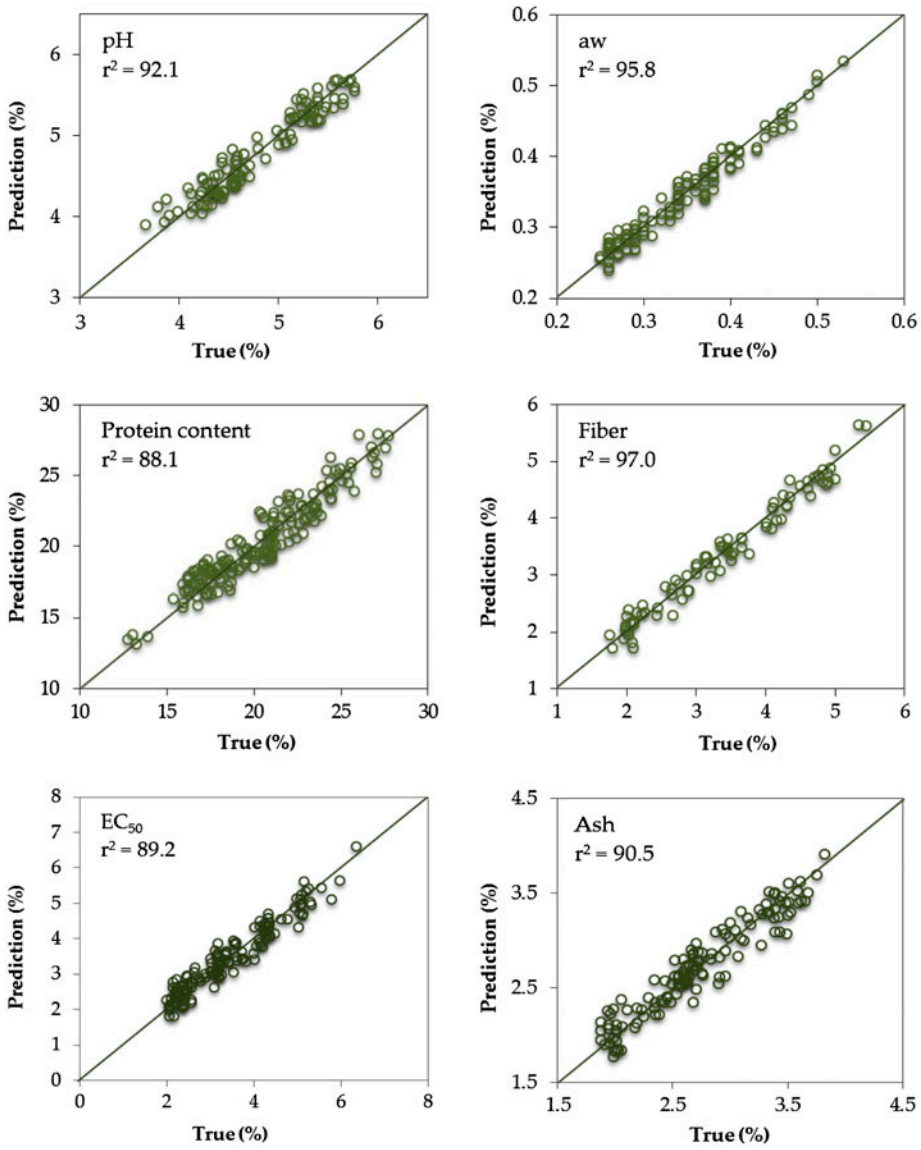

Figure 4. FTIR-ATR PLSR predicted vs. laboratory determined in pollen samples, for pH, aw, protein content, fibre content, EC_{50} and Ash.

Cascant et al. (2016) developed models with middle and near infrared (ATR-MIR and NIR) using PLS regression and found good correlation between the total phenolic compounds content of the different samples. These authors did not work with the region between 2240 and 1880 cm^{-1} due to the high absorbance of ATR crystal. However, in the present work it all regions were tested separately and together and for total phenolic compounds characterization it was only possible to use the regions between 2787 to 2459 cm^{-1} and 2135 to 1809 cm^{-1} (with $r^2 = 2.6$ and RPD = 3.7). Indeed, according to the literature (Martín-Alberca, Zapata, Carrascosa, Ortega-Ojeda, & García-Ruiz, 2016) these bands might correspond to vibrations of cyanate and thiocyanate groups and C≡N groups. More research was need to better clarify the use of FTIR-ATR for total phenol content in pollen. Given the obtained results for total phenol content on bee pollen the author decided to present the calibration values in Table 2.

Figure 4 shows the better FTIR-ATR PLSR calibration models representation obtained with data predicted with reference method. All analytical determinations have significant correlation coefficient between the predicted and the determined content values that varied between 74.8 and 97.0.

This work suggests that it is possible to use the FTIR-ATR spectroscopy to rapidly analyse the different mixtures of pollen and that it is possible to calibrate models with other standard methods that are not usually applied due to their cost and delay.

This work aimed to be a first approach on the use of FTIR-ATR spectroscopy in the evaluation of bee pollen's quality and as a fast and non-expensive methodology that allows characterizing the mixtures of pollen in order to

correctly label this product with high variability. As far as we know, it is the first time that this matrix was used with FTIR-ATR and that it was proved that it could be used for this propose. However, more research is needed using different bee pollen mixtures from different countries, with different characteristics and using more samples in order to develop more accurate models that may be applied in a quality control laboratory.

Conclusions

According to the data hereby presented, it can be considered that the FTIR-ATR method has an acceptable accuracy when applied in screening analysis for the determination of pH value, ash content, carbohydrates percentage, lipid content, protein content, reducing sugar and fibre content of bee pollen samples.

Furthermore, this technique has the advantage of requiring a reduced sampling path length, besides being simple, fast and more cost-effective for the correct characterization and labelling this product with a higher variability according to the lot.

Therefore, more studies are required in order to increase the consistency of these results to obtain an RPD higher than 5 for all parameters, allowing its consistent use in quality control laboratories.

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