

Quantification of mineral composition of Brazilian bee pollen by near infrared spectroscopy and PLS regression

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ABSTRACT

Bee pollen consumption has increased in the last years, mainly due to its nutritional value and therapeutic applications. The quantification of mineral constituents is of great importance in order to evaluate both, the toxicity and the beneficial effect of essential elements. The purpose of this work was to quantify the essential elements, Ca, Mg, Zn, P and K, by diffuse reflectance spectra in the near infrared region (NIR) combined with partial least squares regression (PLS), which is a clean and fast method. Reference method used was ICP OES.

The determination coefficients for calibration models (R^2) were above 0.87 and the mean percent calibration error varied from 5 to 10%. For external validation R^2 values were higher than 0.76. The results indicated that NIR spectroscopy can be useful for an approximate quantification of these minerals in bee pollen samples and can be used as a faster alternative to the standard methodologies.

1. Introduction

Bee pollen is collected from flowers by honeybees that transport it to the hive and pack in the honeycomb mixing with secretion from salivary glands and honey. The mixture created is the bee bread, the main protein source for bee colony and for royal jelly. Bee pollen, as honey and propolis, is an apitherapeutic that contains about 250 substances including sugars (fructose and glucose), proteins, minerals, vitamins, lipids and flavonoids (Komosinska-Vashev, Olczyk, Kafmierzczak, Mencner, & Olczyk, 2015; Costa, Morgano, Ferreira, & Milani, 2017). However, the chemical composition depends on the local of the collect, climate and season of the year. According to Komosinska-Vashev et al. (2015), bioelements are present in about 1.6% including macronutrients (calcium, magnesium, phosphorus, potassium and sodium) and micronutrients (iron, copper, zinc, manganese, silicon, and selenium).

The presence of these essential elements in human diet is necessary in order to avoid deficiencies, but toxic effects can occur if consumed above the required level. Therefore, the analytical control of daily intake of minerals is of great importance. These minerals have been quantified in bee pollen samples from diverse locations in the world (Campos, Firgerio, Lopes, & Bogdanov, 2010; Morgano, Martins, Rabonato, & Milani, 2012; Cosmulescu, Trandafir, & Nour, 2015; Szczesna, 2007; Krejcova, Ludvikova, Cernohorsky, & Pouzar, 2012;

Somerville & Nicol, 2002). The inductively coupled plasma optical emission spectrometry (ICP OES) and inductively coupled plasma mass spectrometry (ICP-MS) are the most accurate techniques for determination of mineral elements in foods (Krejčová et al., 2012; Somerville & Nicol, 2002), but involves previous preparation of samples such as dissolution and digestion that can be time consuming. Infrared spectrometry and X-ray fluorescence methods are green alternatives for direct determination of the mineral composition of food samples. Infrared spectroscopy combined with chemometrics has been extensively applied to determine and quantify organic nutrients in food; however, it is believed that vibrational spectroscopy has a lack of sensitivity for mineral components (Schmitt, Garrigues, & Guardia, 2014), in the sense that metal detectability by vibrational spectroscopy depends probably, of the metal association with organic matter. Nevertheless, there are several publications in this field (Cozzolino et al., 2008; Gonzalez-Martin, Hernandez-Hierro, & Gonzalez-Cabrera, 2007; Lebot, Champagne, Malapa, & Shiley, 2009; Masoum, Alishahi, Shekarchi, & Farahmand, 2011; Moros et al., 2008; Plans et al., 2012; Wu, He, Shi, & Feng, 2009).

Recently, near infrared spectroscopy (NIR) and partial least square regression (PLS) were applied to a set of 154 bee pollen samples collected in twelve different locations from Brazil, in order to quantify ash, lipid, protein, glucose, fructose and free acidity (Costa et al., 2017). Brazil has a great potential for apiculture due to its diversified flora and

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favorable climate, but the current production is not sufficient to national demand, since the pollen consumption has actually increased in Brazil (SEBRAE, 2015). Knowing the composition and quality of bee pollen from different regions can help finding the best places to install new apiaries. The minerals calcium (Ca), magnesium (Mg), zinc (Zn), phosphorus (P) and potassium (K) were quantified through ICP OES by Morgano et al. (2012) for the same set of bee pollen samples. According to Morgano et al. (2012), the most abundant elements in the Brazilian pollen composition are Ca, Mg, P and K, whereas zinc, present in minor quantity, is considered an important antioxidant since it is a component of the superoxide dismutase (SOD) antioxidant enzyme (Rauma & Mykkanen, 2000).

The authors found the following percentage contribution of the mineral elements to Brazilian recommended daily intake (RDI) for adults, considering a 25 g portion of pollen: Ca (5.5%), Mg (11.3%), Zn (16.7%), P (17.2%) and K (2.7%). The recommended daily intake (RDI) established by Brazilian regulation *per* portion are (Brazilian Ministry of Health, 2005): Ca (1000 mg); Mg (260 mg); Zn (7 mg) and P (700 mg); the RDI for potassium is 4700 mg (IOM, Institute of Medicine, 2011). The levels of these minerals (Morgano et al., 2012) in the Brazilian bee pollen samples are above or similar to those found in the literature, except for the concentration range of zinc which is below to that found by González-Martín et al. (2015). According to the National Agency of Health Surveillance – ANVISA (1998), any food “with minimum of 15% of the daily recommended intake (DRI) per 100 g of solid food” is considered as a source of minerals while those “with minimum of 30% of the reference DRI per 100 g of solid food” is considered rich in minerals. Thus, the Brazilian pollen is considered as a source of zinc (16.7% of RDI) and phosphorus (17.2% of RDI) for an intake of about 25 g. The results obtained by Morgano et al. (2012) show that Brazil can be a potential pollen producer, especially in the Northeast region where the amounts of minerals are higher and the production remains almost constant throughout the year.

The main objectives of the present study were to verify the potential of near infrared spectroscopy (NIR) combined with partial least square regression (PLS) for the quantification of calcium, magnesium, zinc, phosphorus and potassium in bee pollen samples and to propose a fast, simple and environment-friendly method for the determination and quantification of these minerals in bee pollen.

2. Methodology

2.1. Samples

One hundred and fifty-four dehydrated bee pollen samples (packages with 200–300 g) were acquired from apiculture producers from twelve different regions of Brazil: Bahia (BA), Santa Catarina (SC), São Paulo (SP), Sergipe (SE), Paraná (PR), Minas Gerais (MG), Espírito Santo (ES), Rio Grande do Sul (RS), Distrito Federal (DF), Mato Grosso (MT), Piauí (PI) and Ceará (CE) (Table 1). Before analyses, the samples were ground in a refrigerated mill (M20, IKA Labortechnik, Staufen, Germany) and sieved through a 30-mesh sieve (600 µm).

2.1.1. Reagents and solutions

All analyses were carried out using analytical purity grade reagents and reverse osmosis-purified water (Gehaka, São Paulo, Brazil, 18.2 MΩ cm). Nitric acid (Merck, Darmstadt, Germany), hydrogen peroxide 30% (Merck, Darmstadt, Germany) and hydrochloric acid (Merck, Darmstadt, Germany) were applied in sample treatment.

Analytical curves were prepared by successive dilutions of standard solutions: 10,000 mg L⁻¹ for Ca, K, Mg (Titrisol – Merck, Darmstadt, Germany) and P (Qhemis High Purity, Jundiai, Brazil) and 1000 mg L⁻¹ for Zn (Merck, Darmstadt, Germany) in 5% HCl (v/v). The concentration ranges of standard solutions were: 0.05–2.5 mg L⁻¹ for Zn; 5.0–75.0 mg L⁻¹ for Ca, P and Mg and 25.0–150.0 mg L⁻¹ for K.

2.1.2. Determination of minerals in bee pollen samples

Minerals (Ca, K, Mg, P and Zn) were determined by an axial view ICP OES (Vista MPX, Varian, Mulgrave, Victoria, Australia) after digestion in a closed microwave oven decomposition system (Start D, Milestone, Sorisole, Italy), as described in a previous study (Morgano et al., 2012). Briefly, 0.65–0.70 g of milled bee pollen samples was weighed into a Teflon digestion flask, added by 10 mL of nitric acid and 3 mL of 30% hydrogen peroxide. Digestion was performed in two steps, using 1000 W power at maximum temperature of 200 °C. After that, samples were cooled at room temperature and the content was transferred to a 25 mL volumetric flask with 5% HCl solution (v/v).

The elements were quantified by ICP OES equipped with radio-frequency source of 40 MHz, CCD simultaneous solid state detector, peristaltic pump, seaspray nebulizer connected to cyclonic spray chamber and high-purity argon (99.996%, Air Liquid, São Paulo, Brazil). The system was controlled by ICP Expert software using the method previously reported by Morgano et al. (2012): power (1000 W); auxiliary, nebulizer and cooling argon flow rate (1.5, 0.9 and 15 L min⁻¹, respectively); background correction (2 points); integration and reading time (10 s); replicate number (3) and wavelengths Ca, 317.933 nm; K, 766.491 nm; Mg, 280.270 nm; P, 213.618 nm and Zn, 206.200 nm.

2.1.3. Quality control

All analyses were performed in triplicate and analytical blanks were prepared following the same procedure used for the bee pollen samples. Analytical curves linearity was verified using the coefficient of correlation and the limits of detection and quantification were calculated as proposed by Mermet & Poussel (1995): LOD = (3 × RSD × BEC)/100 and LOQ = 5 LOD, being RSD, the relative standard deviation and BEC, the background equivalent concentration, both determined experimentally (n = 8). Method accuracy and precision were evaluated by recovery experiments and the coefficient of variation (n = 8), respectively.

2.2. Near infrared spectroscopy

The spectra in the near infrared region were recorded from 10,000 to 4000 cm⁻¹ with 4 cm⁻¹ increment using an Antaris II FT-NIR spectrometer (Thermo Fisher Scientific, Verona, USA). The spectra were acquired as the average of sixteen successive scans, yielding 3112 wavenumbers. The signals were generated in reflectance (%R) mode and transformed into absorbance by using log 1/R. The spectra were recorded in triplicate for each sample, and the average spectrum was used for data analysis. Fig. 1 shows a generic spectrum.

2.2.1. NIR spectroscopy data analysis

The data were organized in a matrix format X (154 × 3112) where each row corresponds to a bee pollen sample and the columns correspond to the absorbance (log 1/R) values. Different mathematical pretreatment such as first and second derivatives, multiplicative signal correction (MSC) and baseline correction were tested. Using the Pirouette®4.5 software, the vector y of concentrations was correlated with spectral information through the partial least squares (PLS) regression method on the mean centered data. The number of factors (latent variables) in the models was selected according to the method proposed in the Pirouette software when performing cross-validation. In fact, the F test was used to determine if two PRESS values were significantly different. If there was no significant difference, more parsimonious model was chosen and only those having fewer factors than the minimum PRESS model were compared. The presence of outliers was verified by analyzing the plot of leverage vs. Studentized residuals. After removing outliers, the data sets were randomly split into two subsets: training (calibration) and test (external validation) sets (Table 2: Ca, 106/30; Mg, 112/29; Zn, 111/27; P, 118/30; K, 100/36 samples for calibration/test sets, respectively). Then, the PLS models

Table 1

Mineral concentrations (in mg per kg) of 154 bee pollen samples collected from eleven Brazilian States and Federal District. Adapted from Morgano et al. (2012)

State ^a	N ^b	Statistic	Calcium	Magnesium	Zinc	Phosphorus	Potassium
			(mg kg ⁻¹)				
BA	37	Mean ± SD	3135 ± 746	1444 ± 341	52 ± 8	5239 ± 744	5960 ± 1588
SC	30	Mean ± SD	1691 ± 376	966 ± 337	38 ± 8	4458 ± 1144	4683 ± 1818
SP	23	Mean ± SD	1346 ± 336	884 ± 215	54 ± 11	4397 ± 909	4434 ± 1840
SE	18	Mean ± SD	3479 ± 829	1597 ± 222	53 ± 13	6886 ± 980	7376 ± 1123
PR	10	Mean ± SD	1511 ± 280	1315 ± 827	39 ± 9	4760 ± 909	4205 ± 2272
MG	10	Mean ± SD	1401 ± 295	789 ± 303	41 ± 15	3399 ± 747	3182 ± 811
ES	10	Mean ± SD	1814 ± 527	915 ± 446	40 ± 9	3257 ± 707	3509 ± 1235
RS	9	Mean ± SD	1838 ± 334	994 ± 245	47 ± 6	4658 ± 1078	3869 ± 1167
DF	3	Mean ± SD	1723 ± 29	1384 ± 77	43 ± 1	5078 ± 425	7044 ± 1693
MT	2	Mean ± SD	2310 ± 138	1744 ± 124	43 ± 5	3691 ± 687	4993 ± 123
PI	1	Mean ± SD	3724	1627	76	5778	6542
CE	1	Mean ± SD	3511	1668	56	6439	6413
Total	154	General mean ± SD	2215 ± 984	1179 ± 455	47 ± 12	4828 ± 1309	5089 ± 1981
		Interval	828–4670	348–3621	5–76	2177–8165	1431–9910

^a BA (Bahia); SC (Santa Catarina); SP (São Paulo); SE (Sergipe); PR (Paraná); MG (Minas Gerais); ES (Espírito Santo); RS (Rio Grande do Sul); DF (Distrito Federal); MT (Mato Grosso); PI (Piauí); CE (Ceará).

^b N: number of samples.

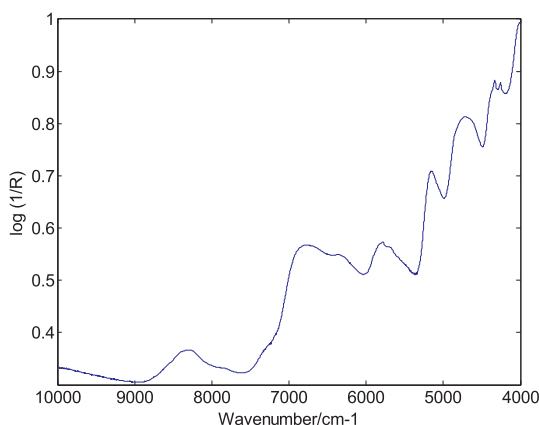


Fig. 1. Generic NIR spectrum of bee pollen samples.

were rebuilt for the training sets and after, the predictions were performed for the test sets. The final regression models were assessed by the values of coefficient of determination for the training sets (R^2 Cal), standard error of calibration (SEC), standard error of cross validation ($SECV$) and the relative error ($RE\%$). The prediction ability of the models was evaluated by external validation sets (test sets), through the coefficient of determination for the test sets (R^2 Pred), the standard error of prediction (SEP) and the residual predictive deviation (RPD). The analytical quality of the models was evaluated by the limits of detection (LOD) and quantification (LOQ) (Ferreira, 2015). In order to verify some critical statistical assumptions concerning the residual

distribution, besides the graphical evaluation of the residuals of the calibration models, the Durbin-Watson (DW) statistical test with a significance level $\alpha = 0.05$, was performed (Krämer, 2011). The equations applied to obtain the validation parameters are shown in Supplementary Material, Table S2.

3. Results and discussion

3.1. Quality control and method performance of experimental determination

The method performance was confirmed by analytical figures of merit as linearity, accuracy, precision and limits of detection and quantification. The analytical curves linearity was verified by the correlation coefficient (r) values close to 1 for all minerals ($r = 1.000$ for Ca and Zn, 0.995 for Mg, 0.995 for K and 1.000 for P). The limits of detection (LOD) and quantification (LOQ) for ICP method were below 0.006 and 0.03 mg L⁻¹, respectively, except for P (0.009 and 0.04 mg L⁻¹, respectively). Due to the lack of reference certified material for bee pollen, the accuracy was evaluated by recovery tests considering mineral levels in bee pollen: Ca (20.05 and 31.00 mg L⁻¹), K (20.05 and 36.00 mg L⁻¹), Mg (30.00 and 50.00 mg L⁻¹), P (25.00 and 40.00 mg L⁻¹) and Zn (0.80 and 1.00 mg L⁻¹). Mean results ranged from 76% to 101%, mostly close to 100%. For determining the precision of the method, the tests were performed with 8 replicates under the same conditions. The coefficient of variation was calculated for the 8 independent analytical replicates and the results ranged between 1.5 and 5.6% (Morgano et al., 2012).

Table 2

Parameters for evaluation and validation of the best PLS models obtained.

y	Training/test sets ^a	Pre-treatment ^b	F	Out	R^2			SEC	$SECV$	SEP	Mean $RE\%$	RPD
					cal	val	pred					
Ca	106/30	1D + MSC	7	18	0.93	0.78	0.85	235	398	337	10.1	2.1
Mg	112/29	1D	7	13	0.90	0.80	0.79	131	179	180	10.2	2.2
Zn	111/27	1D + MSC	7	16	0.87	0.76	0.76	4.0	5.2	4.6	7.0	2.0
P	118/30	2D + MSC	7	6	0.96	0.87	0.80	265	462	608	5.0	2.8
K	100/36	2D + MSC	9	18	0.97	0.77	0.76	303	883	864	5.2	2.1

^a Original data matrix dimensions 154 × 3112. y, dependent variable: analyzed bee pollen minerals. The results are shown in original units.

^b 1D: first derivative; 2D: second derivative; MSC: multiplicative signal correction. F: number of factors. out: outliers.

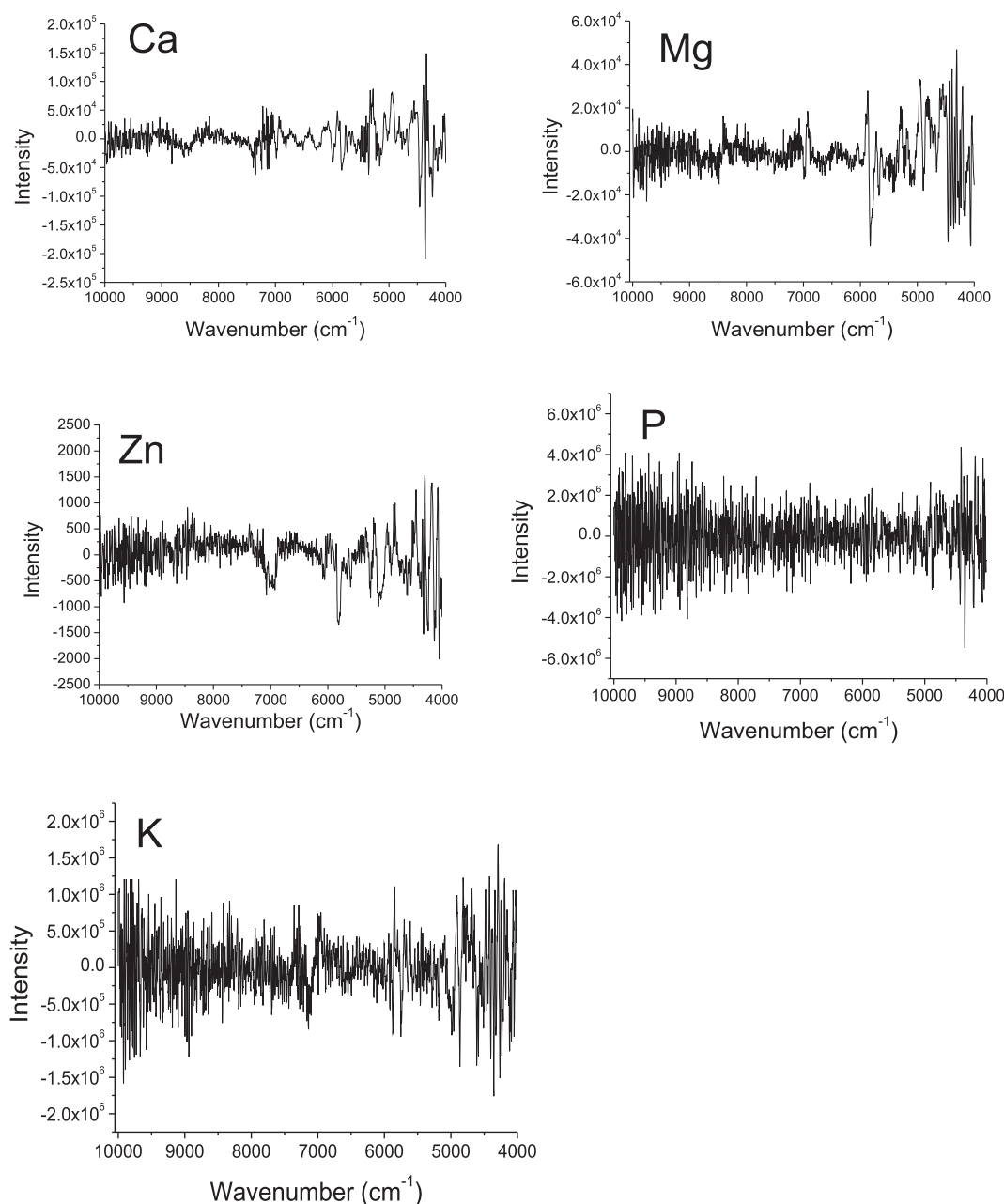


Fig. 2. Regression vectors for PLS regression models built for mineral constituents of bee pollen samples.

3.2. Partial least square regression – PLS

PLS regression models were built with the training sets for Ca, Mg, Zn, P and K. Different pre-processing methods were tested to the mean spectra and the best results were obtained by combining multiplicative signal correction to first and second derivatives. Specific pretreatments and statistical results for the final models are summarized in Table 2.

The spectral pretreatment in the regression models for calcium, magnesium and zinc were first derivative (Supplementary Material, Fig. S1), such that the regression vectors presented similar profiles. For phosphorus and potassium the data were transformed by the second derivative (Supplementary Material, Fig. S2) that confers a different aspect to the regression vector spectra (Fig. 2). For the first three PLS models, it is observed signals between 4000 and 7000 cm^{-1} in the regression vector that could be correlated to the organic constituents of bee pollen and to the corresponding mineral components, considering the possible metal-organic matter associations. The NIR technology can

be applied to mineral quantification, due to the association of these elements with the organic materials (González-Martín et al., 2015). Although it is generally believed that application of vibrational spectroscopy is quite limited for mineral element determinations, there are several studies published in this field (Schmitt et al., 2014; González-Martín et al., 2007, 2015; Sauvage, Frank, Stearne, & Millikan, 2002).

The coefficients of determination (R^2) for calibration displayed in Table 2 were higher for P and K, which were the most abundant constituents among the quantified minerals. The mean percent calibration error ($RE\%$) were lower than 7% for Zn, P and K. The reference vs. predicted values from calibration and validation sets for the PLS models are shown in Fig. 3. Linearity is observed in all plots, but the best fitted models were obtained for phosphorus and potassium, which presented the lowest $RE\%$: 5.0 and 5.2, respectively. The agreement between experimental and predicted contents of calcium and magnesium are satisfactory, with $RE\%$ around 10%. The analyzed mineral contents presented high range of variation (Table 1) that can explain, in part, the

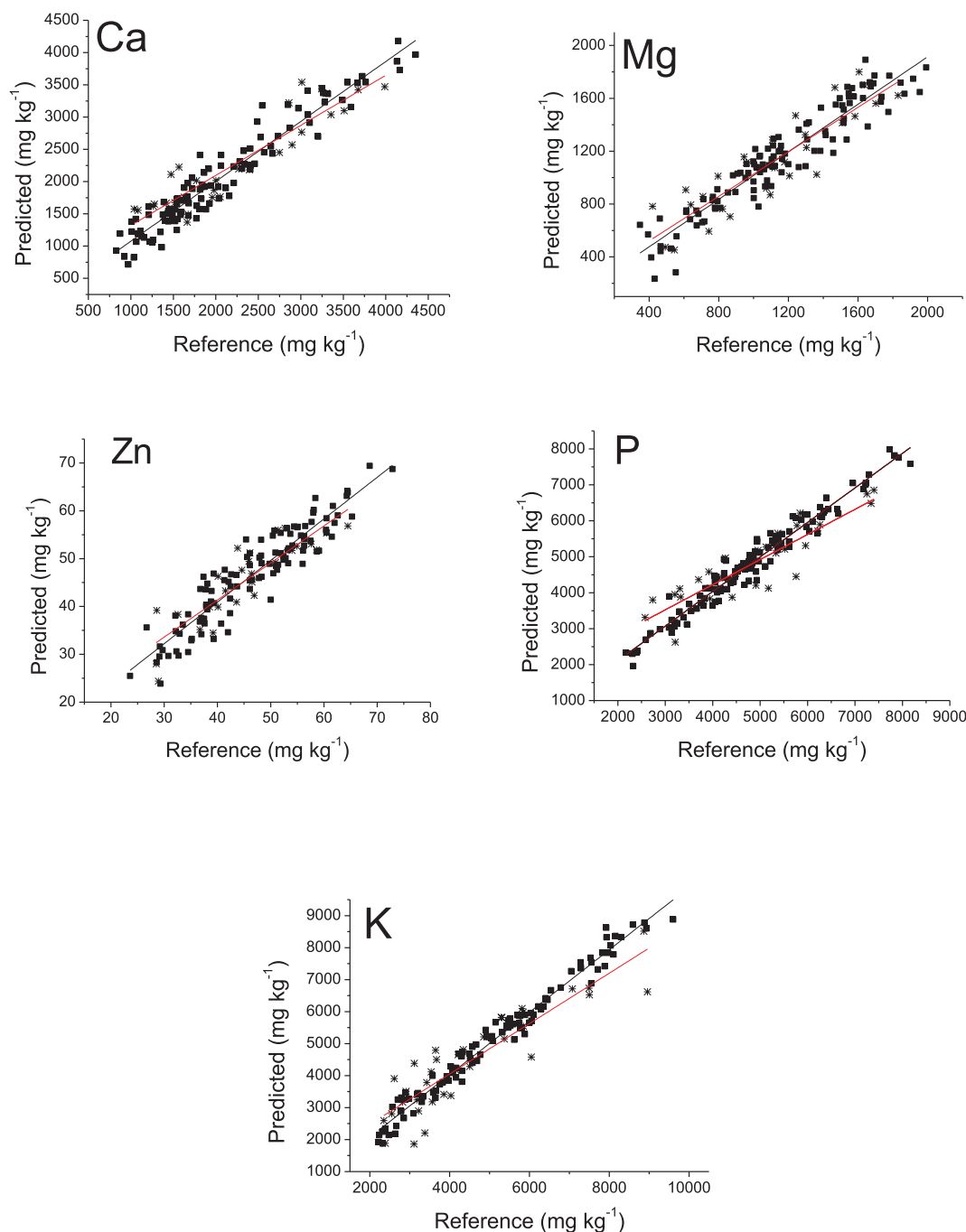


Fig. 3. Plots of reference vs. predicted values obtained from PLS models built for mineral contents in bee pollen samples. Calibration set (■). Validation set (*).

difficulty to obtain better results. However, the results found in this article are similar to those obtained by [González-Martín et al. \(2015\)](#), in which the determination of these minerals were carried out by NIR spectroscopy and modified PLS for a set of 71 samples of propolis from different sources (Galicia, Castilla-León, Chile), using ICP OES for mineral quantification. The authors found the following coefficients of determination (R^2) for calibration: Ca (0.83), Mg (0.70), Zn (0.87), P (0.94) and K (0.95). The values found in the present work ([Table 2](#)) were slightly better: Ca (0.93), Mg (0.90), Zn (0.87), P (0.96), and K (0.97).

The linearity of the method was assessed by visual inspection of the residuals from PLS models vs. predicted values ([Supplementary Material, Figs. S3–S7](#)). The points are satisfactorily distributed in a horizontal line centered at the origin, indicating their random behavior,

the absence of systematic trends in the residuals distribution and that the models are not biased. Besides, the Durbin-Watson (DW) test ([Krämer, 2011](#)) was applied to detect the presence of autocorrelation in the residuals from the regression analyses. The obtained values at 95% of confidence were greater than the critical value of 1.3 and the associated p values were greater than 0.05 for all minerals, and so, there is no statistical evidence that the residuals are autocorrelated ([Supplementary Material, Table S3](#)).

The predictive capacity of the models was assessed by the coefficient of determination for the test set (R^2 Pred) and by the residual predictive deviation (RPD). This parameter is defined as the ratio of standard deviation of reference data in calibration set to standard error of cross-validation. According to [Saeys, Mouazen and Ramon \(2005\)](#), a value for RPD between 2.0 and 2.5 makes approximate quantitative

Table 3

Figures of merit for the PLS models built for the minerals analyzed in bee pollen samples.

Figures of merit (mg kg ⁻¹)	Calcium	Magnesium	Zinc	Phosphorous	Potassium
SEN	7.5e-07	1.6e-06	5.0e-05	4.3e-08	1.6e-08
LOD	203	61	1.3	273	721
LOQ	618	185	3.8	828	2.2e + 03

SEN: sensitivity; LOD: limit of detection; LOQ: limit of quantification.

predictions possible. For values between 2.5 and 3.0, and above 3.0, the prediction is classified as good and excellent, respectively. The RPD values calculated for all of the models were above 2.0. Phosphorus presented the best result for RPD (2.8), followed by Mg with RPD equal 2.2. The values for (R^2 Pred) were between 0.76 and 0.85.

The results indicated that in general the models built for these minerals are capable to make approximate predictions, except for phosphorus, whose RPD value indicates that good predictions can be made.

3.3. Figures of merit

In order to improve the validation of the results, figures of merit were calculated. Sensitivity, limit of detection (LOD) and limit of quantification (LOQ) were estimated by the conventional method (Ferreira, 2015). The meaning of these figures of merit was included in Supplementary Material, Table S2, and the results, in Table 3. The results for LOD and LOQ were compared to the minimal concentrations for each mineral (the concentrations range are displayed in Table 1). The limits of detection found are quite below the minimal values found for all of the minerals. With respect to the limit of quantification, although the values are high, they are below the minimal concentrations for all minerals in the samples of the training sets. These results indicated that NIR-PLS method is useful to detect and quantify Ca, Mg, Zn, P and K.

4. Conclusions

The results confirmed that NIR spectroscopy associated to PLS regression can be applied to the quantification of minerals in bee pollen samples with a wide range of contents of Ca, Mg, Zn, P and K. The obtained coefficients of determination (R^2) for calibration, internal and external validation indicated that the models are validated and the best PLS model was that built for phosphorous (P), which is one of the minerals present in higher amounts in the analyzed bee pollen samples.

According to the residual predictive deviation (RPD) results, the built PLS models are capable to make good predictions for phosphorus (P), and an approximate prediction to the other ones.

In addition, this work confirms that the method based on vibrational spectroscopy and chemometrics can be successfully applied for mineral quantification in bee pollen samples, probably due to mineral – organic matter association.

Conflict of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.foodchem.2018.02.017>.

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