Peer-reviewed version available at *Sensors* **2017**, *17*, 1647; doi:10.3390/s17071647

*Article*

# Flavonoid and Antioxidant Capacity of Propolis Prediction Usingnear Infrared Spectroscopy

**Eddy Betances-Salcedo [1], Isabel Revilla [2], Ana M. Vivar-Quintana [2] and M. Inmaculada González-Martín [1,*]**

[1] Department of Analytical and Food Chemistry, Faculty of Chemistry, University of Salamanca, Plaza de la Merced 37008, Salamanca, Spain; e-mail: eddybetances2011@hotmail.com

[2] Food Technology, University of Salamanca, E.P.S. de Zamora, AvenidaRequejo 33, 49022, Zamora, Spain; e-mail: irevilla@usal.es (I.R.); avivar@usal.es (A.M.V.-Q.)

* Corresponding author: inmaglez@usal.es

**Abstract:** Propolis is a bee product widely used as a dietary supplement and included in sweets or baby foods due to its well-known antioxidant and nutritional properties that are directly correlated with its phenolic composition. For this reason, this study analysed the total contents of flavones and flavonols, flavanones and dihydroflavonols, and the antioxidant capacity by using the methods of ABTS and linoleic acid/β-carotene in 99 samples of propolis from Spain and Chile. A rapid method was developed for quantifying these parameters in raw propolis using near infrared (NIR) spectroscopy with an optical fibre probe of remote reflectance applied directly to the ground up sample. The models developed allow the determination of the total of flavones and flavonols (0-183 mg rutin/ g propolis), of the total of flavanones and dihydroflavonols (9-109 mg pinocembrin/ g propolis extract), and the antioxidant capacity by the ABTS method (0-3212 nmolesTrolox/ mg of propolis) and of linoleic acid/β-carotene (22-86% inhibition). The NIR spectroscopy models were applied in external validation to different samples of the calibration group, which led to the conclusion that the methods developed provide significantly identical data to the initial chemical data of reference.

**Keywords:** propolis; NIR spectroscopy; flavonoids; antioxidant capacity

## 1. Introduction

Propolis is a natural substance that bees collect from plant shoots and exudates and which they mix with wax and salivary secretions of*Apis*melliferas[1-3]. These substances have a resinous appearance and a pleasant sweet aroma. In accordance with the time of year when they are collected and their botanical origin they vary in colour (yellow, greenish, grey-brown, brown, reddish, and even black), taste (bitter, slightly spicy, or insipid) and consistency, beingbrittle at low temperatures and viscous at higher temperatures; these aspects influence their properties [4,1].The detailed chemical composition of propolis is highly complexand is characterised by the presence of biologically active compounds such as polyphenols [5], which include flavonoids, phenolic acids, and theiresters[6].The varied composition of propolis gives it multiple therapeutic properties that make it for example antioxidant, antimicrobial (antibacterial, antifungal, and antiviral), anti-inflammatory,

Peer-reviewed version available at Sensors 2017, 17, 1647; doi:10.3390/s17071647



antitumoral (cytostatic), healing, immunostimulating, anaesthetic, anti-ulcerous, hypotensive, anticariogenic, anti-allergic, and antiparasitic[7-10].Numerous studies exist on the spectrophotometric determination of flavonoids in propolis types such asflavonesand flavonolsusing as a reference quercetin or rutin, applying the method proposed by Bonvehí*et al*., (1994)[11], which has been adopted by other researchers[12,13]. The determination offlavanones anddihydroflavonolsin propolis by means of spectrophotometric methods[14]is based on the reaction of these compounds to 3.4-dinitrophenylhydrazine (DNP) to obtain coloured yellowphenylhydrazone usingpinocembrin as a reference [12,15].As for the individualised quantification of these compounds, it is carried out by chromatography[12,16-18].The antioxidant capacity of raw propolis can be determined by various methods. In numerous studies therefore the inhibiting activity of the ABTS radical is measured usingTroloxas a reference[19-22].In the same manner the determination of the antioxidant capacity, measured as an inhibiting activity of the linoleic acid/β-carotene radical using Trolox as a method of reference, has been applied to determination in propolis by the authors[19, 23-27].NIR spectrometry could hence be an effective and rapid method for the quality determination of propolis. However, there are few studies evaluating the potential of near infrared spectroscopy (NIR) for quantitative analysis of propolis. Among them, a study characterises the propolis of Jalisco in Mexico by means of UV-Vis fluorescence-FTIR spectroscopy techniques, considering that the FTIRspectrum will change depending on the geographical area and the season of the year[28].Moreover, Visible/Near infrared spectroscopy has been applied for the analysis of chrysin and galangin in Chinese propolis[29],to detect propolis adulteration with Poplar balata[30], to identify beeswax in the propolis[31],and to determine the mineral composition[32],the quantification of phenyl ester of caffeic acid or CAPE[33],and more recent applications of the quantification of pesticides in propolis[34].The objective of this study was to develop a quick method to quantify in propolis the composition of flavones andflavonols, flavanones, and dihydroflavonols, and antioxidant capacity by the methods of ABTS and linoleic acid/β-carotene using near infrared spectroscopy (NIR) with areflectancefibre-optic probe applied directly to the ground up sample of propolis, with samples from Spain and Chile.

## 2. Materials and Methods

### *2.1 Samples*

99 samples of propolis were directly collected by beekeepers in Chile (the Bio-Bio region, 52 samples) and Spain (Galicia, 14 samples and Castilla y León, 33 samples). The samples were collected mostly with a mesh and by using the scraping technique from different beekeepers. They were ground up in a Foss Knifetecc 10095 grinder (Höganäs, Sweden), their NIR spectra were recorded, and all samples were kept frozen until used in the laboratory.When using the solutions of the propolis, extracts are prepared according to the method proposed by[11-13,18,35], with slight modifications. Ten milliliters of methanol were added to a 1 g aliquot of sample, and extraction was subsequently carried out in an ultrasonic bath for 15 min. The methanol sample was centrifuged (1500 rpm) for 10 min at 20ºC. The supernatant was filtered through Whatman grade No.4 filter paper and the liquid

Peer-reviewed version available at Sensors 2017, 17, 1647; doi:10.3390/s17071647



filtrated was transferred to a 10 mL volumetric flask. This methanolic extract was diluted 1:100 with methanol for its analytical determination.

### *2.2.Chemical methods*

#### *2.2.1.Flavones and flavonols*

The content of flavones andflavonols is quantified as described[11-13,18,35],with minor modifications. 0.5 ml of a solution of $AlCl_3$ in ethanol is added to 2ml of propolis alcoholic extract. After 30 min at ambient temperature, the absorbance at 425 nm is measured. The results were expressed in milligrams of quercetin or rutin (used as a reference) per gram of propolis, using for the purpose the calibration lines drawn up with each of said standards.

#### *2.2.2.Flavanonesanddihydroflavonols*

The total quantification of flavanones anddihydroflavonols is carried out according to the method described by Popova*et al.* (2004)[12], with minor modifications. A 1 ml aliquot of sampleof propolis alcoholic extract and 2 ml of the DNP solution (2.4-dinitrophenylhydrazine) (solution: 1 g DNP in 2 ml of 96% sulphuric acid, diluted to 100 ml with methanol) is heated at 50ºC for 50 min. After it has cooled to ambient temperature, 10% potassium hydroxide (KOH) in methanol (w/v) to 10 ml is added to the solution. 1 mL of the resultant solution is diluted to 50 mL with methanol in a volumetric flask and the absorbance is measured at 486 nm. The results are expressed as milligrams of pinocembrin (used as a reference) per grams of propolis extract using the pertinent calibration curve to do so.

#### *2.2.3.Antioxidant activity, inhibiting capacity of the ABTS radical*

The total antioxidant capacity was determined by the ABTS method, which is based on the reduction of the 2.2-azinobis(3-ethylenebenzothiazoline-6-sulfonic acid) radical cation. Scavenging of the ABTS·+ radical was monitored by the decrease in absorbance at 734nm by spectrophotometry[36]. The water-soluble vitamin E analogue Trolox (6-hydroxy-2,5,7,8-tetramethylchorman-2-carboxylic acid) was used as standard. To prepare the ABTS radical cation, an ABTS solution was oxidized in water by treatment with potassium persulphate (molar ratio=1:0.35) for 12–16 h in the dark, and then diluted in a 2-mL cuvette with 0.1 M potassium phosphate buffer, pH 7.4, prior to the assays, to give an absorbance of 0.7 + 0.02 at 734 nm. A suitable amount of the sample (20 µL) was added to the reagent and the mixture was incubated at 25 ºC. Absorbance was recorded each minute for 10 min using a Shimadzu spectrophotometer (Columbia, Maryland). Appropriate solvent blanks were run in each assay. The percentage of inhibition of absorbance at 734 nm was calculated and plotted as a function of the concentration of Trolox to give the Total Equivalent Antioxidant Capacity (TEAC).

#### *2.2.4. Inhibiting activity of the linoleic acid/ß-carotene radical*

In order to assess the antioxidant capacity of propolis by using linoleic acid/β-carotene method,it is necessary to proceed according to the method described by Emmons *et al.* (1999)[37], with some modifications. Theβ-carotene (3 mg) is dissolved in 30 ml of chloroform and 3 ml are added to 40 mg of linoleic acid and 400 mg of Tween 40. The chloroform is eliminated under a rotary evaporator and 100 ml of distilled water is added; and the solution is mixed well. The aliquots (3 ml) of the linoleic acid/β-caroteneemulsion are mixed with 50 µL of propolis ethanol extract and are incubated in a thermostatic water bath at

50ºC. The oxidation of the emulsion is monitored spectrophotometrically by measuring the absorbance at 470 nm during a 60-min period.The results are expressed as the inhibition percentage of the spectrophotometric signal which is calculated by using the following formula [($A_0$-$A_1$/ $A_0$)*100], in which($A_0$) is absorbance at time zeroand ($A_1$) is absorbance after 60 min.

### *2.3. NIR Spectroscopy*

A Foss NIRSystem 5000(DK-3400 Hillerød, Denmark), with a standard 1.5 m, 210/210 bundle fiber-optic probe, Ref nº R6539-A, was used. The spectral range was set at 1100-2000 nm since above this value (2000nm) significant attenuation of the signal occurred due to strong absorption of the OH groups present in the fiber optic. The probe employed a remote reflectance system and used a ceramic plate as a reference. The window was made of quartz with a 5 cm × 5 cm surface area. The NIR spectrum was obtained for each of the samples by applying the remote reflectance fiber-optic probe to ground up propolis. The spectra were recorded at 2 nm intervals, and 32 scans were taken for both the reference and the samples. All samples were analyzed in triplicate in order to minimize sampling error. For subsequent statistical analysis 70 propolis samples were randomly selected for the calibration set, while the remaining 29 samples formed the validation set.

### *2.4. Chemometric methods. NIR-chemometric methods*

The models of calibration were developed by using the data obtained from analytical determinations and the spectral data obtained from NIR spectra99 samples were assessed, of which 70 constitute the calibration group and 29 samples the external validation set.The samples were selected at random. The quantification of the different analytical parameters was performed using the modified partial least squares (MPLS) regression method. Partial least squares (PLS) regression is similar to principal component regression (PCR), but uses both reference data (chemical, physical, etc.) and spectral information to form the factors useful for fitting purposes[38]. MPLS is often more stable and accurate than the standard PLS algorithm. In MPLS, the NIR residuals, obtained after each factor and at each wavelength, were calculated and standardized (dividing by the standard deviations of the residuals at each wavelength) before the next factor was calculated. The scattering effects were removed using the multiplicative scatter correction (MSC), standard normal variate (SNV), DeTrend (DT) or SNV–DT. Moreover, the mathematical treatments were tested in the development of the NIRS calibrations by using a nomenclature of 2,4,4,1 in which the first digit is the number of the derivative, the second is the gap over which the derivative is calculated, the third is the number of data points in a running average or smoothing, and the fourth is the second smoothing. When developing the MPLS equations, cross-validation is recommended in order to select the optimal number of factors and to avoid overfitting[39,40], and the calibration set is divided into several groups for the cross-validation. Each group is then validated using a calibration based on the other samples. Finally, any validation errors generated are combined into a root mean square error of cross-validation SECV[41].

## 3. Results and Discussion

### *3.1.Chemical analyses and spectral information.*

The composition of the 99 samples of propolisanalysed is shown in Table 1, which includes the contents of flavones andflavonols, flavanones and dihydroflavonols,the inhibiting activity of the ABTSradical and the antioxidant activity on linoleic acid oxidation. It shows the minimum and maximum values, the mean, and the standard deviation (SD) for each of the constituents by regions and countries.In the quantification of flavones and flavonols, quercetin and rutinhave been used as a reference owing to the fact that various authors use both compounds for their determination; in this study both have been used so as to compare the results obtained with the bibliographical sources consulted.

Table 1 - Chemical data on flavones andflavonols, flavanones, anddihydroflavonols, the inhibiting activity of the ABTS radical and the antioxidant activity on linoleic acid oxidation.

| **Chemical data obtained by Spectrophotometry** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Countries** | **España** | | | | | | **Chile** | | |
| **Regions** | **Galicia** | | | **Castilla y León** | | | **Bio-Bio** | | |
| Constituents | Min-Max | Mean | SD | Min-Max | Mean | SD | Min-Max | Mean | SD |
| Total flavones + flavonols (mg quercetin / g propolis) | 10.4 - 58.7 | 25.8 | 13.7 | 8.0 - 49.9 | 31.0 | 14.6 | 0 - 63.3 | 24.1 | 13.6 |
| Total flavones + flavonols (mg rutin / g propolis) | 28.2 - 149.6 | 61.5 | 36.3 | 19.9 - 190.3 | 88.5 | 38.9 | 0 - 161.1 | 57.3 | 35.6 |
| Total flavanones + dihydroflavonols (mg pinocembrin / g propolis extract) | 31.8 - 73.9 | 49.4 | 9.6 | 31.7 - 81.6 | 56.7 | 15.3 | 27.0 - 149.7 | 75.8 | 34.3 |
| ABTS (nmolesTrolox/mg propolis) | 1552.1 - 2012.6 | 1777.1 | 195.1 | 1197.7- 2649.3 | 1907.7 | 384.2 | 641.2 - 8215.4 | 3863.5 | 1911.2 |
| Linoleic acid / β-carotene ( % inhibition) | 72.7-83.3 | 78.0 | 3.7 | 21.6-82.2 | 56.5 | 21.1 | 54.7-88.1 | 70.0 | 7.1 |

It can be appreciated that the content in flavones andflavonols,whether quercetin or rutin is used as a reference, is found in larger amounts inCastilla y León (8.0-49.9 mg quercetin/g of propolis), (19.9-190.3 mg rutin/g of propolis), followed by Galicia (10.4-58.7 mg quercetin/g of propolis), (28.2-149.6 mg rutin/g of propolis)andChile (0-63,3 mg quercetin/g of propolis), (0-161.1 mg rutin/g of propolis). In the case of the content offlavanonesand dihydroflavonols referring to pinocembrin,the highest content is to be found in Castilla y León (31.7-81.6 mg pinocembrin/g of propolis), followed by Galicia (31.8-73.9 mg pinocembrin/g of propolis)andChile (27.0-149.7 mg pinocembrin/g of propolis).However, in the case of the antioxidant activity determined by the ABTS method the highest value is to be found in the Chilean samples with (641,2-8215,4 nmolesTrolox/mg propolis), followed by Castilla y León (1197.7-2649.3 nmolesTrolox/mg propolis) andGalicia (1552.1-2012.6 nmolesTrolox/mg propolis). In the same manner, the

Peer-reviewed version available at Sensors 2017, 17, 1647; doi:10.3390/s17071647



inhibiting capacity of linoleic acid/β-carotene is greater inChile (54.7-88.1% inhibition), in this case followed by Galicia (72.7-83.3% inhibition),and the lowest value occurs in Castilla y León (21.6-82.2% inhibition).The values found for flavones and flavonolswere in generalhigher than those found in Chinese samples[19],by authors who used quercetin as a reference, while the values of this study are lower than those found in propolis from Mexicowhen using rutin as a reference[13].

For the contents of flavanones and dihydroflavonolsresults found in Spanishpropolissamples are higher than those found in propolis from Mexico[13], Portugal [15], and Argentina[25].As far as antioxidant activity is concerned, the closest ABTS values to those found in this study were found in Turkish propolis[20].Finally, the results of the antioxidant capacitydetermined by thelinoleic acid/β-carotene method of this study are in line with those found in various locations inChina [19], and similar to those found byKumazawain countries such as Argentina[23], Australia, Chile, China, and Hungary, which are higher than propolis values fromBrazil[26] andKorea[27],(Table 1).

*3.2. NIR calibration equations*

The NIR calibration models are carried out by using the chemical data (flavones andflavonols, flavanones anddihydroflavonols; the inhibiting activity of the ABTSradical and theantioxidant activity onlinoleic acid oxidation obtained by spectrophotometry)and the NIR spectra of the samples. The NIR data are divided into two established groups: 70 samples serve to constitute the calibration set and 29samples are used for the external validation set, chosen always at random. The mean composition of each of the parameters in the calibration and external validation sets are shown in Table 2.

Table 2. Chemical data of reference for flavones and flavonols; flavanones and dihydroflavonols; the inhibiting activity of the ABTS radical and the antioxidant activity on linoleic acid oxidation for the subsets of calibration and external validation in raw propolis.

| **Constituents** | **Calibration set (N=70)** | | **Externalvalidation set (N=29)** | |
|---|---|---|---|---|
| | **Min-Max** | **SD** | **Min-Max** | **SD** |
| Total flavones + flavonols (mg quercetin / g propolis) | 0-74.3 | 15.0 | 10.1-72.2 | 14.7 |
| Total flavones + flavonols (mg rutin / g propolis) | 0-190.3 | 39.5 | 19.9-184.8 | 39.1 |
| Total flavanones + dihydroflavonols (mg pinocembrin / g propolis extract) | 31.7-221.2 | 30.5 | 27.0-109.3 | 21.6 |
| ABTS nmolesTrolox/mg propolis | 903.9-6791.6 | 1668.5 | 641.2-8215.4 | 1853.4 |
| Linoleic acid/β-carotene (% inhibition) | 15.8-86.8 | 13.3 | 31.1-83.0 | 13.1 |

Prior to the application of the Modified Partial Least Squares (MPLS)regression model, the samples with a value of H (the Mahalanobis distance) greater than 3 are eliminated. Subsequently the MPLS regression is carried out; those samples with T values exceeding 2.5 are eliminated from the set because they are different from the population from a

Peer-reviewed version available at Sensors 2017, 17, 1647; doi:10.3390/s17071647



chemical point of view. The results of the NIRcalibration models obtained for each constituent can be seen in Table 3 with the indication of the number of samples (N) used (after eliminating the samples because of criterion H and criterion T), together with the best of the different mathematical treatments, the range of concentration, standard deviations for each parameter, RSQ values, and calibration errors (SEC). The equations obtained allow the determination of theflavones and flavonols (taking bothquercetin and rutin as a reference), flavanonesanddihydroflavonols, the inhibiting activity of the ABTS radical, and the antioxidant activity on linoleic acid oxidation.

Table 3 - NIR calibration data of the 70 samples of each of the flavones and flavonols, the flavanones and dihydroflavonol, and the inhibiting activity of the ABTS radical and the antioxidant activity on linoleic acid oxidation.

| Constituents | Mathematicaltreatment | N | Min-Max | SEC | SECV | SD | RSQ | RMSEP | Regression line |
|---|---|---|---|---|---|---|---|---|---|
| Total flavones + flavonols (mg quercetin / g propolis) | Standard MSC 2,4,4,1 | 65 | 0-72.0 | 9.5 | 11.8 | 14.4 | 0.63 | 23.4 | $C_{NIR} = 1.00\ C_{Ref} + 1.78$ |
| Total flavones + flavonols (mg rutin / g propolis) | Detrend only 0,0,1,1 | 65 | 0-183.4 | 24.1 | 29.4 | 37.9 | 0.62 | 8.9 | $C_{NIR} = 0.98\ C_{Ref} + 2.33$ |
| Total flavanones + dihydroflavonols (mg pinocembrin / g propolis extract) | Standard MSC 2,4,4,1 | 62 | 9.89-109.4 | 10.2 | 13.4 | 16.6 | 0.68 | 9.5 | $C_{NIR} = 1\ C_{Ref} + 0.00$ |
| ABTS (nmolesTrolox/mg propólis) | Detrend only 2,10,10,1 | 63 | 0-3212.7 | 386. 1 | 449.3 | 707.7 | 0.87 | 119.7 | $C_{NIR} = 0.99\ C_{Ref} + 44,03$ |
| Linoleic acid / β-carotene (% inhibition) | SNV only 1,4,4,1 | 66 | 22,7-86,8 | 72,3 | 139 | 15,0 | 0,65 | 9,41 | $C_{NIR} = 0,96\ C_{Ref} + 1,9$ |

N= number of samples worked. SEC= Standard calibration error. SECV= cross-validation standard error. SD= standard deviation. RSQ= correlation coefficient. RMSEP = mean square error of prediction. $C_{NIR}$ = NIR concentration. $C_{Ref}$= Reference concentration.

The results obtained indicate that it is possible to determine by means of NIR technology, given the high RSQ values and the small calibration errors, theflavoneandflavonolcontentindependently of the standard compound used for calibration, and the flavanoneanddihydroflavonol total in concentrations of the same kind as in spectrophotometry. For the determination of antioxidant activity using the ABTSmethod, the concentration margin is lower (0-3212.6 nmoles of Trolox/mg of propolis), while in the case of thelinoleic/β-carotene method the margin of application of the model regarding the chemical data of reference clearly widens(22.7-86.8% inhibition).

### *3.3. Internal validation (prediction)*

The models obtained by NIR calibration are assessed by cross-validation. The set of calibration samples was divided into a series of subsets(establishing seven cross-validation groups). The prediction process involves taking six of these sets for the celebration set and one for the prediction set. The process is repeated for each subset so that all the samples pass the calibration set and the prediction set.It can be seen from Table 3 that the cross-validation errors (SECV) are of the same kind as those of calibration. This table indicates the regression lines of NIR calibration compared with the reference data obtained by spectrophotometry.This method allows the validation of the models obtained and also the checking of their prediction capacities. The correlations of the values obtained in the laboratory (Ref) with regard to those predicted by NIR with a fibre-optic probe of theflavones and flavonols (referring tomg of quercetin or of rutin/g of propolis), flavanones anddihydroflavonols (referring tomg pinocembrin/g of propolis), the inhibiting activity of the ABTS radical (nmolesTrolox/mg propolis) and the antioxidant activity onthe linoleic acid (% inhibition)are shown in Figure. 1. These data tell us that theNIR models obtained can be used to predict these parameters in unknown samples. NIR technology with a fibre-optic probe may become an alternative to the chemical methods used. This spectroscopic method has great potential owing to its low cost as it does not require the treatment of the samples compared with chemical methods.

### *3.4. External validation*

Once the NIR calibration equations have been obtained for the determination of the composition of the total of flavones and flavonols, flavanones and dihydroflavonols, the inhibiting activity of the ABTSradical and the antioxidant activity on linoleic acid oxidation in propolis, it is necessary to proceed to the stage of external validation, which consists of the application of the equations to a set of29samples that do not belong to the calibration set. The procedure is as follows: theNIR spectra are recorded in triplicate and the spectral mean is taken.

The NIR calibration equationsobtained in the carrying out of the study are applied to said spectra so as to predict the values for each of the parameters; subsequently they are compared with the results predicted by means of NIR technology with the laboratory chemical data of these samples.The Student t assay is used to compare both methods (spectrophotometry and NIR). The *p* values range from 0.41 for ABTS method and1.00 for linoleic acid/β-carotene method, which indicates that there are no differences between the results obtained by NIR spectroscopy and the spectrophotometric data.Inaddition,the measures of the values of the Root Mean Square Error Prediction (RMSEP)that fall between3.89 and 68.48 respectively indicate that these are acceptable values (Table 4).

Peer-reviewed version available at Sensors 2017, 17, 1647; doi:10.3390/s17071647



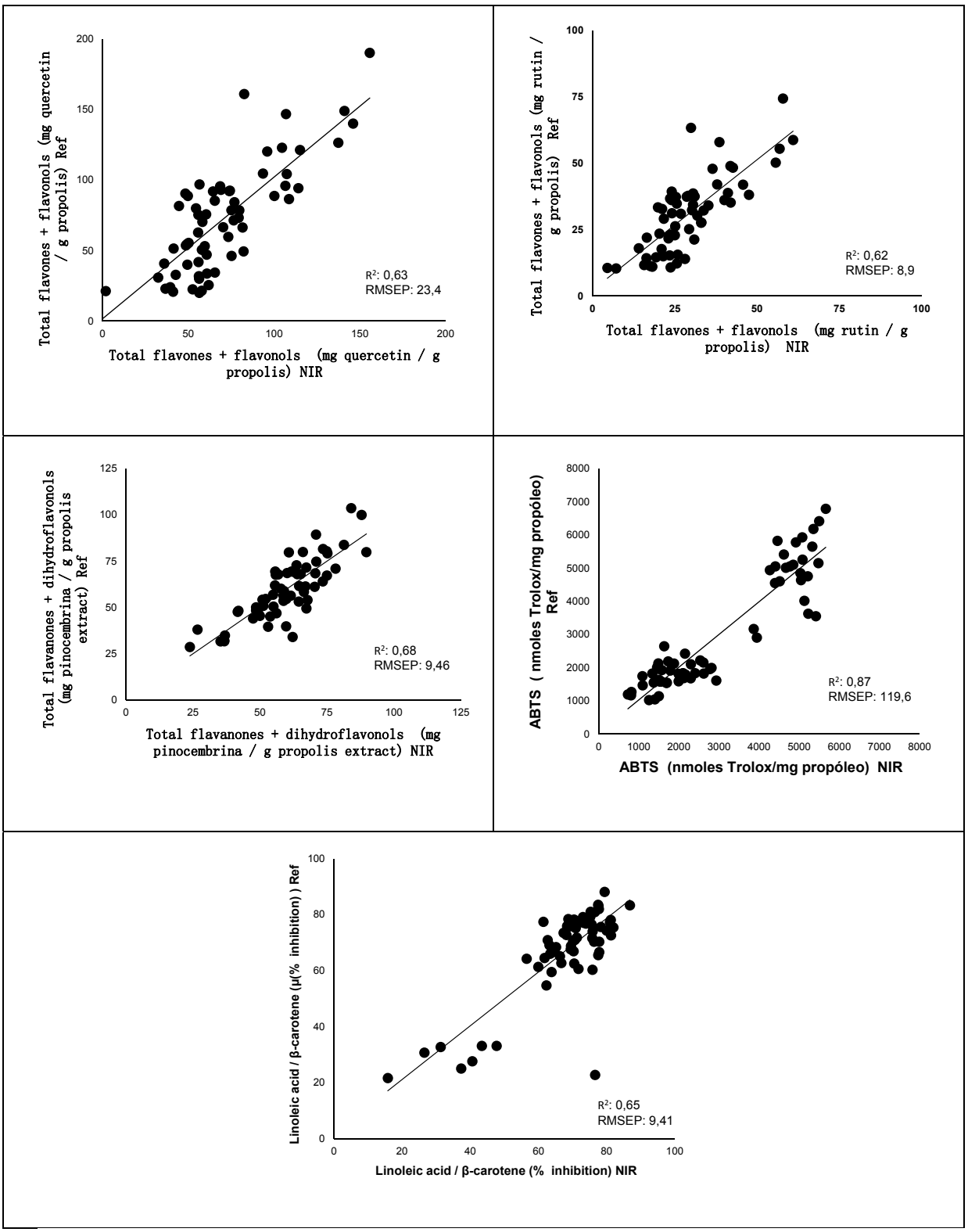


Figure 1. Comparison of the reference values with the values predicted by calibration equations NIR. RSQ multiple correlation coefficients; RMSEP = mean square error of prediction.

Table 4 - Data of external validation (29 samples), the level of significance, residual means and the Root Mean Square Error (RMSE) of flavones and flavonols, flavanones and

Peer-reviewed version available at Sensors 2017, 17, 1647; doi:10.3390/s17071647



dihydroflavonols, and the inhibiting activity of the ABTS radical and the antioxidant activity on linoleic acid oxidation in propolis.

| Constituents | *p* ( Level of significance ) | Residual mean | RMSEP |
|---|---|---|---|
| Total (flavones + flavonols) (mg quercetin / g propolis) | 0.97 | 98.8 | 6.6 |
| Total (flavones + flavonols) (mg rutin / g propolis) | 0.97 | 74.1 | 3.9 |
| Total (flavanones + dihydroflavonols) (mg pinocembrin / g propolis extract) | 0.59 | 26.7 | 4.1 |
| ABTS (nmolesTrolox/mg propólis) | 0.41 | 45.2 | 68.5 |
| Linoleic acid / β-carotene (% inhibition) | 1.00 | 20.4 | 3,8 |

It can be emphasised that up to now we have found no research implementing NIR technology in propolis for the determination of these parameters.

## 4. Conclusion

In view of the results, NIR methodology can be used to predict the total contents offlavonesandflavonols, the sum offlavanonesanddihydroflavonols, and the inhibiting activity of the ABTSradical and the antioxidant activity on linoleic acid oxidation in propolis with values comparable to spectrophotometry. The most determinant aspect of this methodology is that it can be developed and applied to any type of unknown propolis of different origins without prior treatment and without destruction of the samples, i.e. from the direct application of the fibre-optic probe after grinding up the propolis.

**Acknowledgment**.

This study was made possible by funds from Project KCCN/463AC01 of University of Salamanca.

## Author Contributions

Eddy Betances-Salcedo**:** performed the experiments

Isabel Revilla**;**Ana M. Vivar-Quintana : analyzed the data and wrote the paper.

Mª Inmaculada González-Martín**:** conceived and designed the experiments; wrote the paper.

## Conflicts of Interest

"The authors declare no conflict of interest".

## References

1. Farré, R.; Frasquet I.; Sánchez A. El propolis y la salud. *Ars Pharm*.**2004**, *45*, 21-43.
2. Dussart, E.; Bartholomé, Y. Elaboración de subproductos de la Miel y las Colmenas. IICA, Managua., Nicaragua, 2007; pp. 1-51.
3. Pineda, J.; Principal, J.; Barrios C, Milla D, Solano Y and Gil E, Propiedad fungistática in vitro de propóleos sobre tres aislamientos de Colletotrichum

gloeosporioides. *Zootecnia trp*.**2010**, *28*, 83-91.

4. Alarcón,R. Estudio Químico de Propóleos. Tesis Pedagogía en Biología, Química y Ciencias Naturales. Facultad de Filosofía y Humanidades, Universidad Austral de Chile, 1989, p. 38.
5. Bankova, V.Recent trends and important developments in propolis research. *Evid.Based Complement.Altern. Med*.**2005**, *2*, 29-32.
6. Gómez-Caravaca, A.M.; Gómez-Romero, M.; Arráez-Román, D.; Segura-Carretero, A.;Fernández-Gutiérrez, A. Advances in the analysis of phenolic compounds in products derived from bees. *J. Pharm. Biomed. Anal*.**2006**, *41*, 1220-1234.
7. Grange, J.M.; Davey, R.W. Antibacterial properties of propolis (bee glue). *J. R. Soc. Med.***1990**, *83*, 159-160.
8. Marcucci, M.C.Propolis: chemical composition biological properties & therapeutic activity. *Apidologie***1995**, *26*, 83-99.
9. Kujumgiev, A.;Tsvetkova, I.;Serkedjieva, Y.;Bankova, V.;Christov, R.; Popov, S. Antibacterial, antifungal and antiviral activity of propolis from different geographic origin.*J. Ethnopharm*.**1999**, *64*, 235-240.
10. Sforcin, J.M.;Bankova, V.Propolis: is there a potential for the development of newdrugs?.*J. Ethnopharmacol.***2011**, *133*, 253-260.
11. Bonvehí, J.S.; Coll, F.V. Phenolic composition of propolis from China and from South-America.*Z. Naturf.***1994**, *49*, 712-718.
12. Popova, M.; Bankova, V.;Butovska, D.;Petkov, V.;Nikolova. B. Validated methods for the quantification of biologically active constituents of poplar-type propolis. *Phytochem. Anal.***2004**, *15*, 235-40.
13. Valencia, D.; Alday, E.; Robles, R.; Garibay, A.; Gálvez, J.; Salas, M.; Jiménez, M.; Velázquez, E.; Hernández, J.; Velázquez, C. Seasonal effect on chemical composition and biological activities of Sonoran propolis.*Food Chem.***2012**, *131*, 645-651.
14. Nagy, M.; Grancai, D. Colorimetric determination of flavanones in propolis. *Pharmazie***1996**, *51,* 100–101.
15. Falcão, S.;Freire, C.; Vilas, M.A. Proposal for Physicochemical Standards and Antioxidant Activity of Portuguese Propolis.*J. Am. Oil Chem. Soc.***2013**, *90*, 1729-1741.
16. García, M.; Medina, R.; Hidalgo, P.; Delgado, M.; Truffin, E.; Gómez, R. Actividad in vitro del Propóleos frente a Patógenos Bacterianos aislados de Infecciones Humanas. *Lat.Am. J. Pharm.***2007**, *26*, 100-102.

17. Hostettmann, K.;Wolfender, J.; Rodríguez, S. Rapid detection and subsequent isolation of bioactive constituents of crude plant extract. *Planta méd.***1997**, *63*, 2-4.

18. Popova, M.;Silic, S.;Kaftanoglu, O.; Bankova, V. Antibacterial activity of Turkish propolis and its qualitative and quantitative chemical composition. *Phytomed.***2005**, *12*, 221-228.

19. Ahn, M.; Kumazawa, S.;Usui, Y.; Nakamura.J.;Matsuka, M.B.; Zhu, F.C.; Nakayama, T. Antioxidant activity and constituents of propolis collected in various areas of China. *Food Chem.***2007**, *101*, 1383-1392.

20. Gülçin, S.;Bursal, E.;Hilal, M.;Bilsel, M.; Goren, A. Polyphenol contents and antioxidant activity of lyophilized aqueous extract of propolis from Erzurum, Turkey. *Food Chem. Toxicol.***2010**, *4*, 2227-2238.

21. Graça, M.;Nunes, S.;Anahi, S.;Cavaco, A.;Antunes, M. Phenols and antioxidant activity of hydro-alcoholic extracts of propolis from Algarve, South of Portugal. *Food Chem. Toxicol.***2010**, *4*, 3418-3423.

22. Debbache, N.;Atmani, D.;Atmani, D. Chemical analysis and biological activities of Populusnigra, flower buds extracts as source of propolis in Algeria. *Ind. Crops Prod.***2014**, *53*,85-92.

23. Kumazawa, S.;Hamasaka, T.; Nakayama, T. Antioxidant activity of propolis of various geographic origins. *Food Chem.* **2004**,*84*, 329-339.

24. Chaillou, L.; Nazareno, M. New method to determine antioxidant activity of polyphenols. *J. Agric. Food Chem*.**2006**,*54*, 8397-8402.

25. Chaillou, L.; Nazareno, M. Bioactivity of propolis from Santiago del Estero, Argentina, related to their chemical composition. *LWT - Food Sci. Technol*.**2009**, *42*, 1422-1427.

26. Oldoni, T.L.; Cabral, I.; Regitano, M.; Rosalen, P.; Ikegaki, M.; Nascimento, A.; Alencar, S. Isolation and analysis of bioactive isoflavonoids and chalcone from a new type of Brazilian propolis.*Separ.Purif. Techn.***2011**, *77*, 208-213.

27. Shimomura, K.; Inui, S.; Sugiyama, Y.; Kurosawa, M.; Nakamura, J.; Choi, S,J.;Ahn, M.R.; Kumazawa, S. Identification of the Plant Origin of Propolis from Jeju Island, Korea, by Observation of Honeybee Behavior and phytochemical Analysis. *Biosci.Biotechnol. Biochem.***2012**, *76*, 2135-2138.

28. Delgado-Aceves, M.; Andrade-Ortega, J.A.;Ramírez-Barragán, C.A. Physical-chemical description of propolis collected in La Primavera forest, Zapopan, Jalisco state. *Rev. Mexicana de CienciasForestales*.**2015**,*6*, 74-87.

29. Nie, P.;Zhengyan, X.; Da, S.; He, Y. Application of visible and near infrared

spectroscopy for rapid analysis of chrysin and galangin in chinesepropolis. *Sensors***2013**, *13*, 10539-19549.

30. Xu, L.; Yan, S.M.;Cai, C.B.; Yu, X.P. Untargeted detection and quantitative analysis of poplar balata (PB) in Chinese propolis by FT-NIR spectroscopy and chemometrics. *Food Chem.***2013**, *141*, 4132-4137.

31. Hogendoorn, E.A.;Sommeijer, M.J.;Marjo, J.;Vredenbregt, M.J. Alternative method for measuring beeswax content in propolis from the Netherlands. *J. Apic. Sci.***2013**, *57*, 81-90.

32. González-Martín, M.I.; Escuredo, O.; Revilla, I.; Vivar-Quintana, A.M.; Coello, M.C.; Riocerezo, C.P.;Moncada, G.W. Determination of the Mineral Composition and Toxic Element Contents of Propolis by Near Infrared Spectroscopy.*Sensors***2015**, *15*, 27854-68.

33. Venegas, Y.; Peña, C.;Pastene, E.; Contreras, D.A new near-infrared method for simultaneous determination of caffeic acid phenethyl ester and antioxidant activity of propolis samples.*J. Apic. Res.***2015**, *55*,8-18.

34. González-Martín, M.I.; Revilla, I.; Vivar-Quintana, A.M.; Betances-Salcedo, E.V. Pesticide residues in propolis from Spain and Chile. An approach using near infrared spectroscopy. *Talanta***2017**, *165*, 533-539.

35. Popova, M.; Bankova, V.; Stefan, B.; Tsvetkova, I.; Naydenski, C.; Marcazzan, G.; Sabatini, A. Chemical characteristics of poplar type propolis of different geographic origin.*Apidologie***2007**, *38*, 306-311.

36. Chen, E.H.; Pryce, B.A.;Tzeng, J.A.; González, G.A.; Olson, E.N. Control of myoblast fusion by a guanine nucleotide exchange factor, loner, and its effector ARF6. *Cell***2003**, *114*, 751-762.

37. Emmons, C.; Peterson, D.; Paul, G. Antioxidant capacity of oat (*Avenasativa L.)* extracts. 1. In vitro antioxidant activity and content of phenolic and totalantioxidants. *J. Agric. Food Chem.***1999**, *47*, 4894-4898.

38. Martens, D.A. Nitrogen cycling under different soil management systems. *Adv. Agron.* **2001**,*70*, 143-192.

39. Shenk, J.S.; Westerhaus,M.O.*Analysis of Agricultural and Food Products by Near Infrared Reflectance Spectroscopy.NIRSystems*. USA; Silver Spring Inc.: MD, USA, 1995.

40. Shenk, J.S.; Westerhaus, M.O. Routine Operation, Calibration, Development and Network System Management Manual.NIRSystems.USA; Silver Spring, MD USA. 1995.

41. Davies, A.M.C.; Williams, P.H. Near Infrared Spectroscopy: The Future Waves. NIR Publications.Chichester. England, 1996; pp. 519-523