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Rapid Scanning of the Origin and Antioxidant Potential of Chilean Native Honey Through Infrared Spectroscopy and Chemometrics

Ady Giordano[1] · Mauricio Retamal[1] · Edwar Fuentes[2] · Loreto Ascar[2] · Patricia Velásquez[3] · Karina Rodríguez[3] · Gloria Montenegro[3]

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Abstract
Antioxidant compounds have the ability to trap free radicals; in honey, this capacity is related to the botanical origin of the sample, and therefore, there has been a growing interest in verifying the floral origin of beehive products and its relation with the polyphenolic compounds with potential antioxidant activity. A FTIR spectrum has been use to discriminate floral origin in Chilean monofloral samples and to predict their antioxidant capacity. Forty-nine honey samples from different geographical zones and botanical origin were classified according to melissopalynology analysis, and total phenolic and flavonoid contents were quantified by spectrophotometric methods. Discriminant analysis showed that Quillay (*Quillaja saponaria*), Corcolén (*Azara petiolaris*), and Tebo (*Retanilla trinervia*) honeys showed similarities related to their common geographical origin, while Ulmo (*Eucryphia cordifolia*) presents a differentiate behavior. The FTIR spectra were able to predict phenolic and flavonoid content, establishing the potential of spectroscopic tools for quality control in Chilean beehive industry.

Keywords Honeybee · PCA · Antioxidant · FTIR · Melissopalynology

Introduction

Antioxidant compounds in food have an important role as a health-protecting factor, and they reduce the risk for chronic diseases, but their main characteristic is its ability to trap free radicals. Antioxidant compounds like phenolic acids, polyphenols, and flavonoids scavenge free radicals thus inhibiting the oxidative mechanisms that lead to degenerative diseases. Natural foods rich in compounds like vitamin C, vitamin E, carotenes, and phenolic acids have been preferred as a healthier choice.

Honey has been widely studied, specially his antioxidant, antimicrobial, and homeopathic activities (Ahmed et al. 2018; Alissandrakis et al. 2011; Bueno-Costa et al. 2016; Corbella and Cozzolino 2006; Machado De-Melo et al. 2018), since this product made from the nectar of flowers and plants inherits the natural occurring antioxidant compound present in the nectar. Studies about the properties and applications of honey have distinguished honey from different botanical origins, concluding a relation between the polyphenols and the antioxidant properties (Ciulu et al. 2016; Cornara et al. 2017; do Nascimento et al. 2018; Gašić et al. 2017).

In Chile, native plants used by bees as a nectar source result in the production of honey with particular characteristics. Approximately 95% of the honey produced in Chile is exported in bulk (Barrera and Valdés 2014). Nowadays, 18 different types of native monofloral honey have been identified in Chilean beehive industry, where *Quillaja saponaria* (Quillay) and *Eucryphia cordifolia* (Ulmo) honeys represent approximately 20% of production (Montenegro and Ortega 2013). The differentiation of several types of Chilean honey according to their particular biological origins could improve their competitive values on the international market; currently, between 7000 and 11,000 tons of honey are exported annually, especially to the European Union (Oficina de Estudios y Polìticas Agrarias 2017).

To classify the botanical origin of honey, a melissopalynology analysis can be used, but this method has proven to be complicated and tedious. Moreover, there is a need to assess the antioxidant properties of honey. Spectrophotometric methods are

✉ Ady Giordano
agiordano@uc.cl

[1] Facultad de Química y de Farmacia, Pontificia Universidad Católica de Chile, Santiago, Chile

[2] Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile

[3] Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile, Santiago, Chile

widely used to determine flavonoids and polyphenols in honey even though they give poor information about the composition of these compounds. Liquid chromatography and mass spectrometry have also been used, but unfortunately, these are more laborious and expensive methods in comparison with NIR, that is quick and require a minimal amount of sample and handling (Martínez del Río et al. 2013; Pascual-Maté et al. 2018).

An FTIR spectrum has the potential to bring information on compounds and their functional groups present in honey allowing to discriminate and authenticate different samples (Kasprzyk et al. 2018; Leme et al. 2018; Pascual-Maté et al. 2018). However, it is necessary to process the data using chemometric tools, such as main component analysis, partial least squares, and discrimination analysis to characterize and classify honey samples (Corvucci et al. 2015; Jandric et al. 2015; Popek et al. 2017; Song et al. 2016; Wen et al. 2017; Zhou et al. 2014).

The aim of this work is to apply FTIR analysis and chemometric tools to describe and differentiate two or more botanical origins in Chilean honey samples and predict antioxidant activities like total phenolic or flavonoid content in native honey.

Materials and Methods

Chemicals

Folin–Ciocalteu's phenol reagent, aluminum chloride, sodium chloride, hydrochloridric acid, disodium hydrogen phosphate dihydrate, ethanol, and sodium phosphate monobasic reagent were supplied by Merck (Darmstadt, Germany). Water was purified in a Milli-Q system (Synergy, Millipore, Darmstadt, Germany).

Honey Sampling

Honey samples were taken from local beekeepers from the O'Higgins (Mediterranean climate) and Araucanía (humid temperate climate) regions of Chile. A total of 49 samples were taken, including 20 *Quillaja saponaria* (Quillay), 14 *Eucryphia cordifolia* (Ulmo), 8 *Azara petiolaris* (Corcolén), and 7 *Retanilla trinervia* (Tebo) honey samples. They were stored in darkness and refrigerated at 4 °C until analysis.

Melissopalynology Analysis

The botanical/floral origin of the honeys was determined by a palynological analysis as described in the Chilean Normative for classification of honeybees (Nch2981 n.d. Of2005). To determine the botanical origin of the pollen in honey samples, specific literature (Heusser 1971; Marticorena and Quezada 1985) and a botanical palinotec library in Pontificia Universidad Católica de Chile were consulted. Unifloral honey types were those that come mainly from one species whose pollen composition was at least 45% of pollen from that plant. On the other hand, multifloral honey types were those made from the nectar of several species whose principal pollen did not reach a percentage equal to or greater than 45% and are considered bifloral honey types. These are determined by the fact that their composition is made significantly of pollen from two species (both ≥ 45%) and a similar proportion was found (with a difference of < 5%).

Determination of Total Flavonoids

Honey extracts were obtained based on the Montenegro patent (EP1852017-2010) and Ferreres et al. (1994) procedures. Honey samples were diluted in acidic water (pH 2 with HCl). Then, the dissolution was passed through an open chromatographic column filled with Amberlite XAD-2 and washed with acidic water, distilled water, and ethanol. Residues were concentrated with a vacuum rotary evaporator and redissolved in pure water. Finally, extracts were filtrated (EDLAB CA Syringe filter 0.45 μm) and stored at − 18 °C.

Total flavonoids were determined using Prelipcean et al. (2011) published procedures. Briefly, 1 mL of extract was mixed with aluminum chloride 2% in ethanol and let rest for 1 h at room temperature. Its absorbance was determined at 420 nm (Spectrophometer Agilent 8453; Software UV-Visible Chemstation Rev.A.10.01 Agilent Technologies 95-03). Calibration curve was prepared with quercetin, and the results were expressed in mg QE/100 g of honey.

Determination of Total Phenolic Compounds

Two hundred microliters of the previously described extract was taken and mixed with 50 μL of Folin-Ciocalteu Reagent, 150 μL of sodium carbonate solution (20% *w*/*w*), and 600 μL of ultrapure water. The solution was let to rest for 30 min at dark, and measure the absorbance of the samples at 765 nm. The calibration curve was prepared with gallic acid, and the results were expressed in mg GAE/100 g of honey.

IR Analysis

Honey samples were homogenized before IR analysis. A 200 mg of honey was deposited on KBr pellets of 0.1 mm optical path for their analysis on a Vector 22 spectrophotometer (Bruker Optics Inc., Germany) over the range of 250–4000 cm^{-1}. For each sample, 64 scans were recorded, with a spectral resolution of 2 nm.

Multivariate Analysis

FTIR spectra were centered and standardized before chemometric analysis. Matrix data was constructed by 49 × 1945 (49 samples and 1945 spectral point data). A principal component

Table 1 Melissopalynology analysis of four different botanical origins of Chilean honey samples

Sample	Primary species		Secondary species		Total phenolics content (mg GAE/100 g honey)	Total flavonoids content (mg QE/100 g honey)
	Scientific name	Percentage (%)	Scientific name	Percentage (%)		
Q1	*Quillaja saponaria* (Quillay)	46.6	*Galega officinalis*	19.0	13.2 ± 0.7	2.2 ± 0.2
Q2		68.5		25.0	12.1 ± 0.9	1.9 ± 0.1
Q3		50.9	*Crinodendron patagua*	12.7	14.2 ± 1.1	2.2 ± 0.1
Q4		47.8	*Trifolium repens*	21.7	10.5 ± 1.6	1.4 ± 0.2
Q5		76.3	*Medicago poymorpha*	8.3	12.3 ± 0.1	2.1 ± 0.1
Q6		49.6	*Galega officinalis*	32.3	12.7 ± 0.6	2.5 ± 0.2
Q7		48.2		24.5	8.4 ± 0.1	1.4 ± 0.2
Q8		46.0		17.0	13.3 ± 0.6	1.6 ± 0.5
Q9		62.2		15.2	8.1 ± 0.5	0.4 ± 0.1
Q10		38.9		20.1	12.9 ± 0.1	2.3 ± 0.1
Q11		70.7		15.9	11.7 ± 1.3	1.8 ± 0.4
Q12		40.4		21.2	12.9 ± 0.6	2.1 ± 0.1
Q13		43.9	*Azara* sp.	29.6	16.3 ± 0.1	2.6 ± 0.3
Q14		73.9	*Galega officinalis*	8.6	11.5 ± 1.1	2.5 ± 0.4
Q15		74.9		23.0	12.7 ± 0.6	1.9 ± 0.4
Q16		35.4		33.4	13.7 ± 0.4	2.5 ± 0.2
Q17		46.3		27.7	13.2 ± 0.7	1.9 ± 0.1
Q18		46.9		9.4	12.7 ± 0.4	2.5 ± 0.2
Q19		38.3		24.5	10.7 ± 0.6	1.8 ± 0.1
Q20		46.2		27.0	16.9 ± 0.7	2.7 ± 0.1
U1	*Eucryphia cordifolia* (Ulmo)	45.7	*Lotus pedunculatus*	19.4	180.4 ± 1.5	46.9 ± 1.5
U2		93.5		2.9	199.6 ± 6.7	42.7 ± 7.4
U3		89.1		7.7	185.5 ± 2.2	81.4 ± 3.7
U4		95.6	*Weinmannia trichosperma*	3.8	202.1 ± 4.4	82.3 ± 9.5
U5		89	*Luma apiculata*	6.2	184.6 ± 1.9	75.5 ± 4.3
U6		98		1.9	183.8 ± 2.1	78.4 ± 3.3
U7		97.2	*Weinmannia trichosperma*	2.8	188.6 ± 4.3	90.1 ± 6.9
U8		92.9	*Lotus pedunculatus*	2.6	176 ± 9.5	83.1 ± 5.9
U9		96		2.5	180.4 ± 4.1	70.5 ± 1.3
U10		94.9	*Weinmannia trichosperma*	3.5	181.6 ± 2.6	70.1 ± 2.6
U11		97.2	*Luma apiculata*	1.6	177.3 ± 8.4	83.3 ± 9.4
U12		68.4	*Weinmannia trichosperma*	22.9	180.6 ± 2.1	59.7 ± 0.5
U13		49.1	*Lotus pedunculatus*	41.4	178.4 ± 2.8	66.1 ± 2.6
U14		93.6	*Azara/Salix*	9.1	155.5 ± 2.4	54.1 ± 9.2
C1	*Azara petiolaris* (Corcolén)	51.7	*Galega officinalis*	15.9	66.3 ± 7.4	12.3 ± 0.9
C2		76.6		6.7	153.3 ± 6.1	11.9 ± 0.8
C3		45	*Luma/Myrceugenia*	33	72.9 ± 2.5	8.3 ± 0.6
C4		43.4		39	65.1 ± 2.3	8.7 ± 0.6
C5		58.5	*Brassica* sp.	20.2	48.8 ± 1.1	5.9 ± 0.4
C6		63.9	*Escallonia* sp.	11	128.4 ± 4.4	13.6 ± 0.9
C7		83.6	*Luma/Myrceugenia*	8	135.2 ± 4.3	38.3 ± 3.1
C8		73		7.1	126.2 ± 2.9	10.9 ± 0.7
T1	*Retanilla trinervia* (Tebo)	77.96	*Robinia pseudoacacia*	11.84	157.5 ± 6.6	104.8 ± 1.5
T2		63.14	*Talguenea quinquinervia*	11.3	194.1 ± 1.2	127.1 ± 4.6
T3		45.32	*Myrceugenia*	9.98	204.2 ± 6.5	84.8 ± 1.3
T4		47.82	*Brassica* sp.	13.4	154.2 ± 1.6	56.6 ± 5.5
T5		47.96	*Galega officinalis*	11.1	250.3 ± 8.9	108.6 ± 2.4

Table 1 (continued)

Sample	Primary species		Secondary species		Total phenolics content (mg GAE/100 g honey)	Total flavonoids content (mg QE/100 g honey)
	Scientific name	Percentage (%)	Scientific name	Percentage (%)		
T6		56.15	*Salix* sp.	11.3	179.5 ± 4.2	98.2 ± 5.7
T7		56.23	*Crinodendron patagua*	24.36	178.5 ± 2.2	105.1 ± 4.1

analysis (PCA) was performed on the data to obtain an overall multidimensional view. The number of components required to explain the data adequately was selected by cross-validation.

The matrix of scores generated in the PCA, consisting of 49 rows (samples) and *A* columns, where *A* = number of components extracted by cross-validation, was used to build a classification model using linear discriminant analysis (LDA). The A initial PCs were further reduced by backward elimination on the basis of their partial *F* values in the discriminant models.

A prediction model of total content of polyphenol and flavonoids was created using partial least square method (PLS1). The X-variable was spectra data, and Y-variable was the total flavonoids and polyphenols concentrations. The optimum number of factors to be used within the PLS regression was determined through a full cross-validation procedure, which consisted of systematically removing one of the training samples, and using only the remaining ones for construction of the latent factors and/or regression coefficients. All data was previously centered and standardized.

PCA and PLS1 were built using The Unscrambler v9.7 CAMO software, while Statgraphics Centurion XV software was used for LDA.

Results and Discussion

Classification by Linear Discriminant Analysis

The melissopalynology analysis results are listed in Table 1. Most of the samples were cataloged as monofloral honeys accordingly with the Chilean normative (Montenegro et al. 2008). However, a wide range of secondary species have been observed, especially in honey from the central zone where diverse flora grows. In Quillay honey, the most abundant secondary species is G*alega officinalis* with high percentages up to 33%. Ulmo honey showed *Lotus pedunculatus* as its most abundant secondary species; but in all samples, the other species represent less than the 22% of the contribution. Corcolén and Tebo honeys do not have a characteristic secondary species, representing less than 32% of the botanical origin. Important to highlight is the contribution of introduced species such as *Galega officinalis* (galega), *Trifolium repens* (white clover), and *Brassica* sp. (cabbage, cauliflower, broccoli, brussel sprouts) in samples from Central Chile, a reflection of its agricultural industry. On the other hand, low percentages of endemic species of Chile like *Weinmannia trichosperma* (Tineo) and *Lotus pedunculatos* (*Alfalfa chilota*) were observed in samples from southern Chile.

Total phenolic content and total flavonoids content shown in Table 1 have been used as preliminary screening of antioxidant capacity, since they are widely use in food matrices (Bridi et al. 2015). A significantly lower content of phenolics was observed in Quillay honeys when compared with the other honey samples in this study, and with some international monofloral honeys such as the famous Manuka honey (*Leptospermum scoparium*) or the black forest honey (*Acacia* sp.), with a total polyphenol value reported of 89.9 ± 1.2 mg and 62.7 ± 4.4 GAE/100 g of honey, respectively (Alzahrani et al. 2012).

In accordance with the FC results, Quillay honey presents a lower flavonoid content than the other samples. However, a previous reported study with 26 Chilean honey samples establishes that flavonoid content can vary from 0.014 to 13.80 mg QE/100 g of honey (Muñoz et al. 2007).

IR Honey Samples

Samples were analyzed by IR since it is a relatively easy and quick technique to address honey composition. The fingerprint spectra of a representative sample for each honey type (Quillay, Corcolén, Tebo, and Ulmo) are shown in Fig. 1.

The four types of honeybees have a remarkable band related to water signal at 3600–3000 cm^{-1}, associated to the OH stretching, since water along with fructose and glucose represents 95% of honey composition. The signal at 2900–2800 cm^{-1} should correspond to the C–H stretching of the methyl and methylene groups of the sugar molecules. Also, in all the samples, a peak at 1700–1600 cm^{-1} corresponds to the C=O stretching absorption of aldehyde group from glucose and fructose compounds. As expected, all samples showed a peak at 1450–1400 cm^{-1} assigned to the antisymmetric deformation of methyl and the scissors vibration of methylene groups and a defined 1200–1050 cm^{-1} signal assigned to the stretching vibration absorption of C–OH from typical alcohol and phenol structures (Svečnja et al. 2015).

However, relevant difference can be observed in the fingerprint zone. Quillay, Corcolén, and Tebo honey types showed similar signal in the 2500–2200 and 1800–1500 cm^{-1} spectra regions due to C–H stretching and aliphatic C=O stretching,

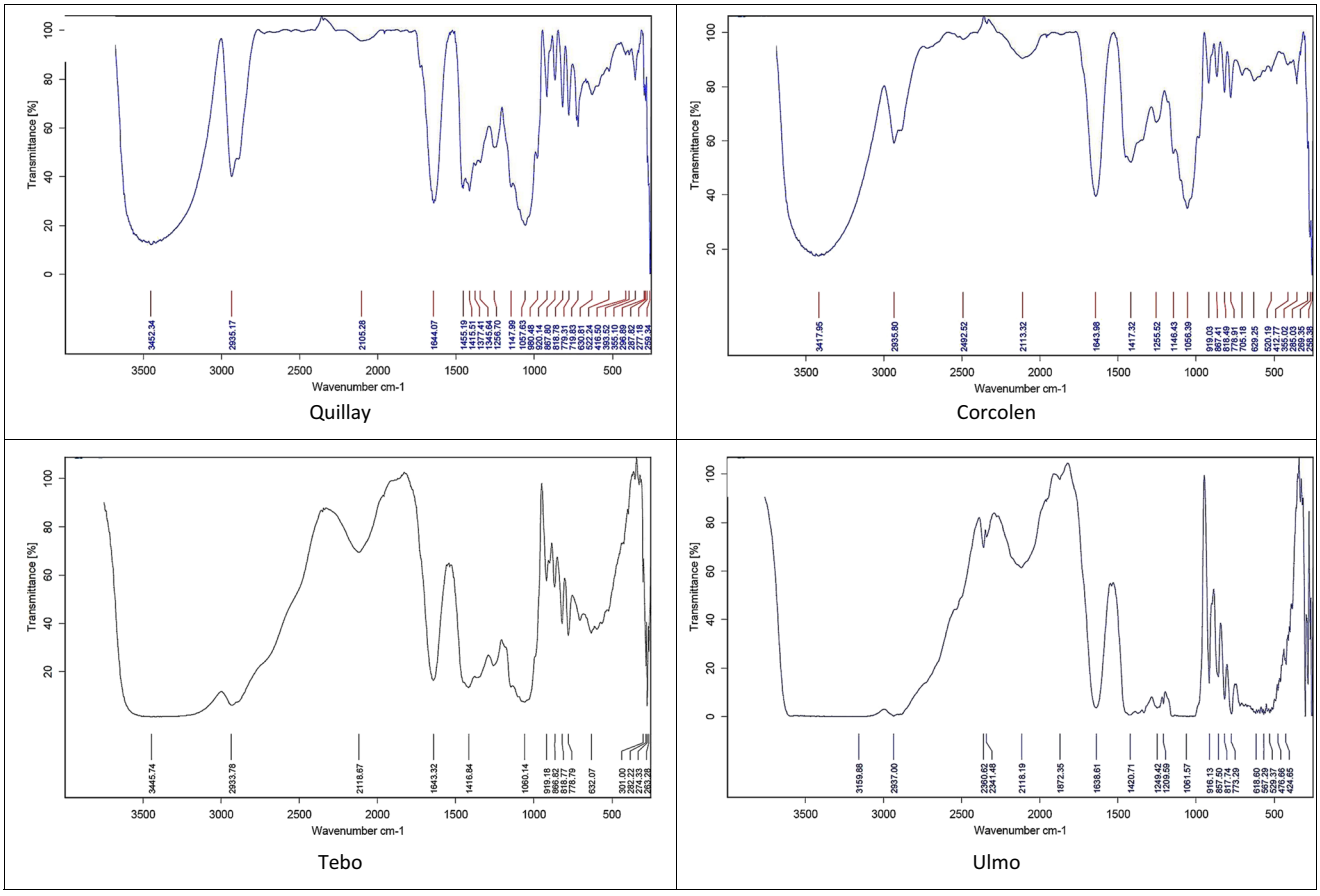

Fig. 1 Representative IR spectra from different botanical origins of four native Chilean honey types

respectively, which may be linked to their common geographical origin (Region of O'Higgins). Ulmo honey shows a more distinctive signal a 1750–1710 corresponding to the stretching vibration absorption of C=O carbonyl group. On the other hand, Ulmo's honey showed saturation in the 900–500 and 1800–1600 cm^{-1} regions, which is characteristic of methylic and phenolic compounds and cetonic groups, respectively. Thi slightly different could indicate that the composition of honey, reflected in the IR spectra, could be related to both its botanical and geographical origin.

Principal Component Analysis

The IR spectra of the samples in the range 4000–400 cm^{-1} were analyzed by PCA, a common statistical tool to analyze data from complex matrices such as honey. Figure 2 shows the score plot for the first two main components that explains 79 and 13% of the variance of data, respectively (both PCs explain 92% variance of data). As it is evident, Ulmo honey forms a distinct group from the rest of the samples. Honey from Quillay and Tebo is distinguishable from one another, yet Corcolén honey appears in both groups; this could indicate that the difference in composition given by botanical origin is less significant than the one given by the geographical origin. Thus, honey from Quillay, Tebo, and Corcolén collected in the same region showed similar IR spectra and appeared to be less differentiated from one another using only this information.

Botanical Origin

In LDA, eight initial PCs were further reduced to seven by backward elimination on the basis of their partial *F* values in the discriminant model. The incorporation of more than seven PCs makes greater misclassification probable since they are only modeling the noise of IR spectra.

Table 2 shows the classification honey by LDA. For Ulmo and Tebo honey types, all samples were correctly classified (7 Tebo and 14 Ulmo honey samples), while for Quillay and Corcolén honey, the classification rate was 80% and 75%, respectively. This misclassification could have been produced by the similarity in the composition of the nectar source due to weather conditions but also could be important to consider that those honey types share botanical composition due to the presence of *Galega officinalis* and *Luma apiculata* as secondary species in most of the samples.

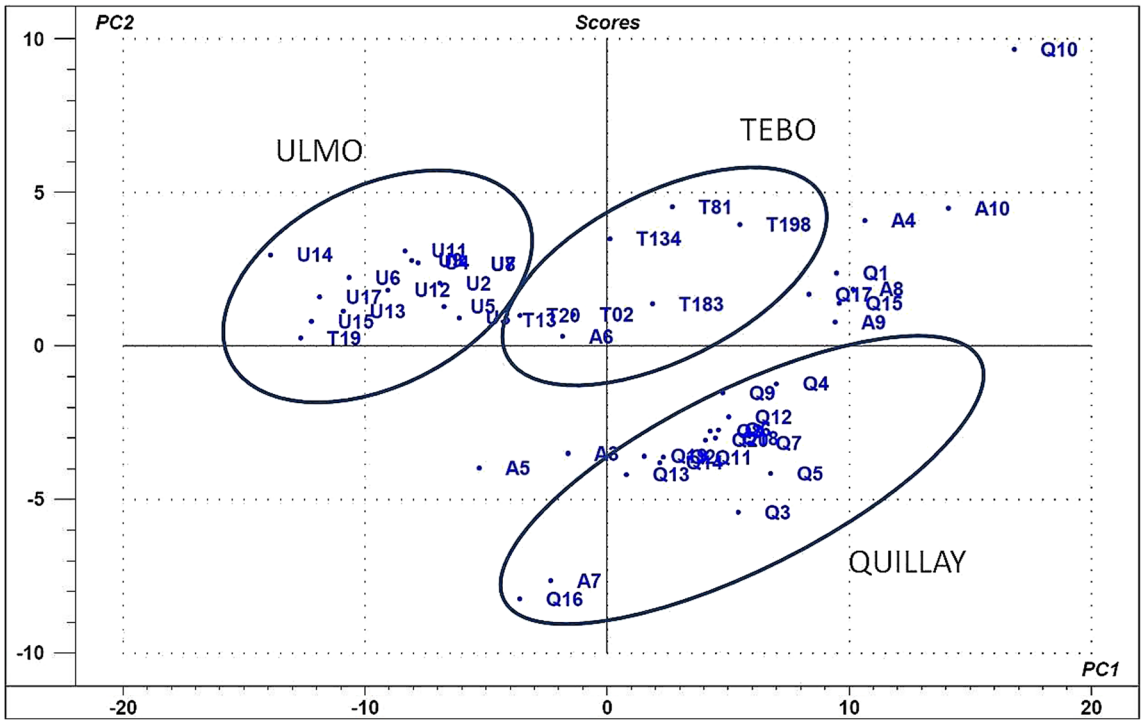

Fig. 2 Score plot of PCA at IR spectra Chilean honey

Geographical Origin

Honey samples were taken from two different Regions with different climate. The central zone of the country has Mediterranean climate and hence helps produce honey from endemic unifloral and native multifloral botanical origins, while the south area of the country is characterized by a humid temperate climate and thus produces both native and non-native unifloral and native multifloral varieties with restricted botanical diversity (Montenegro et al. 2013).

In this regard, vegetation in those regions is diverse and does not necessarily share the same native and endemic species. IR analysis gives a correlation between functional group and concentration of them in honey. There is a good correlation between IR honey spectra and their geographical origin (Fig. 3).

In predicted geographical origin by PLS1 model, regression data showed a good correlation (R-Square near to 0.9): Ulmo honeys can be easily differentiated from the other three honey types, showing that samples from O'Higgins region share similar characteristics, having similar data range (a little compacted).

Prediction of the Antioxidant Potential of Honey

A PLS regression was carried out between IR spectra (X variable) and total phenolic or total flavonoid content (Y variable) for all honey samples. The score plots and the predicted concentrations compared with the measured values obtained experimentally are displayed in Fig. 4. For these models, four and three PLS factors were selected by cross-validation that accounts for the variance in phenolic and flavonoid content. A significant correlation was observed between predicted and measured values for both parameters (R-square = 0.82 and 0.73 in cross-validation, respectively). Conversely, the values of slope obtained were lower than 1.00 (0.84 and 0.77 for polyphenols and flavonoid) in both cases, indicating an underestimation in its content by the IR-PLS1 model.

Table 2 Prediction of classification honeys by discriminant analysis

Botanical origin	Predicted botanical origin				
	Corcolén	Quillay	Tebo	Ulmo	% correct classification
Corcolén ($n = 8$)	6	2	0	0	75
Quillay ($n = 20$)	4	16	0	0	80
Tebo ($n = 7$)	0	0	7	0	100
Ulmo ($n = 14$)	0	0	0	14	100

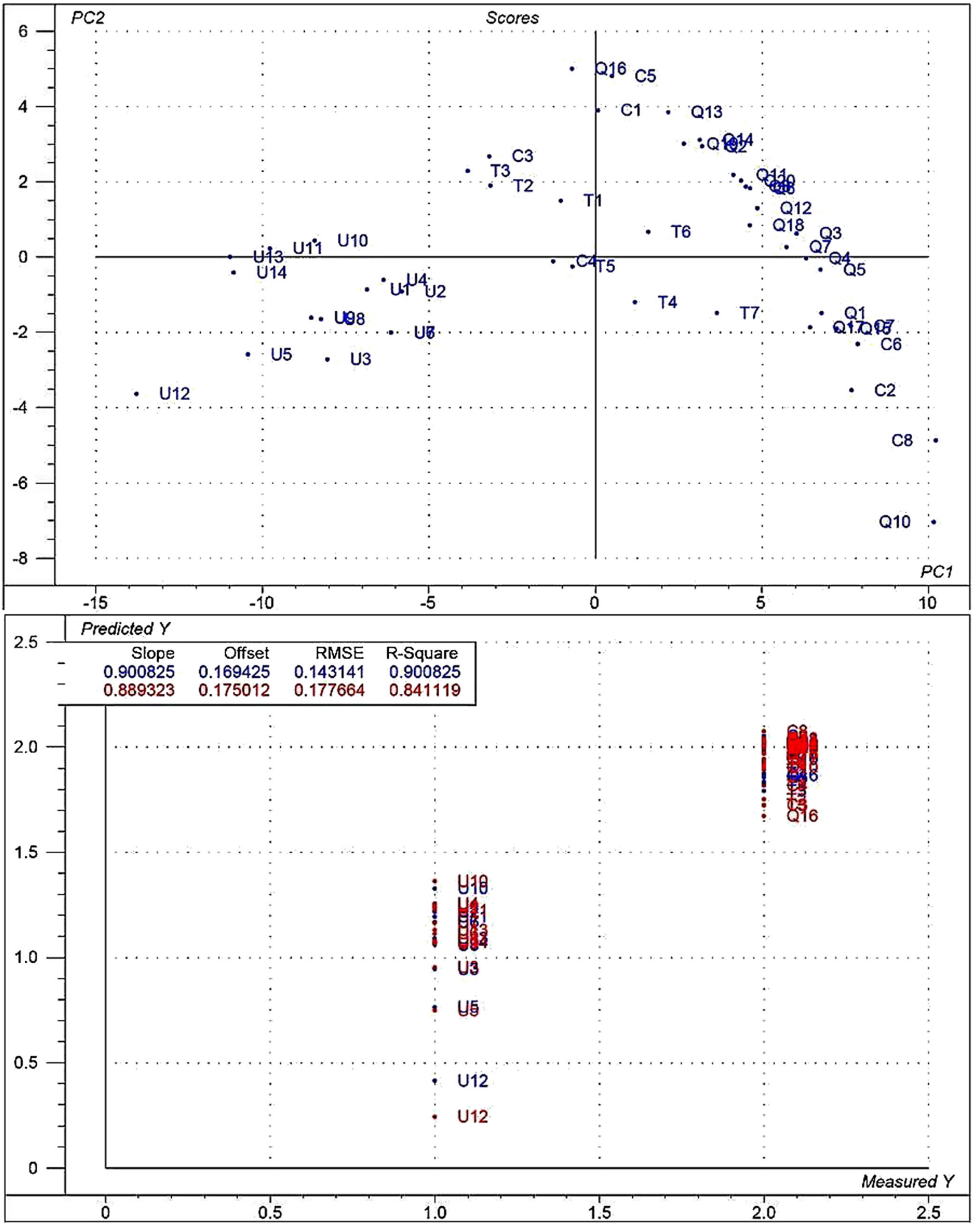

Fig. 3 Score plot and predicted data of geographical-IR spectra correlation by PLS1 model

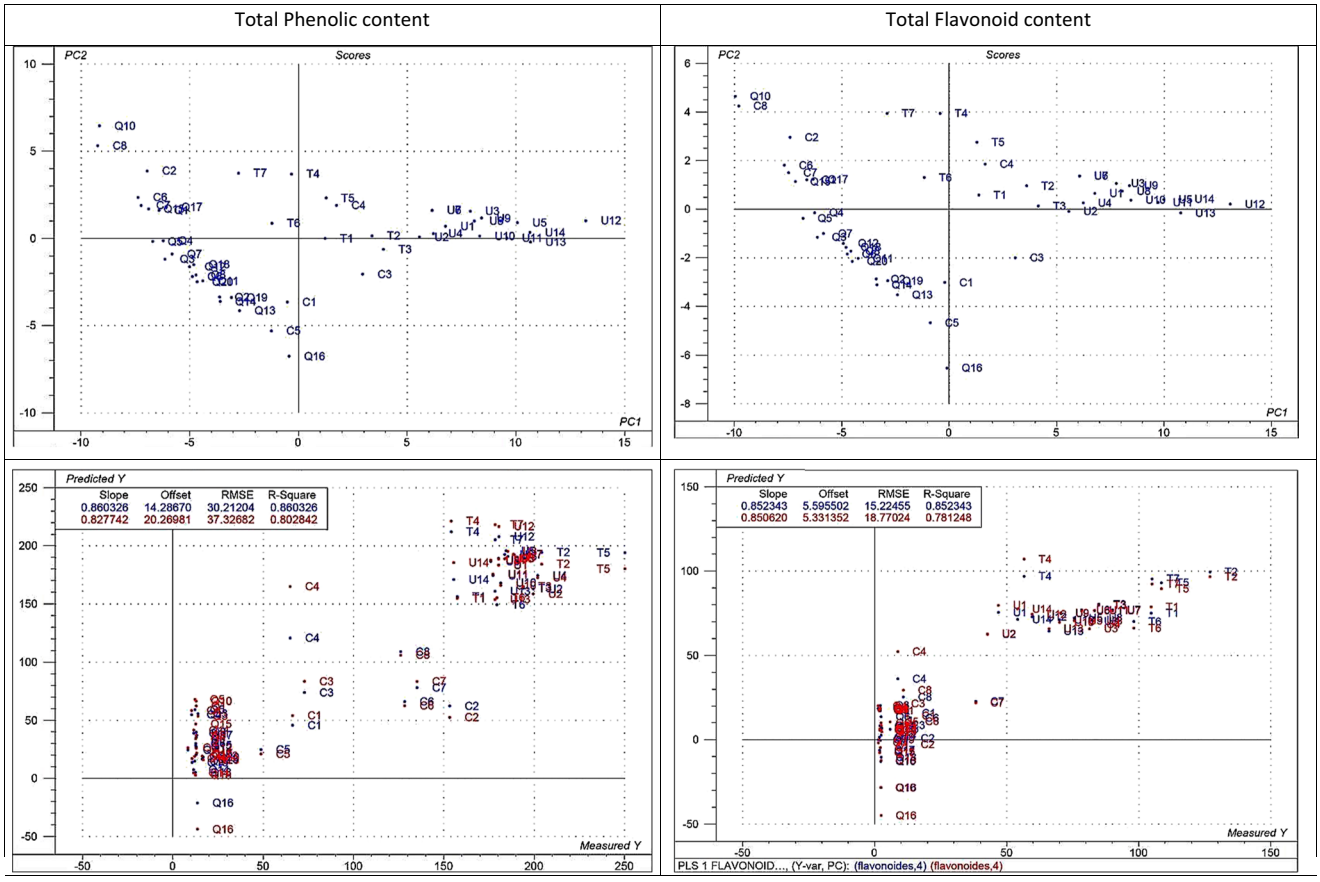

Fig. 4 Prediction of total phenolic and flavonoid contents in Chilean honey types by PLS1 model

However, as seen in Fig. 4, the concentration of both parameters was predicted incorrectly in Quillay honey samples. This honey type had the lowest concentration of polyphenol and flavonoid content (lower than 20 mg GAE or QE/100 g of honey) that could imply that the PLS models are appropriate for honey samples with higher values of flavonoid and phenolic content, as was determined in Ulmo, Corcolén, and Tebo honey samples. RMSE value of PLS model was 30 mg GAE/100 g honey and 15 mg QE/100 g honey for polyphenol and flavonoid content, respectively, and RMSE represents a variance lower than 20% of values founded in Ulmo, Corcolén, and Tebo. These values were actually predicted by the PLS models with some level of dispersion related to the spectral variability of the samples, and considering that both FC method and Prelipcean method values can be overcalculated due to interference such as sugar residues in the extracts (Bridi et al. 2015), suggesting that the error in prediction could be linked to error in the experimental analysis.

Conclusion

IR spectra analysis on Chilean honey samples can offer very important data such as botanical origin or antioxidant activity when chemometric data analysis is applied. Prediction of important properties of honey like botanical origin and total phenolic and flavonoid compounds offers important information allowing us to be aware of the quality of Chilean honey samples in a short time. The PLS models can be used as a quick review of honey samples by NIR to establish antioxidant potential, providing an estimate of the contents of polyphenols and flavonoids. This approach also could be used as a tool to identify unifloral honeys or adulteration in samples.

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Compliance with Ethical Standards

Conflict of Interest Ady Giordano declares that she has no conflict of interest. Mauricio Retamal declares that he has no conflict of interest. Edwar Fuentes declares that he has no conflict of interest. Loreto Ascar declares that he has no conflict of interest. Patricia Velásquez declares that

she has no conflict of interest. Karina Rodríguez declares that she has no conflict of interest. Gloria Montenegro declares that she has no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent Not applicable.

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