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Relationships among botanical origin, and physicochemical and antioxidant properties of artisanal honeys derived from native flora (Catamarca, Argentina)

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Abstract

The present work was designed to increase our scientific understanding of the quality of artisanal honeys (i.e., derived from a single species of flower), as a basis for enhancing their value as an economic product and a natural nutraceutical food. Physicochemical properties of honey samples (n = 39) from Catamarca province, Argentina were analysed by a variety of methods, including high-performance liquid chromatography with diode array detection/mass spectrometry (HPLC-DAD-MS/MS). The samples showed a preponderance of pollen types from native floral species, and high levels of antioxidants. Flavonoid content, polyphenol content, radical scavenging capacity (RSC), acidity, and ash content comprised an array of closely interrelated parameters. Polyphenols and flavonoids were identified in two samples (*Prosopis* honey and *Cercidium* honey), that had a single pollen type abundance > 80%, by HPLC-DAD-MS/MS. The results suggest that *Cercidium praecox* contributed more strongly than other floral species to beneficial antioxidant properties, probably related to caffeoylquinic compounds. Our findings demonstrate the importance of native flora as a renewable natural resource, and the relatively greater contribution of certain floral species to antioxidant properties of honeys.

Introduction

Honey is a sugary food substance produced by honey bees (genus Apis) from flower nectar and pollen (Hussein *et al*., 2011). Honeys are important economic resources in many poorly developed areas of the world, and it is important to characterise properties that may increase their market value. Antioxidant capacity, oxidative status and radical scavenging capacity are notable properties that add value to artisanal honeys (i.e., derived from a single species of flower); there is general consensus that low oxidative status and high antioxidant capacity have several beneficial effects on health. The antioxidant capacity of honey is based on a wide range of components, including phenolics, peptides, organic acids, enzymes and Maillard reaction products (Gheldof *et al*., 2002).

It has been proposed that honey has a potential therapeutic role in the treatment of diseases because of anti-inflammatory, antimicrobial, and antioxidant properties; polyphenols and flavonoids which act as antioxidants, are two main bioactive molecules present in honey (Samarghandian *et al*., 2017; Cianciosi *et al*., 2018). The composition of honey depends on the contributions of nectar, pollen and the work of the bees. Polyphenols are provided by the plants, and therefore these molecules have been addressed as possible markers of the botanical origin of a honey (Bertoncelj *et al*., 2007; da Silva *et al*., 2016).

Catamarca is a province of Argentina, located in the northwest of the country (25 - 30 °S and 69 - 65 °W). Honey from Catamarca offers a unique opportunity since it is a zone of reduced anthropogenic activity with native flora from different regions. *Apis*

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mellifera colonies utilise native flora, from where a diversity of pollen is provided. Some of the richest sources of pollen and nectar are *Prosopis* spp., *Larrea* spp., *Geoffraea decorticans* (Gillies ex Hook. et Arn.) Burkart and *Cercidium praecox* (Ruiz et Pav. Ex Hook.) Harms ssp. praecox (Salgado and Pire, 1998). We previously described the prevalence of pollen from native flora (almost 80%) in Catamarca honeys (Costa *et al*., 2013).

In the present work, the relationship between physicochemical properties and antioxidant capacity of honeys, in terms of three parameters: polyphenol levels, flavonoid levels, and radical scavenging capacity (RSC) was investigated. Honey samples were analysed, and a relationship between floral origin, polyphenol and flavonoid content was evaluated. Polyphenols and flavonoids were identified in two samples that had a single pollen type abundance (> 80%) in order to characterise the molecules that could contribute to antioxidant activity and/or botanical origin markers for the honeys of Catamarca.

Materials and methods

Reagents

Guanidine hydrochloride (ultrapure) was purchased from Genbiotech (Buenos Aires, Argentina). Trichloroacetic acid, glacial acetic acid, gallic acid, ethyl acetate, ethyl alcohol, and hydrochloric acid were purchased from Cicarelli (Buenos Aires, Argentina). Acrylamide, 2,4-dinitrophenylhydrazine (DNPH), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich (Steinheim, Germany). N,N′-methylene bis-acrylamide and acrylamide were purchased from Sigma-Aldrich (St. Louis, US). All products were of analytical grade. Methanol used for mobile phase was purchased from Merck (Darmstadt, Germany). HPLC-grade water was produced by a Milli-Q system (Millipore; Bedford, US). Amberlite XAD-2 resin was purchased from Supelco (Bellefonte, US).

Artisanal honey samples

Thirty-nine artisanal honey samples produced by *Apis mellifera* L. colonies were obtained from and collected by beekeepers registered with RENAPA (Argentinean National Registry of Beekeepers) in southeast Catamarca province during summer 2014 and summer 2016.

Physicochemical analysis of honey samples

Water content was determined from refractive index of honey using a table based on Wedmore's formula (Wedmore, 1955). Colour was determined by measuring light transmittance in a colorimeter (Hanna Instruments; Szeged, Hungary) with glycerol as standard. Results were expressed in mm Pfund. Colour intensity was calculated as the difference between spectrophotometric absorbance at 450 and 720 nm (Beretta *et al*., 2005). Ash content was determined by gravimetric method (IHC, 2009). Free acidity was determined by potentiometric titration following homogenisation and filtration using a pH meter (Adwa AD8000 Professional Multi-Parameter pH-mV-EC-TDS-TEMP Meter; Szeged, Hungary) to measure free acidity and expressed as milliequivalents acid per kg (mAE/kg). Total protein content was determined by Bradford's method (Bradford, 1976), as adapted to honey (Azeredo *et al*., 2003; Beretta *et al*., 2005). For the determination of 5-hydroxymethylfurfural (HMF) content, honey sample was dissolved in water and determined using a formula (IHC, 2009).

Total phenolic content was determined by the Folin-Ciocalteu spectrophotometric method (Singleton and Rossi, 1965). Concentrations were calculated using a gallic acid standard curve, and total phenolic content was expressed as mg gallic acid equivalents (mg GAE) per 100 g honey.

Total flavonoid content was determined by a colorimetric method (Zhishen *et al*., 1999). Absorbance of a complex generated by flavonoids and $AlCl_3$ in the presence of $NaNO_2$ was determined at wavelength 510 nm using a spectrophotometer (model UV-Vis Bio-Spec mini, Shimadzu Corp.; Kyoto, Japan). Results were expressed as mg catechin equivalents (mg CE) per g honey.

Radical scavenging capacity (RSC) was evaluated against DPPH radical following the method of Beretta *et al*. (2005).

Melissopalynological analysis

Melissopalynological qualitative studies were performed as previously described (Louveaux *et al*., 1978). A 10-g honey sample was dissolved in 50 mL distilled water, centrifuged at 1,500 g for 10 min, washed with distilled water, and acetolysed (Erdtman, 1960). Pollen sediment was mounted in glycerin jelly and sealed with paraffin. Pollen types were identified by comparison with a reference collection (located in the Palynotheca, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba [UNC]) of plants in the area surrounding the beehives. Herbarium specimens collected during the study were deposited in the Cátedra de Palinología of the Faculty.

HPLC analysis of phenolic compounds

Phenolic compounds were analysed by HPLC-DAD-MS/MS using a 1200 Series HPLC system (Agilent Technologies) connected to a G1315 C Starlight diode array detector (DAD) (Agilent) and mass spectrometer (micrOTOF-Q11 Series; Bruker). Compounds were tentatively identified based on retention times, elution order, UV-vis spectra, and MS fragmentation spectra in comparison with phenolic standards (Table 2). For polyphenol quantification, mass peak areas were obtained from extracted ion chromatograms. Precursor and corresponding fragment ions were deconvoluted using the Dissect algorithm (Data Analysis 4.0, Bruker).

Statistical analysis

Data were expressed as mean ± SE from three separate experiments, each conducted in triplicate. Correlation analysis was performed by the least-squares method. Data were analysed using the Stat Plus software program for Microsoft Windows (Berk Carey, Pacific Grove, CA).

Table 1. Geographical and botanical origins of 39 samples studied.

Sample (code)	Location	Major pollen type (% abundance in individual samples)
01	Nueva Coneta-Capayán	24 *Mimosa ephedroides;* 23 *Larrea divaricata;* 16 *Prosopis* spp.;
02	Nueva Coneta-Capayán	44 *Larrea divaricata*; 20 *Prosopis* spp.; 14 *Mimosa ephedroides*
03	Trampasacha-Capayán	97 *Schinopsis* spp.
04	Alijilán-Santa Rosa	28 *Myrcianthes mato*; 20 *Tournefortia lilloi*; 19 *Aloysia gratissima*;
05	Alijilán-Santa Rosa	63 *Tournefortia lilloi*
06	Alijilán-Santa Rosa	53 *Tournefortia lilloi*
07	Alijilán-Santa Rosa	90 *Tournefortia lilloi*
08	Nueva Coneta-Capayán	60 *Prosopis* spp.
09	Huillapima-Capayán	39 *Prosopis* spp.; 22 *Geoffroea decorticans*; 17 *Larrea* sp.; 17 *Mimosa ephedroides*
10	Nueva Coneta	29 *Larrea divaricata*; 28 *Citrus* spp.; 17 *Cercidium praecox*
11	Huillapima-Capayán	57 *Citrus* spp.; 12 *Larrea divaricata*
12	La Antena	26 *Larrea divaricata*; 19 *Prosopis* spp.; 10 *Cercidium praecox*
13	Aeropuerto Capayán	58 *Larrea divaricata*; 35 *Prosopis* spp.
14	La Bajada- Paclín	16 *Citrus* spp.; 15 *Prosopis* spp.; 13 *Cercidium praecox*; 13 *Parkinsonia aculeata*
15	Fray M. Esquiú	13 *Prosopis* spp.; 10 *Glandularia dissecta*; 9 *Parkinsonia aculeata*
16	Choya-Andalgalá	55 *Mimosa* spp.; 23 *Acacia* spp.
17	Choya-Andalgalá	47 *Adesmia* sp.; 19 *Mimosa ephedroides*
18	Medanitos-Tinogasta	48 *Prosopis* spp.; 12 *Larrea divaricata*
19	Chumbicha-Capayán	57 *Cercidium praecox*; 13 *Schinus* sp.; 10 *Larrea* sp.
20	Chumbicha-Capayán	21 *Prosopis* spp.; 18 *Larrea divaricata*; 15 *Lycium* sp.
21	Chumbicha-Capayán	17 *Prosopis* spp.; 15 *Larrea cuneifolia*
22	Chumbicha-Capayán	80 *Cercidium praecox*
23	Chumbicha-Capayán	25 *Prosopis* spp.; 15 *Larrea divaricata*; 12 *Xinemia americana*; 11 *Adesmia* sp.
24	Chumbicha-Capayán	40 *Senna aphylla*; 29 *Cercidium praecox*
25	La Costa-Capayán	82 *Prosopis* spp.
26	Recreo-La Paz	13 *Geoffroea decorticans*; 12 *Larrea divaricata*; 10 *Mimosa* spp.; 10 *Ziziphus mistol*
27	Recreo-La Paz	16 *Prosopis* spp.;13 *Ziziphus mistol*; 12 *Mimoziganthus carinatus*; 9 *Xinemia americana*
28	Recreo-La Paz	18 *Ziziphus mistol*; 16 *Prosopis* spp.; 9 *Larrea* sp.
29	Balcozna- Paclín	48 *Parkinsonia aculeata*; 15 *Ziziphus mistol*; 13 *Baccharis* sp.
30	Palo Labrado- Paclín	57 Zanthoxylum coco; 21*Mimosa* spp.
31	Palo Labrado- Paclín	23 *Larrea divaricata*; 20 *Parkinsonia aculeata*; 19 *Mimosa ephedroides*
32	La Higuera	36 *Ziziphus mistol*; 12 *Baccharis* sp; 10 *Tournefortia lilloi*; 9 *Schinopsis* spp.
33	Choya-Andalgalá	51 *Prosopis* spp; 18 *Senna aphylla*
34	Alijilán-Santa Rosa	27 *Tournefortia lilloi*; 11 *Myrcianthes mato*; 11 *Larrea* sp.
35	Nueva Coneta-Capayán	34 *Prosopis* spp.; 26 *Larrea divaricata*; 9 *Cercidium praecox*; 8 *Mimosa* spp.
36	Nueva Coneta-Capayán	40 *Prosopis* spp.; 10 *Larrea divaricata*; 11 *Capparis atamisquea*; 10 *Vicia* spp.
37	La Antena- Valle Viejo	28 *Prosopis* spp.; 15 *Larrea divaricata*
38	Aeropuerto- Capayán	41 *Prosopis* spp.; 15 *Larrea divaricata*
39	Coneta- Capayán	31 *Prosopis* spp.; 27 *Mimosa ephedroides*; 9 *Larrea divaricata*; 9 *Schinus* sp.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
Prosop	82	60	51	48	41	40	38	35	34	31	28	25	21	20	19	17	16	16	16	15	13	11	11	9	8	8	7	6	4	4	3	2	1	1
Larrea	58	44	28	26	26	23	18	17	15	15	15	15	14	12	12	12	11	11	11	10	10	9	9	9	8	7	6	5	2	1	1			
Mimo	56	27	24	21	19	19	17	14	10	8	8	6	6	6	6	5	5	5	3	3	2	2	2	2	1	1	1	1						
Schin	13	10	10	9	8	7	7	7	7	6	5	5	5	4	4	4	4	4	3	2	2	2	2	2	1	1	1							
Cerc	80	57	29	17	13	12	10	10	9	9	8	6	5	5	4	4	4	4	3	3	3	2	2	1	1	1								
Bacc	13	12	8	5	3	3	2	2	2	2	1	1	1	1	1	1	1	1	1	1														
Acce	23	8	4	3	3	2	2	2	2	2	2	1	1	1	1	1	1	1																
Aloy	19	8	7	7	7	7	7	5	3	3	3	3	3	3	2	2	1																	
Cap	11	5	5	5	4	4	4	4	4	3	2	2	1	1	1	1																		
Euc	8	4	4	4	4	3	2	2	2	1	1	1	1																					
Park	48	21	13	9	5	5	4	2	2	2	1	1																						
Celt	6	5	4	3	2	2	2	2	1	1	1	1																						
Lyci	15	5	5	4	2	2	2	2	2	1	1	1																						
Geo	22	15	13	9	8	7	6	5	5	4	2																							
Gom	5	5	4	2	2	1	1	1	1	1	1																							
Zizi	36	18	15	13	10	6	5	3	2	1	1																							
Schino	97	11	9	5	4	4	3	3	1																									
Bras	4	3	3	2	2	1	1	1																										
Aste	7	5	2	2	1	1	1	1																										
Meli	7	3	2	2	1	1	1	1																										
Senn	40	18	10	8	7	5	1	1																										
Citrus	57	28	16	2	2	1	1																											
Con	9	8	7	6	3	1	1																											
Tourn	90	63	53	27	20	10																												
Ammi	5	3	3	2	2	1																												
Chen	3	2	2	1	1	1																												
Hypt	3	1	1	1	1	1																												

Figure 1. Abundance and frequency of pollen types in honey samples. Pros = *Prosopis* spp.; Larrea = *Larrea* sp.; Mimo = *Mimosa* spp.; Schin = *Schinus* sp.; Cercid = *Cercidium* praecox; Bacc = *Baccharis* sp.; Acca = *Acacia* spp.; Aloy = *Aloysia gratissima*; Cap = *Capparis atamisquea*; Euc = *Eucalyptus* sp.; Park = *Parkinsonia aculeata*; Celt = *Celtis ehrenbergiana*; Lyci = *Lycium* sp.; Zizi = *Ziziphus mistol*; Geo = *Geoffroea decorticans*; Gom = *Gomphrena pulchella*; Schino = *Schinopsis* spp.; Bras = *Brassica* sp.; Aste = *Asteraceae*; Meli = *Melilotus albus*; Senn = *Senna aphylla*; Con = *Condalia microphylla*; Citrus = *Citrus* spp.; Tour = *Tournefortia lilloi*; Ammi = *Ammi visnaga*; Chen = *Chenopodium* sp.; Hypt = *Hyptis mutabilis*. Each value represents percent of relative abundance in one sample. Top row: number of samples in which pollen type was detected. Numbers in cells: pollen type abundance (as percentage) in a sample.

Results and discussion

Geographical and botanical origins of samples

Melissopalynological analysis of the 39 honey samples revealed an almost exclusive preponderance of pollen types from native flora (Table 1 and Figure 1). *Prosopis*, *Larrea*, *Mimosa*, *Cercidium*, and *Schinus* were the most frequently present genera; *Prosopis* was absent in only five of the 39 samples (Figure 1). Abundance of the pollen types was highly variable, ranging from trace to 82%; 14 samples were categorised as monofloral honeys because they contained > 45% of a single pollen type (Figure 1). *Schinus* was unique in that its abundance was never > 13% in any of the 27 samples that contained it. The only exotic (non-native) pollen types observed were *Eucalyptus*, *Melilotus albus*, *Brassica* sp. and *Citrus*.

Botanical origins of the 39 samples were consistent with those reported for honeys from this geographical area; we observed previously that *Prosopis* spp., *Larrea divaricata*, *Schinopsis* spp., *Condalia microphylla*, *Sarcomphalus mistol, Cercidium praecox*, and *Geoffroea decorticans* are the most frequent and predominant pollen types in honeys from the arid Chaco region of central Argentina (Costa *et al*., 2013; 2016).

Physicochemical characteristics of samples

Values of polyphenol content, protein content, flavonoid content, RSC, colour, ash content, HMF content, acidity, and absorbance at 600 nm are summarised in Table 2. The values for all samples values were within the range considered acceptable for international honey marketing (Codex Alimentarius, 2001).

Values of HMF content and colour (Beretta *et al*., 2005) showed the greatest dispersal (SE > 10% of mean) (Table 2, column "Mean ± SE"). The other parameters were more homogenous; the most homogeneous were OD 600 nm and polyphenol content (SE ~2% of mean).

We performed multivariate statistical analysis on these data to look for possible correlations among the tested parameters (Table 2). Polyphenol content was correlated with flavonoid content, ash content, acidity, RSC, and colour. Flavonoid content was correlated with ash content, acidity, RSC, and colour. RSC and colour were both correlated with ash content and acidity. These findings indicate that polyphenol content, flavonoid content, ash content, acidity, RSC, and colour comprise an array of interrelated physicochemical parameters.

A multivariate analysis of principal components

Table 2. Physicochemical parameters and their correlation matrix (p values).

Content	Mean ± SE	Polyphenol	Protein	Flavonoid	SRC	Colour*	Ash	HMF	Acidity
Polyphenol (mg GAE/100 g)	106 ± 2								
Protein (g/kg)	0.43 ± 0.02	0.876							
Flavonoid (m CE/kg)	4.6 ± 0.3	0.001	0.069						
RSC (mol GAE/kg × 10-6)	32 ± 1	0.006	0.384	0.010					
Colour (Beretta) (O.D., arbitrary units)	22 ± 2	0.001	0.260	0.001	0.574				
Ash (g/kg)	2.6 ± 0.2	0.001	0.638	0.001	0.020	0.001			
HMF (mg/kg)	20 ± 2	0.562	0.524	0.347	0.930	0.491	0.349		
Acidity (mAE/kg)	26 ± 2	0.001	0.098	0.001	0.014	0.001	0.001	0.984	
O.D. 600 nm (arbitrary units)	0.39 ± 0.01	0.268	0.001	0.437	0.416	0.042	0.327	0.463	0.622

*Colour measured as in Beretta *et al*. (2005).

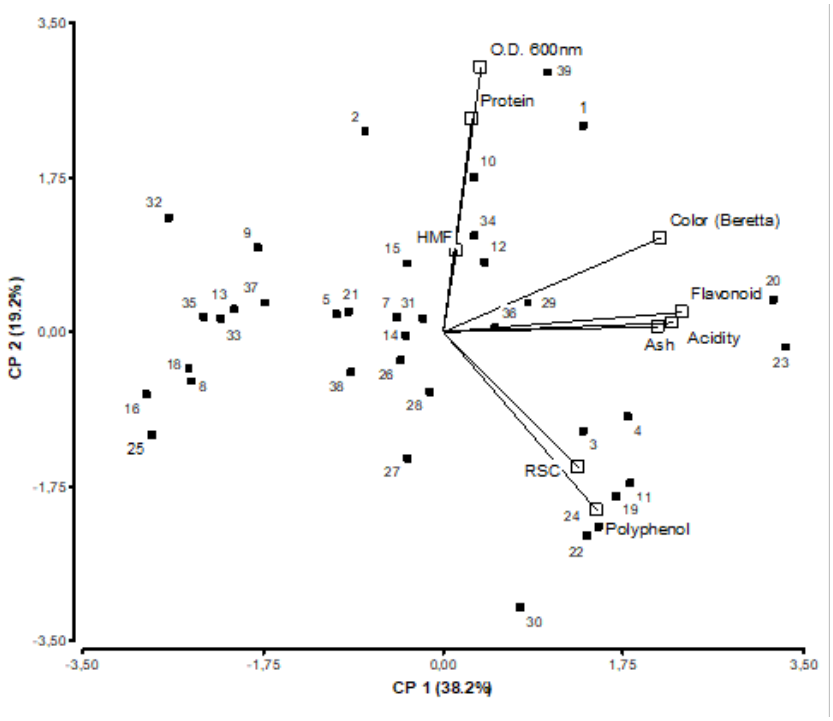

Figure 2. Principal component analysis of the eight variables as in Table 2 plus optical density at 600 nm (O.D. 600 nm) for the 39 samples of honey. Samples are numbered as in Table 1 (black squares) and variables with lines and open squares.

was performed and the biplot (Figure 2) depicts the observations and variables of multivariate data. The results of the principal component analysis corroborated that flavonoid content, ash content, acidity, and colour were intimately related while polyphenol and RSC were both correlated and close to the first array of variables. Lastly, colour measured at O.D. 600 nm, protein content and HMF were strongly correlated between them. Most of the samples that contained *Tournefortia lilloi* were near to these set of variables.

This is the first report of a close interrelationship among all the above properties of honey, although partial relationships among some of them have been described previously. Correlations have been reported between total phenolic content and colour intensity of Malaysian honeys from *Apis* spp. and *Trigona* spp. bees (Kek *et al*., 2014), between phenolic content and RSC of Italian honeys (Giorgi *et al*., 2011), and among total phenolic content, reducing antioxidant power, and colour parameter *L** of Slovenian honeys (Bertoncelj *et al*., 2007). A study of Burkina Fasan honeys found that RSC was positively correlated with polyphenol content and proline content, but negatively with total flavonoid content (Meda *et al*., 2005). Recently, a positive correlation coefficient between total phenolic, DPPH, FRAP and β-Carotene methods was found showing that phenols are responsible for the antioxidant capacity of honey (Gül and Pehlivan, 2018). All these studies found correlations between different methods of measuring a physicochemical characteristic or

two or three physicochemical characteristics of honey samples; to our knowledge, there is no report about a close relationship between the polyphenol content, flavonoid content, ash content, acidity, RSC, and colour forming a matrix of seven interrelated variables.

Most studies had focused their analysis on honeys produced from cultivars; the 39 honeys assessed in the present work were from wild flora of a preserved natural environments with little anthropogenic influence. Thus, the correlation data presented here are from samples that had a typical pollen array of arid Chaco (Costa *et al*., 2013) with 27 types having more than 8% of the total pollen content (Table 1).

When the multivariate analysis of principal components was performed including the content of the five most abundant pollen types, and the coefficients of the correlation matrix analysed, some weak association between the content of some pollen types and physicochemical characteristics were found; the most important were for *Cercidium praecox* and RSC polyphenols, acidity, and to a lesser extent to flavonoid content (R = 0.41, 0.39, 0.28 and 0.17, respectively). *Mimosa* also showed correlation with flavonoid content, RSC and polyphenol content (R = 0.32, 0.29 and 0.17, respectively).

Correlation between botanical origin and physicochemical characteristics

To examine in detail a possible correlation between botanical origins of honeys with the array of closely interrelated physicochemical parameters described in the preceding section, we performed correlation analyses between polyphenol content and flavonoid content, and the abundance of each pollen type in individual samples (Table 3). Positive correlations were found between *Cercidium praecox* pollen abundance and polyphenol content (slope = 4 ± 1; $p < 0.01$) and between *Schinus* sp. abundance and flavonoid content (slope = 0.7 ± 0.3; $p < 0.01$). *Prosopis* spp. abundance showed negative correlations with polyphenol content and flavonoid content (slopes = -6.2 ± 1.7 and -3.7 ± 1.5; $p < 0.0001$ and 0.02, respectively). Thus, of the five most frequently observed pollen types, *Prosopis*, *Cercidium* and *Schinus* abundances were correlated with some of the antioxidant parameters tested, whereas *Mimosa* and *Larrea* abundances showed no correlation with RSC, polyphenol content, or flavonoid content. Pollen types with lower incidences also showed no such correlations (data not shown).

Table 3. Relationship of pollen type abundance with polyphenol content and with flavonoid content.

Pollen type	Polyphenol content	Flavonoid content	p <*	p <**
Prosopis spp.	-6.2 ± 1.7	-3.7 ± 1.5	0.0001	0.02
Larrea spp.	-2.1 ± 1.2		0.10	
Cercidium praecox	4 ± 1		0.01	
Schinus sp.		0.7 ± 0.3		0.01

Identification of major polyphenols from monofloral honey samples

The honey samples with the highest content of *Cercidium praecox* and *Prosopis* spp. pollens (codes 22 and 25; Table 1) were selected for HPLC-DAD-MS/MS identification of polyphenols; reliable identification was considered feasible because of the high abundance (80% and 82%, respectively) of the main pollen type. Total polyphenol content (mg GAE/100 g) was 173 ± 1 and 55.8 ± 0.4, and total flavonoid content (m CE/kg) was 5.1 ± 0.3 and 1.4 ± 0.1, respectively, in samples 22 and 25.

The compounds were extracted by non-ionic polymeric resin (Amberlite XAD-2) and separated on a reversed-phase column by HPLC gradient elution coupled with ESI-MS. This methodology has been specifically recommended for honey analysis (Tomás-Barberán *et al*., 2001), and was effective for extraction of polyphenols by HPLC coupled with UV detection, MS, or both (Istasse *et al*., 2016). HPLC-DAD-MS/MS analysis revealed differing polyphenol composition of the two honey extracts (Table 4).

Several compounds were detected in *Cercidium* honey, of which the two most prominent (retention time [RT] = 11.8 and 12.2 min) had m/z product anions at 488 and 326 that generated fragments of 236 and 164 in the MS2 spectrum, respectively. The short RT indicated high polarity, and the UV spectrum with broad peak between 290 and 320 nm suggested that the two compounds are related to the caffeoyl family (Martucci *et al*., 2014).The molecular ion $[M–H]^-$ m/z 326 was identified as p-coumaroyl tyrosine (also known as dideoxyclovamide) (Clifford and Knight, 2004), and the $[M–H]^-$ m/z 488 produced a principal fragment of m/z 236 plus the same dideoxyclovamide minor fragments, indicating neutral losses of 162 arbitrary mass units (amu). The $[M–H]^-$ m/z 488 could be dideoxyclovamide with attached hexose, since neutral losses of 162 amu are indicative of aromatic attachment. *Cercidium* honey had high proportions of dideoxyclovamide and its derivatives, which have not been reported in other honeys. The other unidentified compound had $[M–H]^-$ m/z 231 and produced a 174 amu fragment. Cyclobrassinone ($C_{11}H_8N_2O_2S$) was reported to have

this m/z and fragmentation pattern when analysed by LC-DAD-ESI-MS in negative mode (Pedras *et al*., 2006). This compound co-eluted with caffeoylquinic acid molecular anion (m/z 353) having the same RT as authentic standard and the same characteristic fragments. Components that eluted at RT 24.9, 26.3, and 26.7 with $[M–H]^-$ m/z 199 and 201 had many of the characteristics of royal jelly aldehyde compounds with the same m/z, as reported from authentic royal jelly samples (Abdelnur *et al*., 2011). Another molecule possibly originating from the bee is an unidentified $[M–H]^-$ m/z 231 compound that could be 10,11-dihydroxy dodecanoic acid (Schmidt *et al*., 2015). Naringenin derivatives were also present, having a major peak at RT 26.3 with a fragmentation pattern consistent with naringenin chalcone.

Prosopis honey contained several polyphenols often detected in other honeys (7-hydroxyflavanone, apigenin, naringenin chalcone, pinocembrin), which were identified by comparison with authentic standards (Table 4).

The two characterised honeys were monofloral, from different species of the same family (Fabaceae), and had many compounds in common (naringenin chalcone, chrysin, pinocembrin, isorhamnetin-3-methoxy). The presence of isoflavones in the monofloral *Prosopis* honey is consistent with the documented expression of isoflavone synthase genes in Fabaceae (Picmanov *et al*., 2012). Several of the *Prosopis* honey compounds (isorhamnetin, caffeic acid derivatives, apigenin) were found in *Prosopis farcta* flowers (Harzallah-Skhiri and Ben Jannet, 2005).

The main compound we identified in *Cercidium* honey, dideoxyclovamide ($C_{18}H_{16}NO_5$), was previously reported in cocoa (*Theobroma cacao*) beans (Sanbongi *et al*., 1998).This compound was described originally from African blackwood (*Dalbergia melanoxylon*) (Van Heerden *et al*., 1980) and later found in red clover (*Trifolium pratense*) (Tebayashi *et al*., 2000), and like *Cercidium*, both are members of the Fabaceae family.

Table 4. Major compounds identified by HPLC-DAD-MS/MS analysis in monofloral *Cercidium* and *Prosopis* honey extracts.

RT (min)	$[M-H]^-$ (m/z)	MS2/MS fragment ions	$[M-H]^-$ (m/z)	MS2/MS fragment ions	peak area %	tentative assignment
monofloral *Cercidium* praecox honey						
11.8	488	308/236/164			10.9	dideoxyclovamide glucoside derivative?
12.2	326	206/164			21.5	dideoxyclovamide
15.0	291	273/177			4.5	epicatechin?
18.1	231	174	353	264/231/191	6.0	n.i./caffeoylquinic acid
18.3	231	174	353	165	8.0	n.i./caffeoylquinic acid
24.9	199	181/151			4.9	rjaldehyde
26.3	271	253/197	201	183	7.6	naringenin chalcone-rj aldehyde
26.7	201	183	223	195/183	4.9	rj aldehyde-flavanone
30.5	253	209	329	314/201/171	3.8	chrysin/isorhamnetin-3-methoxy
32.5	255	213/171	283		4.5	pinocembrin - izalpinin
33.9	285	285			16.8	n.i.
34.3	253	209	283		6.6	chrysin - izalpinin
monofloral *Prosopis* spp. honey						
20.4	361	223,0			4.7	n.i.
24.0	255	227/211/183			5.7	16:0-carboxylate anion
24.5	221	195/175			4.7	flavone
25.8	271	253/225/197/177			15.3	Naringenin chalcone
26.8	269	197	343	271/179	10.2	apigenin /trimethoxyflavone
29.7	239	197			26.0	7-hydroxyflavanone
30.2	327	279/239/179			6.5	pinobanksin-3-O-propionate
32.3	253		329	314/299	5.8	chrysin/isorhamnetin-3-methoxy
32.9	255				9.1	pinocembrin
33.7	253	tz 209			5.5	chrysin
34.5	269				6.3	galangin

RT: retention time, n.i.: not identified, rj: royal jelly.

Conclusion

An important finding from the analysis of physicochemical characteristics and antioxidant properties of honey samples from a defined geographic area was the close association among polyphenol content, flavonoid content, ash content, acidity, RSC, and colour, which comprised an array of interrelated parameters. This finding suggests that certain properties of honey that are often considered unfavourable should be reconsidered, since dark, acidic honey samples with high ash content were also the richest in polyphenols. Thus, honeys derived from native flowers in arid regions such as the Chaco should be re-evaluated, because dark colour and high ash content reflect strong antioxidant properties. This conclusion is relevant to two important topics: (i) the need to preserve native forests or open habitats as renewable natural resources, and (ii) the fact that certain floral species contribute more strongly than others to antioxidant properties of honeys (e.g., *Prosopis*, *Cercidium*, *Schinus* honeys), thus suggesting that reforestation with these species should be promoted. In this context, the high incidence of *Prosopis* pollen type in monofloral honeys indicates the desirability of identifying compounds that may serve as important botanical markers or beneficial biomolecules. It is noteworthy that the major compounds identified in *Prosopis* monofloral honey (7-hydroxy flavanone, naringenin chalcone, apigenin, trimethoxyflavone) have closely related structures. These compounds are known biocides and flavonoids having low antioxidant capacity. In contrast, *Cercidium* monofloral honey has high antioxidant capacity, most likely related to high content of caffeoylquinic derivatives; e.g., dideoxyclovamide was identified as a major antioxidant in cocoa extracts.

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