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Palynological and physicochemical characteristics of three unifloral honey types from central Argentina

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Abstract

The characteristics of 59 unifloral honeys of *Condalia microphylla* Cav. ("piquillín"), *Centaurea solstitialis* L. ("yellow starthistle") and *Prosopis* spp., from La Pampa, Argentina, were studied. Pollen features (abundance and frequency of pollen types) and some physicochemical parameters (colour, electrical conductivity, free acidity, glucose content, glucose:water ratio, moisture and pH) were determined. Two different but related sets of calculations were done: the first involved single-factor variance analysis, while the second set involved two multivariate methods, principal component analysis and cluster analysis. Variance and multivariate analysis allowed differentiation of the three honey types according to their physicochemical properties. The variables that best explained this differentiation were pH, electrical conductivity, colour, glucose content and the glucose:water ratio. Pollen analysis showed that the pollen frequency traditionally used (> 45%) for a botanical origin assignment in honey was not valid for the unifloral honeys studied. Therefore, pollen analysis should be combined with the above physicochemical analysis order to obtain a successful classification of these unifloral honeys.

Additional key words: botanical origin, *Centaurea solstitialis*, *Condalia microphylla*, melissopalynology, multivariate analysis, pollen analysis, *Prosopis* spp.

Resumen

Características palinológicas y fisicoquímicas de tres tipos de mieles uniflorales del centro de Argentina

Se estudiaron las características de 59 mieles monoflorales de *Condalia microphylla* Cav. ("piquillín"), *Centaurea solstitialis* L. ("abrepuño amarillo") y *Prosopis* spp., provenientes de La Pampa, Argentina. Se determinaron caracteres polínicos (abundancia y frecuencia de tipos polínicos) y algunos parámetros fisicoquímicos (color, conductividad eléctrica, acidez libre, contenido de glucosa, relación glucosa-agua, humedad y pH). Se utilizaron dos tipos de análisis estadísticos: análisis de varianza de un factor y análisis multiJT*-0.000aaaaléct.omponentes principales yléct.onglomer0aaa. Los análisis de varianza y multivariados permitieron distinguir las tres clases de mieles de acuerdo a las propiedades fisicoquímicaa. Las variables que mejor explicaron esta diferenciación fueron pH, conductividad eléctrica, color, glucosa y relación glucosa-agua. El análisis polínico demostró que la frecuencia de polen tradicionalmente utilizada para definir una miel monofloral (> 45%) no es válido para las mieles estudiadas. Por ello, el análisis polínico relacionado con las propiedades físico-químicas mencionadas permitiría una adecuada clasificación en estas mieles monoflorales.

Palabras clave adicionales: análisis de polen, análisis multivariados, *Centaurea solstitialis*, *Condalia microphylla*, melisopalinología, origen botánico, *Prosopis* spp.

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Abbreviations used: APC (absolute pollen contents), CA (cluster analysis), PC (principal component), D (dominant), EC (electrical conductivity), GOD-POD (glucose oxidase – peroxidase), G:W (glucose:water ratio), LSD (least significant difference), M (minor), PCA (principal component analysis), S (secondary), T (trace).

Introduction

Honey is a natural substance produced by bees (*Apis mellifera* L.) from flower nectar, and from honeydew. The composition and properties of honey depend on the botanical origin of the nectar or secretion used. Consequently, its composition is influenced by many factors and is subject to variation. Several studies have attempted to establish suitable parameters for honey from the same botanical source. Honey is characterized by its palynological, chemical and physical properties.

Pollen analysis appears to be the most frequently used method of honey identification (Louveaux and Vergeron, 1964; Louveaux *et al*., 1978; Anklam, 1998; von der Ohe *et al*., 2004), although in some honeys it is difficult to establish their exact origin (von der Ohe, 1994; Hermosin *et al*., 2003).

Flower honey is considered to be unifloral when the pollen frequency of the main plant source is greater than 45% (Louveaux *et al*., 1978). This value is not valid for honeys with over, or under, represented pollen types (Maurizio, 1972; Moar, 1985; Accorti *et al*., 1986; Persano Oddo *et al*., 1988; Serra Bonvehí, 1989; Bryant and Jones, 2001). Other authors have suggested and used physicochemical analysis, complemented by pollen analysis, as an additional criterion for characterization of unifloral honeys (Bogdanov *et al*., 1997, 1999; Anklam, 1998; Ruoff *et al*., 2007).

Argentina is the third honey producer in the world after China and the United States and La Pampa province is the fifth honey producer in Argentina (SAGPyA, 2008). The central region of Argentina has the greatest production of honey with abundant unifloral honeys corresponding to adventitious or cultivated exotic plants such as *Centaurea* spp., Brassicaceae, *Melilotus* spp., *Eucalyptus* spp. and some native species such as *Prosopis* spp., *Condalia microphylla* Cav. and *Scutia buxifolia* Reissek (Tellería, 1988; 1992, 1996; Naab, 1993; Valle *et al*., 1995; 2004; 2007; Andrada *et al*., 1998a; 1998b; Naab *et al*., 2001; Andrada and Tellería, 2002; Basilio *et al*., 2002, Fagúndez and Caccavari, 2003; 2006). The three honey types presented in this work were produced in the Pampean Phytogeographical Region and the Espinal Phytogeographical Province (Valle *et al*., 1995; Naab *et al*., 2001; Andrada and Tellería, 2002).

Although palynological studies have been carried out on Argentinean honeys there are few reports on the relationship between their botanical origin and their physicochemical properties (Naab and Torroba, 1993; Andrada, 2001; Baroni *et al*., 2002, 2004; Cometo *et al*., 2003; Tamame and Naab, 2003; Malacalza *et al*., 2005).

As honey is a complex natural food, clear characterization of honey samples requires the use of several parameters. To establish the combinations of these parameters closely related to the origin of honey quality control methods and multivariate statistical analysis need to be used. These methods will help to evaluate honey samples in their totality and give more precise classifications (Anklam, 1998; Ruoff *et al.*, 2007).

The objective of this work was to characterize unifloral honeys from three different botanical sources produced in La Pampa Province, Argentina. This was done using data from melissopalynological and physicochemical analysis to attempt the classification of honey samples according to their botanical origin.

Material and methods

Study area

Unifloral honeys from *Prosopis* spp. and *Condalia microphylla* are from the Caldén District of Espinal Phytogeographical Province. Honeys from *Centaurea solstitialis* L. are from Pampean Province (Cabrera, 1994).

The Caldén District - usually called Caldenal - spreads over the central semiarid region of Argentina. The vegetation basically consists of open woodlands with a poor shrubby stratum and an herbaceous stratum rich in Poaceae.

"Caldén" (*Prosopis caldenia* Burk.) is the dominant tree species. *Prosopis flexuosa* D.C. and *Geoffroea decorticans* (Gillies *ex* Hook. & Arn.) Burkart are subordinate tree species. Shrubs in the region are *Prosopidastrum angusticarpum* R.A. Palacios & Hoc, *Condalia microphylla*, *Discaria americana* Gillies & Hook, *Larrea divaricata* Cav., *Lycium chilense* Miers and *Chuquiraga erinacea* D. Don. The most important species in the herbaceous stratum are different species of the genus *Stipa*, followed by *Piptochaetium napostaense* (Speg.) Hack, *Glandularia hookeriana* Covas & Schnack, *G. pulchella* (Sweet) Tronc., *Erodium cicutarium* (L.) L' Hér. ex Aiton, *Sphaeralcea crispa* Baker and *Nierembergia aristata* Sweet, among others.

In central Argentina *Centaurea solstitialis* ("yellow starthistle") is a winter annual or biennial adventitious species. In natural areas and on rangelands it forms dense impenetrable stands that displace desirable vegetation. Thus, yellow starthistle is a principal nectar source during the summer in disturbed areas of steppe and caldenal.

Sample collection

Fifty nine (n = 59) typical honey samples, from *Apis mellifera*, were collected by beekeepers in 2003, 2004 and 2005. They were obtained by centrifugation and stored at room temperature until analyzed.

The honeys were harvested from different areas of La Pampa Province, Argentina (Fig. 1).

Samples of three different botanical origins were selected after a preliminary pollen analysis. The honey samples were placed into different floral groups: *Prosopis* spp. (n = 18), *Condalia microphylla* "piquillín" (n = 8) and *Centaurea solstitialis* "yellow starthistle" (n = 33) according to the dominant pollen present (Louveaux *et al*., 1978). The *Prosopis* genus has a high percentage of interspecific hybridization, generally in sympatric areas (Palacios and Bravo, 1981; Mollard *et al*., 2000). Further, flowering in these species overlaps (Genise *et al*., 1990).

Sensory analysis (crystallization type) was considered as a complementary criterion. Some *Prosopis* spp. and *Condalia microphylla* honeys were rejected, in the first case because of creamy crystallization and the second because of non-crystallization. Both cases showed atypical characteristics of these honey types.

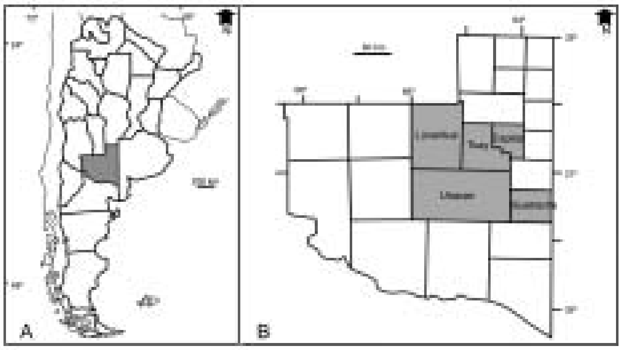

Figure 1. A. Geographical location of La Pampa Province, Argentina; B. La Pampa Province subdivided into departments. The study area is gray.

Pollen analysis

The pollen spectrum of the honey samples was determined using the acetolytic method (Erdtman, 1960) and the method of Louveaux *et al*. (1978). Honeys from central Argentina show very few honeydew elements; therefore they were not calculated (Tellería, 1996; Andrada, 2001; Fagúndez and Caccavari, 2003). The different pollen types were identified by comparing them with a reference collection, made from plants of the area. The preparations were deposited in the Palynological Collection of the Facultad de Agronomía, Universidad Nacional de La Pampa. The identified pollen was classified, according to frequency, into four classes: dominant (> 45%) = D, secondary (16-45%) = S, important minor (3-15%) = M, trace (<3 %) = T (Louveaux *et al*., 1978). To determine frequency class, 1000 pollen grains were counted.

The absolute pollen content (APC) of the honey samples (i.e., the number of pollen grains in 10 g of honey) was calculated using tablets of *Lycopodium* spores (Stockmarr, 1971). Following Louveaux *et al*. (1978) five groups were considered: Group I (honey low in pollen < 20,000/10 g), Group II (normal honey 20,000-100,000/10 g), Group III (honey rich in pollen 100,000-500,000/10 g), Group IV (honey extremely rich in pollen 500,000-1,000,000/10 g), Group V (pressed honeys >1,000,000/10 g). Quantitative analysis of honey samples with over - or under-represented pollen was conducted according to Moar (1985) who suggested standard honey as *Trifolium repens* L. honey, which has 45% of dominant pollen and is in Group II. Moar (1985) also explained how to estimate an adjusted absolute pollen content and an adjusted minimal pollen percentage for a honey sample to be classified as unifloral.

Physicochemical analysis

Physical and chemical analyses followed international recommendations (Bogdanov *et al*., 1997; AOAC, 1999).

The honey samples were analyzed using the following methods:

– Colour was determined with a Coleman spectrophotometer by reading the absorbance in aqueous solutions at 635 nm (10 g honey in 20 mL water). Table 1 shows honey colours and their absorbance and mm Pfund values, obtained using the following algorithm (Bianchi, 1990):

$$\text{mm Pfund} = -38.7 + 371.39 \times \text{Absorbance}.$$

Table 1. Honey colour expressed in absorbance and mm Pfund values

Honey colour	Absorbance	mm Pfund
Water white	0.104 - 0.125	0 - 8
Extra white	0.125 - 0.148	8 - 16.5
White	0.148 - 0.195	16.5 - 34
Light extra amber	0.195 - 0.238	34 - 50
Light amber	0.238 - 0.333	50 - 85
Amber	0.333 - 0.411	85 - 114
Dark amber	> 0.411	> 114

– Electrical conductivity (EC, mS cm^{-1}) was determined with a Luftman C400 conductivity meter in 20 (w/v) aqueous honey solution (dry matter basis).
– Free acidity: acidic components were neutralized with a standard solution of sodium hydroxide in aqueous honey solution (10 g in 75 mL of double distilled water).
– Glucose content was determined by the glucose oxidase – peroxidase method (GOD-POD). Absorbance was measured at 595 nm using in a Metrolab 1700 spectrophotometer.
– Glucose:water ratio (G:W) was obtained from water and glucose content percentage (White *et al*., 1962).
– Moisture was determined with an Abbe-type refractometer. The refractive index was correlated using Chataway Charts.
– Active acidity (pH) was determined, in aqueous solution, with a Horiba B-213 pH meter.

Statistical analysis

Analysis of variance and multivariate analysis were performed using Statgraphics Plus 3.1 software. Differences among means were determined for significance at P = 0.05 using the least significant difference (LSD) test. Principal components analysis (PCA) and cluster analysis (CA) were used to reduce the dimensions of the 7 x 59 data matrix, to determine relationships between physicochemical properties (variables) and honey samples (objects) through optimal graphical 2-D and to define groups between unifloral honeys. To determine similarities among samples and variables, the Euclidean distance and group average method were used.

Results

Pollen analysis

Table 2 shows the frequency of occurrence of pollen types in the three unifloral honeys. A total of 71 pollen types, from 35 plant families, were identified in the honey samples analysed.

In *Prosopis* spp. honeys 43 pollen types were identified with 5 to 20 types per sample. Brassicaceae and *Schinus* spp. were present as secondary pollen. In the minor importance class were *Centaurea solstitialis*, *Vicia* spp., *Eucalyptus* spp., *Condalia microphylla* and *Lycium* spp.

In *Condalia microphylla* honeys 31 pollen types were identified with 5 to 18 types per sample. These honeys were characterized by *Vicia* spp. and *Eucalyptus* spp. as secondary pollen and Brassicaceae, *Schinus* spp., *Prosopis* spp. and *Larrea* spp. pollen being of minor importance.

In *Centaurea solstitialis* honeys 53 pollen types were identified with 7 to 21 types per sample. These unifloral honeys were characterized by *Schinus* spp., *Helianthus* spp. Brassicaceae, *Eucalyptus* spp. and *Condalia microphylla* as secondary pollen and *Ammi* spp., *Carduus* spp., *Melilotus* spp., *Prosopis* spp., Chenopodiaceae-Amaranthaceae and Asteraceae (type echinulate) as pollen of minor importance. Pollen types of nectarless or anemophilous taxa, namely the Poaceae, Chenopodiaceae-Amaranthaceae, *Cupressus* spp., *Juglans regia* L., *Fraxinus* spp., *Typha* spp., *Celtis* spp. and *Ulmus* sp. were found as traces in the three unifloral honeys.

Table 3 shows the adjusted (APC_{adj}, DP_{adj}) and non-adjusted (APC, DP) values for absolute pollen content and the percent dominant pollen for each honey type.

The absolute pollen content indicated that pollen richness is a characteristic of *Condalia microphylla* honeys (Group III), whereas *Prosopis* spp. honeys belong to Group II and *Centaurea solstitialis* honeys to Group I respectively. The percent dominant pollen adjusted (DP_{adj}) according to Moar (1985) indicated that the *C. solstitialis* honeys require 31.5% of their pollen to be considered unifloral. The *Condalia microphylla* and *Prosopis* spp. honeys would require the 64.5% and 75% respectively.

Physicochemical analysis

Table 4 shows the results (mean, standard deviation and range) obtained from physicochemical analysis of the

Table 2. Pollen types and frequency in *Centaurea solstitialis*, *Prosopis* spp. and *Condalia microphylla* honeys. D, dominant pollen (> 45%); S, secondary pollen (16-45%); M, minor important pollen (3-15%) and T, trace pollen (< 3%)

		Centaurea solstitialis				***Prosopis* spp.**				***Condalia microphylla***			
Family	**Pollen type**	**D**	**S**	**M**	**T**	**D**	**S**	**M**	**T**	**D**	**S**	**M**	**T**
Anacardiaceae	*Schinus*		1	5	19		2	3	9			1	6
Apiaceae	*Ammi*			5	12				4				
	Foeniculum								4				
Asteraceae	*Baccharis*				1				3				
	Carduus			1	29				12				5
	Centaurea solstitialis	33						5	11				7
	Chuquiraga erinacea												1
	Cirsium				7				2				
	Cyclolepis								1				
	Gaillardia								1				1
	Helianthus		2	6	14				14				2
	Hyalis				5								1
	Matricaria				1				2				
	Senecio				7				1				4
	Type echinate				1								
	Type echinulate			1	2								
	Type Mutisieae				4								
	Type Taraxacum				7								
	Xanthium-Ambrosia				5								
Brassicaceae			8	23	1		1	8				3	7
Cactaceae	*Opuntia*								1				
Caryophyllaceae					1								
Chenopodiaceae-Amaranthaceae			1	18				5			2		
Cistaceae					1								
Convolvulaceae	*Convolvulus*				1								
Cucurbitaceae	*Cucurbita*				5								
Cupressaceae	*Cupressus*				1								1
Cyperaceae									1				1
Ephedraceae	*Ephedra*								1				
Euphorbiaceae	*Euphorbia*												1
Fabaceae	*Acacia*				1				1				
	Geoffroea decorticans												1
	Glicine max				4								
	Lathyrus				1				4				
	Lotus				1								
	Medicago				3				9				3
	Melilotus			16	17				11				6
	Prosopidastrum angusticarpum			9				6					
	Prosopis			6	2	18						1	8
	Prosopis strombulifera				2								3
	Rhynchosia												1
	Trifolium				3								
	Vicia				19			2	13		1		2
Juglandaceae	*Juglans regia*								1				
Lamiaceae	*Marrubium vulgare*				1				2				
Malvaceae	*Sphaeralcea*				2				3				1
Myrtaceae	*Eucalyptus*		3	15	14			1	1		2	1	4
Oleaceae	*Fraxinus*								1				
Oxalidaceae	*Oxalis*				2								
Pinaceae	*Pinus*								1				
Poaceae	*Zea mays*				2				2				
Poaceae					12				6				3
Polygalaceae	*Monnina*				3								3
Portulacaceae	*Portulaca*				1								1
Ranunculaceae	*Clematis montevidense*				1				1				

Table 2. (Cont.)

Family	Pollen type	*Centaurea solstitialis* D	S	M	T	*Prosopis* spp. D	S	M	T	*Condalia microphylla* D	S	M	T
Rhamnaceae	*Condalia microphylla*		2	1	17			4	9	8			
Rosaceae	Type *Acaena*								3				
Rubiaceae	*Gallium aparine*				1								
Solanaceae	*Lycium*				3			1	11				3
	Solanum				1				1				
Tamaricaceae	*Tamarix gallica*				4				5				1
Typhaceae	*Typha*												1
Ulmaceae	*Celtis*								1				
	Ulmus				2								
Verbenaceae	*Acantolippia seriphioides*				2								2
	Aloysia								1				
	Phyla				2								
	Verbena-Glandularia				5								
Zygophyllaceae	*Larrea*				8				2			3	7
	Tribulus terrestris				4				1				
Morphological types								2					

honey samples. All parameters showed high discrimination power in these honeys. However, moisture content only differentiated *Prosopis* spp. honeys from the others.

In terms of colour, pH, free acidity and EC *Condalia microphylla* honeys had the highest values while *Centaurea solstitialis* and *Prosopis* spp. honeys had the highest glucose content and G:W ratio.

Multivariate analysis (CA and PCA)

Cluster analysis showed that there were two clusters at a linkage distance level of 6 corresponding to the different botanical origins (Fig. 2). From right to left, the first cluster is composed of *Condalia microphylla* honey samples. The second cluster has two sub-clusters, one composed of *Centaurea solstitialis* samples and the other of *Prosopis* spp. samples.

Table 3. Quantitative pollen analysis (mean ± SD) of honey samples. Non-adjusted absolute pollen content (APC), adjusted absolute pollen content (APC_{adj}), dominant pollen (DP) and adjusted dominant pollen (DP_{adj}) for each honey type. Any mean in a row followed by different letters are significantly different

	C. solstitialis	*Prosopis* spp.	*Condalia microphylla*
APC	14,948 ± 10,855a	67,569 ± 47,481b	129,331 ± 117,898b
APC_{adj}	10,625	41,905	70,501
DP	63.31± 12.36	72.56 ± 7.49	82.55 ± 15.55
DP_{adj}	31.5	64.5	75.0

Multivariate CA of variables using the group average method and squared Euclidian distance showed two distinct groups. One group was pH, conductivity, colour and free acidity and the other group was glucose content, G:W ratio and moisture (Fig. 3).

To cluster the three botanical types of honey based on their physicochemical properties, a standardized PCA was applied. Principal component 1 (PC_1) and PC_2 accounted for 76.8 (i.e., 53.1 + 23.7) of the total variance.

Fig. 4 shows the correlation circle where moisture content is located near the origin of two PCs; this variable does not influence group formation in Fig. 5. The first component was positively correlated to colour, EC and pH and negatively related to glucose content and the G:W ratio. The second component was positively correlated to pH and EC and negatively correlated with free acidity. *Condalia microphylla* honeys had high, positive, PC_1 scores (reflecting dark colour and high values of EC, free acidity and pH). *Centaurea solstitialis* and *Prosopis* spp. honeys had high negative PC_1 scores (reflecting high glucose content and G:W ratios). The last variable is related to fast granulation observed in the *Centaurea solstitialis* and *Prosopis* spp. honeys, while *Condalia microphylla* honeys had low, or no granulation (personal observation). Despite *Prosopis* spp. and *Centaurea solstitialis* honeys appearing very close on the biplot, they still formed two different groups; the first on the positive side and the second on the negative side.

Table 4. The physicochemical parameters of the honey samples. Upper line: mean ± SD. Lower line: range (minimum and maximum). Any mean in a row followed by different letters are significantly different

Group of honey	***Centaurea solstitialis* (n = 33)**	***Prosopis* spp. (n = 18)**	***Condalia microphylla* (n = 8)**
Colour (absorbance at 635 nm)	0.163 ± 0.06 a	0.125 ± 0.04 c	0.504 ± 0.14 b
	0.078 - 0.290	0.079 - 0.288	0.305 - 0.675
Electrical conductivity (mS cm^{-1})	0.182 ± 0.040 a	0.315 ± 0.031 c	0.841 ± 0.242 b
	0.113 - 0.278	0.268 - 0.372	0.351 - 1.109
Free acidity (meq kg^{-1})	21.375 ± 4.981 a	9.534 ± 1.348 c	25.728 ± 4.532 b
	13.4 - 32.98	8.46 - 13.64	22.02 - 36.38
Glucose content (%)	31.14 ± 2.70 a	37.20 ± 2.22 c	24.89 ± 2.14 b
	24.12 - 35.81	34.12 - 40.96	22.28 - 28.16
Glucose:Water ratio	1.97 ± 0.18 a	2.15 ± 0.17 c	1.62 ± 0.17 b
	1.54 - 2.54	1.83 - 2.45	1.47 - 1.98
Moisture (%)	15.83 ± 0.67 a	17.37 ± 0.95 b	15.40 ± 1.10 a
	14.7 - 17.5	16.0 - 18.8	14.2 - 17.6
pH	3.57 ± 0.23 a	3.90 ± 0.17 c	4.68 ± 0.16 b
	3.19 - 4.06	3.64 - 4.19	4.52 - 5.02

Discussion

The absolute pollen content indicates that high pollen richness is characteristic of *Condalia microphylla* honeys while lowest pollen richness is typical of *Centaurea solstitialis* honeys. The quantitative pollen analysis, according to Moar (1985), suggests that in *Condalia microphylla* honeys pollen is over-represented, and would require 75% dominant pollen to be classified as a unifloral honey. In *Centaurea solstitialis* honeys pollen is under-represented in samples, so they could be considered unifloral if the dominant pollen is over 31%.

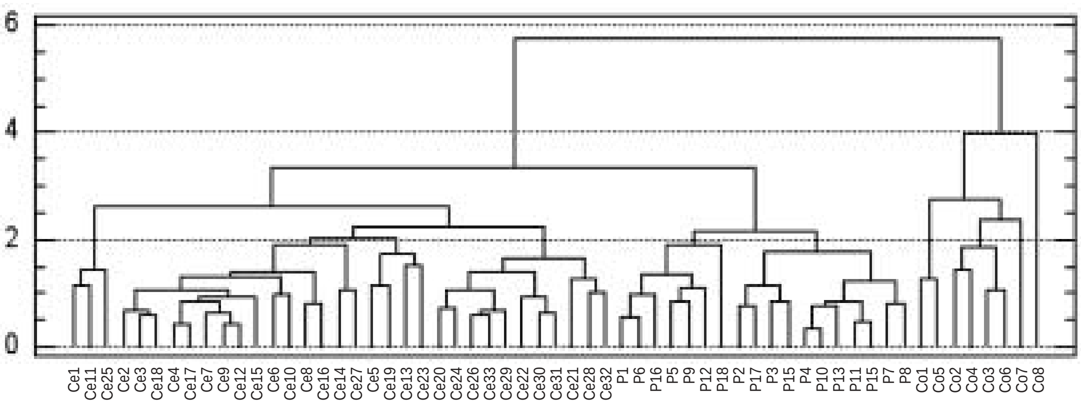

Figure 2. Dendrogram of cluster analysis (CA) of honey samples (group average method, Euclidean distance). Ce, *Centaurea solstitialis*; P, *Prosopis* spp.; Co, *Condalia microphylla*. Ordinate shows distance units.

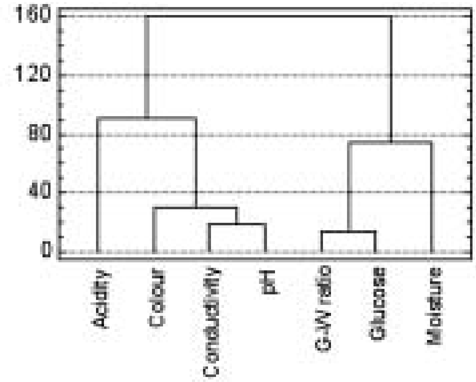

Figure 3. Dendrogram of cluster analysis (CA) of physicochemical variables (group average method, squared Euclidean distance). Ordinate shows distance units.

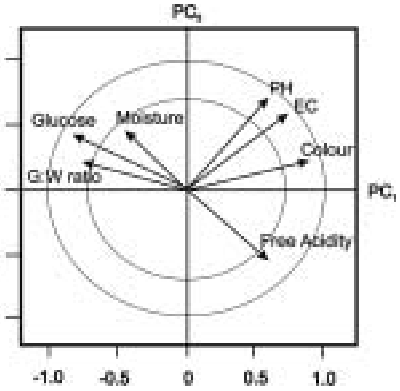

Figure 4. Principal component analysis; correlation among physicochemical variables PC_1 (principal component 1), PC_2 (principal component 2).

Prosopis spp. honeys belong to Group II and would require 64% dominant pollen to be considered a unifloral honey because their dominant pollen is slightly overrepresented in the samples. Thus, the usual pollen frequency of > 45% to assign honey botanical origin is not valid for the unifloral honeys studied in this work.

With regard to physicochemical analysis all the variables analyzed are widely recognized in the evaluation of the botanical origin of honey,

Honey colour is closely linked to botanical origin is used for honey classification. Generally, colour is related to sensory properties such as flavour and odour. Several factors can influence honey colour such as floral source, mineral content and storage conditions (Thawley, 1969; Salinas *et al*., 1994). *Prosopis* spp. honeys were a white water colour (± 7.9 mm Pfund) while *Centaurea solstitialis* honeys were white (± 22 mm Pfund) and *Condalia microphylla* honeys were dark and ranged from light amber to dark (> 140 mm Pfund). The honey colour of the *C. microphylla* and *Prosopis* spp. honey agreed with the results of Andrada (2001).

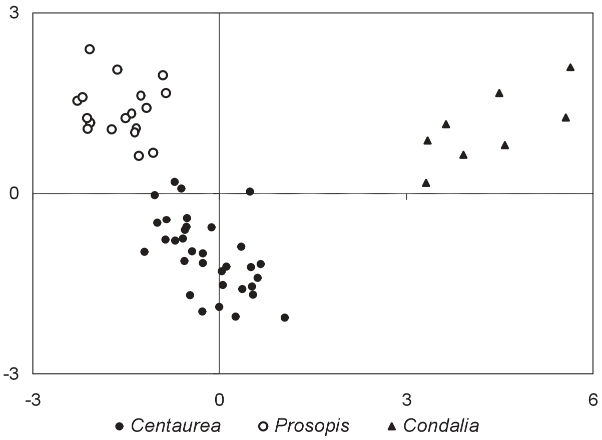

Figure 5. Ordination from principal component analysis of 59 honey samples from three botanical origins by seven physicochemical properties. Samples are located in the space of the two first principal components**.**

Honey EC is closely related to the mineral concentration (total ash), salts, organic acids and protein. The EC varies greatly with honey floral origin because the conductivity and the ash content depend on material collected by the bees during foraging (Terrab *et al*., 2002; Serrano *et al*., 2004; Ouchemoukh *et al*., 2006). The EC results varied widely depending on honey type. The *C. microphylla* honeys had the highest EC values compared with the *Prosopis* spp. and *Centaurea solstitialis* honeys.

Variation in free acidity among different honeys can be attributed to floral origin (El-Sherbiny and Rizk, 1979) or to variation due to the harvest season (Pérez-Arquillué *et al*., 1994). Free acidity differed widely among the three honey types, it was lowest in the *Prosopis* spp. honeys while *Condalia microphylla* honeys had the highest values. Andrada (2001) reported free acidity values in *C. microphylla* honeys which were lower than in these samples. This could be related to the presence of secondary pollen from *Prosopis* spp. in those honeys.

The glucose content of any honey type depends largely on nectar source (Anklam, 1998). Honey samples of different botanical origin had a wide range of glucose content. Values in *Condalia microphylla* honeys were low as found by Tamame and Naab (2003).

The granulation rate and the tendency to granulate are directly related to parameters such as the glucose, water and fructose content (White *et al*., 1962; Manikis and Thrasivoulou, 2001). The average ratio of glucose:water is a criterion for the prediction of granulation tendency; its application to these honeys showed it was a good predictor of granulated and non-granulated honeys as found by Manikis and Thrasivoulou (2001) in Greek honeys. The low G:W ratio in the *C. microphylla* honey samples (≤1.7) confirms that these are less prone to granulation and would remain liquid for longer periods. The *Prosopis* spp. and *Centaurea solstitialis* honeys presented G:W ratio ≥2.1. However, *Prosopis* spp. samples showed faster crystallization than *C. solstitialis* honeys.

In non adulterated honeys the moisture content depends on botanical origin, harvest season, processing techniques and storage conditions. The moisture content of the samples indicated a proper degree of maturity in agreement with international requirements (Codex Alimentarius, FAO-OMS, 1990). The relatively high moisture values in *Prosopis* spp. honeys could be due to the early, spring harvest. Basilio and Nöetinger (2002) reported a similar moisture content in *Prosopis* spp. honeys from the Chaco Region of Argentina. On the other hand, the low moisture in the *Centaurea solstitialis* honeys can be related to the low relative humidity of the semiarid climate of the study area as found by Andrada (2001).

Floral honeys are acidic, with a pH of 3.0 to 4.3 (Bogdanov *et al*., 1999). The pH values in these samples accorded with the acceptable range for floral honeys. *Condalia microphylla* honeys had significantly higher pH values than the other honeys.

The relationship between lower crystallization tendency and high values for pH, EC and free acidity seen in *C. microphylla* honeys has been reported in other honeys (Thawley, 1969; Peña Crecente and Herrero Latorre, 1993; Salinas *et al*., 1994; Sethi and Singh, 1996; Bogdanov *et al*., 1999; Devillers *et al*., 2004; Corbella and Cozzolino, 2005).

The multivariate analysis offered gave quantitative results for the classification of unifloral honeys in agreement with Ruoff *et al.* (2007).

The PCA and CA showed that selected chemical parameters (colour, EC, free acidity, glucose content, G:W ratio, moisture and pH) provided enough information to develop a botanical classification for honeys studied. Consequently, determination of the chemical properties of unifloral honeys can be a useful tool to complement melissopalynological studies. Quantitative pollen analysis showed that the usual pollen frequency (> 45%) for a correct botanical origin assignment in honey was not valid for the unifloral honeys studied.

All the analyzed honeys had excellent quality properties according to Argentinean and International standards (Codex Alimentarius, FAO-OMS, 1990; Bogdanov *et al*., 1997).

The results of this study allowed the classification of three central Argentinean unifloral honeys and justify the use of these parameters with other Argentinean unifloral honeys.

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