Interciencia
ISSN: 0378-1844
interciencia@ivic.ve
Asociación Interciencia
Venezuela

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Interciencia, vol. 38, núm. 7, julio, 2013, pp. 528-534
Asociación Interciencia
Caracas, Venezuela

CONTRIBUTION TO THE PALYNOLOGICAL, PHYSICOCHEMICAL AND ORGANOLEPTIC CHARACTERIZATION OF *Mulinum spinosum* (Apiaceae) HONEYS FROM PATAGONIA, ARGENTINA

Pia V. Aloisi, Alicia E. Forcone and Marisa Amadei

SUMMARY

The market demand for beekeeping products differentiated by origin and the interest to increase their commercial value have led to studies to determine the botanical and geographical origins of honeys from different regions. The aim of this work was to contribute to the knowledge of unifloral honeys. Honey samples classified as unifloral from Mulinum spinosum *(Cav.) Pers. produced in the most southern apicultural area of the Argentinean Patagonia, have been analyzed for various physicochemical parameters (water content, pH, free acidity, diastase activity, electrical conductivity, ashes, hydroxymethylfurfural, sugar composition, polyphenol content and color) and evaluated from the organoleptic point of view.* M. spinosum *honey showed predominantly extra light amber and light amber color tones and sweet flavor. All the samples presented moderate pollinic richness, corresponding to Group II and III of the Maurizio classification, with a relative frequency of* M. spinosum *pollen ranging from 52 to 87%. From the physicochemical point of view this honey was characterized by low values of moisture, hydroxymethylfurfural and free acidity. The monosaccharides glucose and fructose were the main sugar and small amounts of di- and tri-saccharides were also present. The studied honeys were found to meet the requirements of international honey standards and the results contribute to the knowledge of unifloral honeys produced in Argentina and the world.*

Introduction

Honey is a nutritious food produced by bees, mainly *Apis mellifera* L., from nectar or exudations excreted by some plant-sucking insects, gathered, modified and stored in honey combs (Codex Alimentarius Commission, 2001). The composition and properties of honey depend on the botanical origin of the nectar or secretion used. If the nectar from which the honey is derived is gathered mainly from flowers of one specific plant species, the product is called unifloral (White, 2005). These honeys are characterized by its particular pollen content and by their organoleptic, physical and chemical properties (Accorti *et al*., 1986; Bogdanov *et al*., 2004; Persano Oddo *et al*., 2004).

The interest in the production of unifloral honey is due to higher consumer preference, as well as the therapeutic or technological use of certain honey types (Ruoff *et al*., 2006). This recent interest increases the demand for reliable determination of the botanical origin, based primarily on pollen content. Although unifloral honeys have defined properties, flora from different geographical regions can result in variations. Therefore, it is important to characterize honeys by their botanical as well as their geographical origin, according to palynological analysis (Von der Ohe *et al*., 2004).

Botanical characterization also requires physical and chemical determinations, which provide a useful complement to palynological analysis. The parameters used in the physicochemical examination are: color, electrical conductivity, moisture, hydroxymethylfurfural (HMF), optical activity, acidity, pH, sugar composition and enzymatic activity (Bogdanov *et al*., 2004).

Moreover, sensory analysis represents a complement for determining the botanical origin of honey, especially for those characteristics that cannot be studied by analytical methods (Piana *et al*., 2004; Von der Ohe *et al*., 2004).

The market demand for beekeeping products of differentiated origins, and the interest of beekeepers in Argentina to increase the added value of them, have led to a large number of studies aimed to determine botanical and geographical origin of honeys from different regions of Argentina (Tellería, 1988; Andrada and Tellería, 2002; Fagundez and Caccavari, 2006; Forcone, 2008; Forcone *et al*., 2009).

The advance of melissopalynological studies in different areas of Argentina, particularly in those regions with an abundant native flora, rich in endemic species, has allowed the detection of new types of unifloral honeys. In the Patagonia region were detected 18 types of these honeys, two of whom are from native plants (Tellería and Forcone, 2000; For-

KEYWORDS / Honey / Unifloral / Melissopalynology / Organoleptic Attributes /

Received: 08/03/2013. Modified: 07/08/2013. Accepted: 22/08/2013.

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CONTRIBUCIÓN A LA CARACTERIZACIÓN PALINOLÓGICA, FÍSICOQUÍMICA Y ORGANOLÉPTICA DE LAS MIELES DE *Mulinum spinosum* (Apiaceae) DE PATAGONIA, ARGENTINA

Pia V. Aloisi, Alicia E. Forcone y Marisa Amadei

RESUMEN

La creciente demanda de los mercados por productos apícolas diferenciados por origen y el interés por aumentar su valor agregado, han incrementado los estudios tendientes a clasificar la miel de las distintas regiones por origen geográfico y botánico. El objetivo de este trabajo fue realizar un aporte al conocimiento de las mieles monoflorales. Muestras de miel clasificadas como monoflorales para Mulinum spinosum *(Cav.) Pers. producidas en la región melífera más austral de la Patagonia argentina fueron estudiadas para varios parámetros fisicoquímicos (humedad, pH, acidez libre, actividad diastásica, conductividad eléctrica, cenizas, hidroximetilfurfural, composición de azúcares, contenido de polifenoles y color) y evaluadas desde el punto de vista organoléptico. Las mieles de* M. spinosum *mostraron predominantemente colores desde el ámbar claro al ámbar extra claro, y sabor dulce. Todas las muestras presentaron una moderada riqueza polínica correspondiente a los Grupos II y III de la clasificación de Maurizio, con una frecuencia relativa de polen de* M. spinosum *variando entre 52 y 87%. Desde el punto de vista fisicoquímico este tipo de miel se caracterizó por bajos valores de humedad, hidroximetilfurfural y acidez libre. Los monosacáridos glucosa y fructosa fueron los principales azúcares y también estuvieron presentes pequeñas cantidades de di y trisacáridos. Las mieles estudiadas cumplieron con los requerimientos internacionales para los estándares de miel y los resultados contribuyen al conocimiento de las mieles uniflorales producidas en Argentina y en el mundo.*

CONTRIBUIÇÃO À CARACTERIZAÇÃO PALINOLÓGICA, FÍSICO-QUÍMICA E ORGANOLÉPTICA DOS MÉIS DE *Mulinum spinosum* (Apiaceae) DE PATAGÔNIA, ARGENTINA

Pia V. Aloisi, Alicia E. Forcone e Marisa Amadei

RESUMO

A crescente demanda dos mercados por produtos apícolas diferenciados por sua origem e o interesse por aumentar seu valor agregado, tem incrementado os estudos tendentes a classificar o mel das distintas regiões por sua origem geográfica e botânica. O objetivo deste trabalho foi realizar uma contribuição ao conhecimento dos méis monoflorais. Amostras de mel classificadas como monoflorais para Mulinum spinosum *(Cav.) Pers. produzidas na região melífera mais austral da Patagônia argentina foram estudadas para vários parâmetros físico-químicos (umidade, pH, acidez livre, atividade diastásica, condutividade eléctrica, cinzas, hidroximetilfurfural, composição de açúcares, conteúdo de polifenóis e cor) e avaliadas desde o ponto de vista organoléptico. Os méis de* M. spinosum *mostraram predominantemente cores desde o âmbar claro ao âmbar extra claro, e sabor doce. Todas as amostras apresentaram uma moderada riqueza polínica correspondente aos Grupos II e III da classificação de Maurizio, com uma frequência relativa de pólen de* M. spinosum *variando entre 52 e 87%. Desde o ponto de vista físico-químico este tipo de mel se caracterizou por baixos valores de umidade, hidroximetilfurfural e acidez livre. Os monossacáridos glucose e frutose foram os principais açúcares e também estiveram presentes pequenas quantidades de di e tri-sacarídeos. Os méis estudados cumpriram com os requerimentos internacionais para os padrões de mel e os resultados contribuem ao conhecimento dos méis uniflorais produzidas na Argentina e no mundo.*

cone, 2008; Forcone *et al*., 2009). These findings raise the need to characterize and determine in each case the pollen percentages established for the designation of unifloral.

Unifloral honey from *Mulinum spinosum* (Cav.) Pers. (Apiaceae) comes from a subshrub commonly known as 'neneo' which is an Argentine-Chilean endemic species (Zuloaga *et al*., 2008). The production area of *Mulinum* honey (Figure 1) is in the extra-Andean Patagonia, in the northwest of Santa Cruz province (Forcone. *et al*, 2009). The region extends from the sub-Andean foothills and descends eastwards to the Atlantic Ocean; its landscape consists mainly of plateaus with the shrub steppe as physiognomic predominant type, being the most southern apicultural area of Argentina. It is comprised in a transition region between the Patagonian Province and grass steppes of the Subantarctic Province (Roig, 1998). Among the most abundant species stand out: *Senecio patagonicus* Hook & Arn., *Mulinum spinosum* (Cav.) Pers., *Colliguaja integerrima* Gillies et Hook, *Adesmia boronioides* Hook., *Berberis heterophylla* Juss., *Schinus marchandii* Barkley and *Acaena magellanica* (Lam.) Vahl (Roig, 1998).

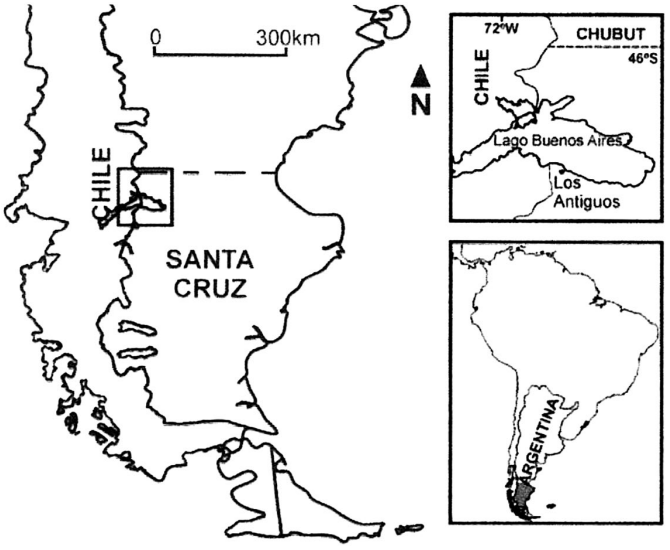

Figure 1. Location of the study area in Santa Cruz province (Argentinean Patagonia).

The objective of the present study was to characterize unifloral honey from *M. spinosum* from the palynological, physicochemical and organoleptic points of view, to broaden knowledge of unifloral honey types produced in Argentina and the world.

Materials and Methods

Sixteen honey samples produced by *Apis mellifera* and classified as unifloral from *Mulinum spinosum* by the Palynology Laboratory of the Universidad Nacional de la Patagonia San Juan Bosco (UNPSJB), were studied. The samples were collected directly from beekeepers in northwest of Santa Cruz Province, Argentina (46.5°S, between 70.5° and 71.7°W) (Figure 1) during the period 2005-2011 and kept at 4ºC. Pollen samples were obtained by centrifugation of the honey as described below.

Pollen analysis

For the pollen qualitative analysis the methods of Louveaux *et al*. (1978) were followed, slightly modified. Twenty grams of honey were dissolved in 100ml of distilled water, centrifuged at 1500g (3000rpm) during 10min at at room temperature, washed and acetolysed (Erdtman, 1960). The pollen sediment was mounted on glycerine-gelatin and sealed with paraffin. To determine the relative frequency (percentage of each pollen type in the pollen content of a sample) 500 pollen grains were counted in each case. Pollen types were classified into four categories: predominant pollen (>45% of the total number of pollen grains); secondary pollen (16-45%); important minor pollen (3-15%) and minor pollen (<3%). When only one pollen type represented >45% of the total number of pollen grains, the sample was classified as unifloral honey (Louveaux *et al*., 1978).

Pollen types were identified by comparing them with a reference pollen collection made from plants from the area surrounding the beehives. The pollen atlases of Heusser (1971) and Markgraf and D'Antoni (1978) were consulted. The reference collection was donated to the Palynotheque of the UNPSJB (Trelew campus). The herbarium specimens were deposited in the Trelew Herbarium (HTW).

Quantitative pollen analysis included the addition of tablets of *Lycopodium clavatum* L. spores (Stockmarr, 1971; Moar, 1985). Ten grams of honey were dissolved in 40ml of distilled water, and two tablets of *L. clavatum* spores (each containing 12000 ±200 spores) were dissolved in 5ml of 5% hydrochloric acid and added. The sediment was concentrated by repeated centrifuging at 1500g (3000rpm), using a 10ml centrifuge tube. The centrifugation was continued until all the sediment was included in one tube. The sediment, without any

TABLE I
FREQUENCY CLASES

Family		Samples															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Anacardiaceae	*Schinus marchandii* F. A. Barkley*					m	m										
Apiaceae	*Conium maculatum* L.					m			m								
	Daucus spp.							m	m						m		
	Foeniculum vulgare Mill.													m			
	*Mulinum spinosum**	62	52	87	61	61	64	63	64	65	58	60	69	55	76	62	73
Asteraceae	Anthemideae	m	m			m						m					
	Astereae*	m	m		m	m	m	m			m	m	m	m	m		m
	Cirsium vulgare (Savi) Ten		m									m		m			m
	Cichorium intybus L.	m	m		m					m		m					
	Cichorieae				m												
	Hypochoeris radicata L.										m						
	*Leucheria achillaeifolia**		m					m	m	m							
	Taraxacum officinale F. H. Wigg.	m	m			m	m	m	m	m	m	m	m			m	
	Senecio spp.*	m	m	m	m	m	m	M	m	m	m	m	m	m	m	m	m
Betulaceae	*Betula* spp.												m				
Boraginaceae	*Phacelia secunda* J. F. Gmel.*		m					m		m	m	m					m
Brassicaceae	Brassicaceae	M	m	m	M	m	M	M	M	M	M	M	M	M	M	M	S
Caprifoliaceae	*Lonicera* spp.													m			
Caryophyllaceae	Caryophyllaceae													m			
Chenopodiaceae	Chenopodiaceae a	m					m										
Convolvulaceae	*Convolvulus arvensis* L.	m		m		m	m	m	m	m	m	m	m	m	m		m
Cyperaceae	Cyperaceae a												m				m
Ephedraceae	*Ephedra* spp.*														m		
Fabaceae	Fabaceae														m	m	
	Adesmia spp.*	m	m							m			M		m	m	
	Lotus spp.								m	m					m		m
	Medicago sativa L.		m		m	m	m	m	m	m	M	m		M	m	m	
	Melilotus sp.	M	M	M	S	S	S	M	S	S	S	S	M	S	M	M	M
	Trifolium spp.	m	m		m	M	M		m		m	m	m	m	m	m	
	Vicia spp.	m	m	m											m		
Geraniaceae	*Erodium cicutarium* (L.) Aiton												m				
Juglandaceae	*Juglans spp.* a														m		
Malvaceae	*Malva* spp.					m	m		m		m	m		m			
Myrtaceae	*Eucalyptus* spp.						m						m				
Oleaceae	*Ligustrum* spp.	m															
Onagraceae	*Oenothera* spp.*													m			
Pinaceae	*Pinus* sp. a																m
Plantaginaceae	*Plantago* sp. a		m	m	m	M	m	m	m	m	m	M	m	M	m	m	m
Poaceae	Poaceae a			m		m	m		m			m	m	m			m
Polygalaceae	*Polygala* spp.*										m					m	
Polygonaceae	*Rumex acetosella* L.*							m						m	m		
Rhamnaceae	*Ochetophila trinervis* (Gillies ex Hook & Arn.) Poepp.*	m			m		m	m		m	m						
Rosaceae	Rosaceae		S			m		m	m			m	m	m			m
	Acaena spp.*	m	m	m		m	m	m	m		m	m	m	m	M	S	m
Schoepfiaceae	*Quinchamalium chilense* Molina*															m	
Typhaceae	*Typha sp.* a	m										m					
Verbenaceae	*Mulguraea-Junellia* spp.*					m					m					m	

For the predominant (>45%) pollen (*Mulinum spinosum*) the numbers refer to found percentages. *: native plants; a: Pollen from wind-pollinated plants; S: secondary pollen (16-45%); M: important minor pollen (3-15%); m: minor pollen (<3%).

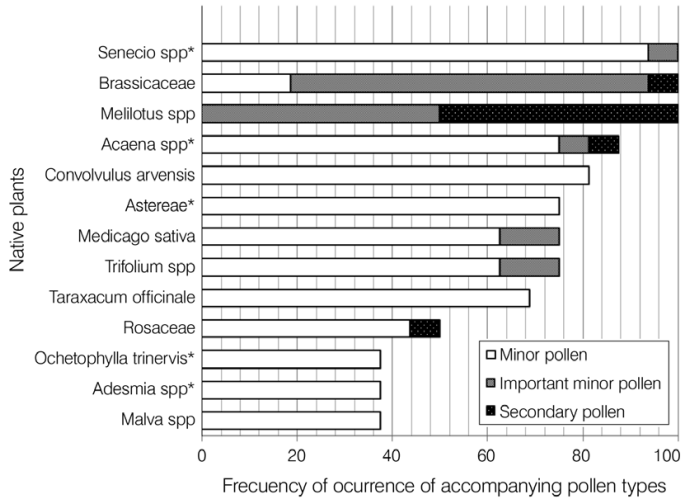

Figure 2. Frequency of occurrence of accompanying pollen types in Mulinum spinosum honeys. * Native Plants

TABLE II
RESULTS OF THE QUANTITATIVE POLLEN ANALYSIS

Samples	NPG	Group (Maurizio)	HDE/P
1	22,220	II	0.01
2	35,111	II	0.01
3	110,666	III	0.00
4	112,838	III	0.00
5	101,443	III	0.00
6	136,000	III	0.00
7	44,959	II	0.00
8	32,621	II	0.01
9	58,750	II	0.01
10	117,030	III	0.01
11	30,973	II	0.01
12	42,491	II	0.00
13	40,452	II	0.00
14	141,425	III	0.00
15	51,325	II	0.81
16	71,628	II	0.01

NPG: Number of pollen grains in 10g of honey; HDE/P: Honeydew index (ratio of honeydew elements to pollen grains of nectariferous plants) Group according to Maurizio (1939).

chemical treatment, was mounted on glycerine-gelatin, and sealed with paraffin. Spores, pollen and honeydew were counted until 500 pollen grains were reached in each case.

Pollen concentration (NPG; number of pollen grains in 10g of honey) was calculated by applying the formula: (pollen counted/*L. clavatum* spores counted) × spores added. Samples were distributed into classes according to the pollen grain content of 10g honey (Maurizio, 1939) as: Group I (<20,000); Group II 20,000-100,000); Group III (100,000-500,000); Group IV (500,000-1,000,000); and Group V (>1,000,000). The honeydew index (Louveaux *et al*., 1978) was calculated as HDE/P (ratio of honeydew elements -HDE- to pollen grains of nectariferous plants -P).

Organoleptic properties

The method presented by Piana *et al*. (2004) was used in the analyses of organoleptic properties. The method is based on the evaluation of the olfactory-gustatory characteristics of honey to identify sensory stimuli on the basis of previously memorized standards.

Physicochemical analysis

The physicochemical analysis performed included color, moisture, hydroxymethylfurfural (HMF), pH, free acidity, ashes, electrical conductivity, diastase activity, total polyphenols and sugars determinations.

Color was determined by the measurement of absorbance at 635nm of 10g of honey diluted in distilled water to a volume of 20ml (Bianchi, 1990). Additional measurements were also carried out by optical comparison with a Lovibond™ tintometer using a Pfund scale (Aubert and Gonnet, 1983). Moisture was determined with an Abbe™ refractometer at 20°C by obtaining the corresponding value of moisture percentage from the Chataway table (AOAC, 1999). Acidity was determined by titration with 0.10M sodium hydroxide (NaOH) until pH reached 8.3 (AOAC, 1999). HMF was measured by spectroscopy (White, 1979). Measurement of electrical conductivity were conducted in a honey solution containing 20% of honey dry matter in 100ml distilled water (Bogdanov *et al*., 1999). The method of Schade modified by White (White, 1979) was used to determine the diastase activity and its results expressed as ml of 1% starch hydrolyzed by the enzyme in 1g of honey in 1h (Codex Alimentarius Commission, 2001).

The levels of polyphenol compounds in the honey samples were estimated with spectrophotometric determination using a modified Folin-Ciocalteu method (Singleton *et al*., 1999). Briefly, 30µl of honey sample ($0.1g \cdot ml^{-1}$) was mixed with 150µl Folin-Ciocalteu's phenol reagent (0.2N). After 2min, 450µl sodium carbonate solution ($0.2g \cdot ml^{-1}$) was added to the mixture. The reaction was kept in the dark for 120min, after which the absorbance was read at 765nm by a SP-300 Plus UV-VIS spectrophotometer (Optima, Tokio, Japan). Gallic acid was used to calculate the standard curve. Phenolic compound levels were measured in triplicate. The results were expressed as mg of gallic acid equivalent (GAE)/100g honey.

Sugars contents were determined using HPLC according to IHC (Bogdanov, 2002) on a high pressure liquid chromatograph (Agilent, Series 1100, Germany) equipped with binary pumps (G1316A), a termostated autosampler (G1329A), a termostated column oven (G1316A) and a refractive index detector (RID) (G1362A), combined with Agilent Chem Station IL 50508 software. The column was Zorbax NH_2, 4.6×250mm, particle size of 5µm.

The amount of sample injected onto column was 5µl. The separation was conducted at a temperature of 35°C with the mobile phase acetonitrile:water (83:17) at a flow rate of $0.65ml \cdot min^{-1}$. For the qualification and quantification of the saccharides, the HPLC chromatograms of the samples were compared to those of commercial standards of fructose, glucose, saccharose, turanose, maltose, trehalose, erlose, melezitose, raffinose, maltotriose and maltotetrose.

All physicochemical results were compared with International Regulatory Standards (Bogdanov *et al*, 1999). Means and standard deviations were calculated by using the software Microsoft® Excel 2007.

Results

Pollen analysis

Qualitative analysis. The relative frequency of *Mulinun spinosum* pollen ranged from 52% to 87% (Table I). Forty six morphological types were determined in the accompa-

TABLE III
PHYSICOCHEMICAL PARAMETERS OF *Mulinum spinosum* HONEYS

Sample	Moisture (%)	Color (mm Pfund)	pH	Free acidity (meq·kg^{-1})	Elect. cond. (mS·cm^{-1})	Ashes (% w/w)	HMF (mg·kg^{-1})	Diastase (DN)	Polyphenols (mg GAE/ 100 g)
1	11.4	61.3	3.7	18	0.29	0.086	0.15	16.8	190
2	17.1	94.3	4.3	21	0.37	0.132	4.19	18.6	130
3	14.9	74.1	4.6	17	0.41	0.155	1.65	17.5	158
4	16.2	79.8	4.6	24	0.34	0.15	3.44	14.1	134
5	15.5	52.5	4.4	25	0.33	0.11	4.34	9.9	130
6	15.6	80.4	4.5	20	0.32	0.1	4.19	5.9	133
7	16.6	30	4.5	19	0.43	0.17	4.49	10	146
8	15.1	71.5	4.5	31	0.5	0	4.34	7.1	130
9	15.3	71.5	4.7	17	0.45	0.178	3.76	7.4	130
10	14.3	97.1	5	22	0.65	0.293	3.64	9.8	163
11	14.7	40	5.3	19	0.41	0.155	2.25	5.6	141
12	16.1	45	4.1	15	0.34	0.115	3.74	5.3	169
13	14.5	40	4.2	19	0.57	0.247	3.52	30.5	113
14	14.4	25	4.1	19	0.39	0.144	0	14.5	152
15	14.6	35	4.1	20	0.44	0.172	1.2	14.5	119
16	0	45	4.1	17	0.34	0.115	0	14.4	151
Mean	15.19	58.9	4.42	20.2	0.41	0.15	2.81	12.6	143.1
Max	17.1	97.1	5.3	31	0.65	0.293	4.49	30.5	190
Min	11.4	25	3.7	15	0.29	0	0	5.3	113
SD	1.35	22.9	0.39	0.1	0.07	1.67	6.52	20	1.7

nying pollen: 39 from insect-pollinated plants and seven from wind-pollinated plants. The most frequent entomophilous types were: *Senecio* spp. (Asteraceae), Brassicaceae and *Melilotus* spp. (Fabaceae); these types were found in 100% of the samples (Table I; Figure 2). Other frequent types were: *Acaena* spp. (Rosaceae*)*, Astereae, *Convolvulus arvensis* (Convolvulaceae), *Medicago sativa* (Fabaceae) and *Trifolium* spp. (Fabaceae; found in 80-90% of the samples), *Taraxacum officinale (*Asteraceae*)* and Rosaceae (50-60% of the samples). From these types Brassicaceae, *Melilotus* sp., *Acaena* spp. and Rosaceae were classified as secondary pollen. *Senecio* spp., *M. sativa* and *Trifolium* spp. were found as important minor pollen together with the tree first mentioned types of pollen, while the remaining types correspond to minor pollen. The most frequent anemophilous types were *Plantago* spp. and Poaceae (Table I).

Quantitative analysis. All the samples showed moderate pollinic richness. The values of pollen grains per 10g of honey were between 22,220 and 141,425 (Table II), with an average of 71,870.75. Of the samples, 62% belonged to Maurizio´s (1939) Group II. The remaining samples were classified in Group III, most of them in the lower limit of that category.

Honeydew indicators were scarce or absent. The HDE/P ratio was <1 in all the samples (Table II).

Sensory analysis

Mulinum spinosum (Cav.) Pers. honey showed light color intensity and amber color tones. Its flavor, sweet and warm, resembled caramel, according to Piana *et al.* (2004). The honey was homogeneous and presented a creamy consistence with fine crystals.

Physicochemical analysis

All the honey samples presented very low values of HMF, with a maximum value of 4.5mg·kg^{-1}. Water content ranged from 11.4 to 17.1% with an average value of 15.19%. Ash content and electrical conductivity were in agreement with international standards, with a mean value of 0.15% w/w and 0.41mS·cm^{-1}, respectively (Table III).

Values of free acidity were low (mean of 20.2mg·kg^{-1}). The average pH value was 4.4 and ranged between 3.7 and 5.3.

With regard to color (Table III), 75% of the samples analyzed ranged mainly between extra light amber (34-45mm Pfund) and light amber (61.3-80.4mm Pfund). Only 12.5% were amber (>94mm Pfund) and 12.5% were white (<34mm Pfund).

The diastase activity recorded for the honey ranged between 5.3 and 30.5 DN with an average of 12.62. Most of the samples showed values between 9 and 19 DN. Only one sample showed a value of 30.5 DN.

M. spinosum honey (Table IV) was rich in fructose and its fructose/glucose ratio was 1.3 and glucose/water ratio 1.9. The values of fructose and glucose were in the range 36.3-40.4g/100g and 24.2-37.4g/100g, respectively. All the samples contained the dissacharides turanose (mean value 2g/100g) and trehalose (mean 0.8g/100g). In addition, the oligosaccharide melezitose was present in six samples and erlose in four samples. Maltotriose was recorded in two samples while raffinose was in only one case. Saccharose, maltose and maltotetraose were not detected.

The total amount of phenolic compounds ranged from 113 mg GAE 100·g^{-1} to 190 mg GAE 100 g^{-1} with an average value of 143.1 mg GAE 100 g^{-1} (Table III).

Discussion

In general, a honey is considered as coming predominantly from a given botanical origin (unifloral honey) if the relative frequency of pollen of that *taxon* exceeds 45%, and if the ratio of the number of honeydew elements (HDE) to that of pollen grain does not exceed three (Von der Ohe *et al*., 2004). The percentages of the *Mulinum spinosum* pollen reported in the present work (52-87%) greatly exceeds the minimum value established for classifying a honey as unifloral, and in all cases the ratio honeydew elements/pollen grains does not exceed 1%. Moreover, the obtained results show a moderate pollen concentration, indicating that

TABLE IV
COMPOSITION AND TOTAL AMOUNT (g/100g) OF MONO-DI AND OLIGOSACCHARIDES IN *Mulinum spinosum* HONEYS

Samples*	Fructose	Glucose	Turanose	Trehalose	Erlose	Melezitose	Raffinose	Maltotriose
2	39.4	26.3	2.9	1.1		0.6		
3	40.3	27.0	2.1	0.7				
4	40.0	24.2	2.4	0.9				
5	37.7	31.4	2.2	0.8			0.1	
6	38.3	28.0	2.3	0.7				
7	40.3	28.2	2.1	0.6				
8	37.9	29.8	2.0	0.7				
9	37.8	29.0	2.1	0.9	0.2	0.2		
10	38.0	8.8	2.1	1.0		0.7		0.6
11	40.4	29.1	2.1	1.0	0.4			
12	40.0	25.8	2.4	0.7		0.2		
13	36.3	31.3	2.4	1.4	0.4	0.5		0.3
14	39.4	34.0	1.8	0.6	0.1			
15	38.3	32.3	1.8	0.7		0.7		
16	37.8	37.4	1.4	0.6				

* Sample 1 was not considered for this analysis because the amount of honey insufficient to perform the assays. Saccharose, maltose and maltotetraose were not detected.

pollen from *M. spinosum* in the pollen content of honey is neither over-represented nor under-represented (Louveaux *et al*., 1978; Von der Ohe *et al*., 2004). These data together with sensory analysis allows inferring that the studied honeys are *M. spinosum* unifloral.

In these honeys, accompanying pollen comes from fodder crops, fruit trees, weed and native plants. The exotic secondary pollens with greater frequency of occurrence in the samples are: Brassicaceae, *Melilotus* sp. (Fabaceae) and Rosaceae, the latter mainly represented by fruit crops.

The most frequently native pollen come from *Acaena* spp. (Rosaceae), Astereae and *Senecio* spp. (Asteraceae); other frequently native pollens are *Adesmia* spp., *Ochetophila trinervis* and *Phacelia secunda*. All these taxa are present in the herbaceous shrub-steppe that characterizes the western district of the Patagonian Province.

Pollen of wind pollinated plants is abundant in *M. spinosum* honeys, most of the anemophilous pollen being from *Plantago* sp. The abundance of anemophilous pollen differentiates extra Andean Patagonian honeys from those originated in other Argentinean areas, where pollen of wind pollinated plants is scarce (Tellería, 1988; Fagúndez and Caccavari, 2006).

The physicochemical characteristics studied in *M. spinosum* honeys could be related to the geographical origin and the climate of the production area, with annual precipitation ~200mm and an average annual temperature of 8°C (Forcone *et al*., 2009). The low values of moisture and HMF measured in the samples could be attributed to the extreme dryness of the region of origin. These results agree with those found in honeys of Chubut Province (Argentinean Patagonia) with similar climate conditions (Aloisi, 2010).

Regarding color, honeys from *M. spinosum* present light tones, varying from white to amber. The clearest samples showed higher percentages of dominant pollen than those that were darker. These results indicate that in these honeys the lightest tones would be related to a greater contribution of nectar from *M. spinosum*.

The characteristics of pollen content and the low values of conductivity indicate that the analyzed honeys originate mainly from nectar. The conductivity values are in the range establish for nectar honeys (Bogdanov *et al*., 1999).

Eight carbohydrates were identified, including two monosaccharides, two disaccharides and four oligosaccharides. The monosaccharides glucose and fructose were present in every sample and were the main sugars in all of them. The prevalence of these monosaccharides agrees with the sugar profile characteristic of blossom honeys (Bogdanov *et al*., 2004). The glucose levels detected in *M. spinosum* honeys correspond to the values determined for unifloral honeys (Persano Oddo and Piro, 2004).

Polyphenols are an important group of compounds with respect to the appearance and the functional properties of honey (Bogdanov *et al*., 2008). These phytochemical constituents are considered to be very important in the assessment of honey flavor quality and, hence, the overall quality of honey and its botanical origin. Different types and quantities of phenolic compounds may also vary according to the floral origin (Sant'Ana *et al*., 2012). The total amounts of polyphenols registered in the studied samples are within the values determined for others unifloral honeys (Bogdanov *et al*., 2008).

Values obtained for *M. spinosum* honey are consistent with the values for other Apiaceae honeys studied in other latitudes, specifically in Morocco (*Ammi visnaga*, *Eryngium campestre*, *Ridolfia segetum*; Terrab *et al*., 2003). Both Apiaceae Moroccan honeys and *M. spinosum* honey are characterized by clear tones ranging from white to amber and a moderate pollen concentration corresponding to Groups II and III of Maurizio's classification. Both types present similar pH, free acidity and electrical conductivity. However, the lower levels of HMF and water content detected in the *Mulinum* honeys can differentiate them from the Apiacea honeys produced in Morocco.

Conclusions

The studied honeys were found to meet the requirements of the international honey standards and the results contribute to the knowledge of unifloral honeys produced in Argentina and in the world.

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