Physicochemical and melissopalynological profiles of *Citrus limon* honey from Tucumán-Argentina. Hesperidin as a suitable marker of floral origin

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ABSTRACT

Geographical Indications (GI) are tools used to add value, protect and recognize the differentiated quality of a product as a consequence of environmental and territorial influence. The objective of this work was to determine physicochemical, melissopalynological and sugar profiles plus chemical markers of Citrus limon monofloral honey produced in Tucumán-Argentina, to the development of the GI "Lemon blossom honey from Tucumán". Samples harvested between August and October 2016, 2017 and 2018 were analyzed. It was established that in addition to Citrus limon, the most frequent pollen species was Salix type. This honey is very light in color (≤ 30 mm Pfund), slightly acidic (≤ 20 meq.kg^{-1}), with moisture values from 15 to 19 g/100g, low diastatic activity (≤ 27 Gothe units), HMF (≤ 3.0 mg.kg^{-1}) and electrical conductivity (≤ 0.32 mS.cm^{-1}). Color inversely correlates with the amount of Citrus limon pollen. The sugar profile showed a higher fructose content than glucose, sucrose and maltose, and intermediate tendency towards crystallization. Hesperidin was easily detected and quantified in all samples. The content of methyl anthranilate was much lower than the international reference for citrus honeys and also, was not detected in some samples. Hence, should be a complementary indicator and hesperidin could be used instead as a chemical marker of genuineness.

Keywords: lemon blossom honey, differentiation, value added, geographical indication, methyl anthranilate, genuineness.

RESUMEN

Las Indicaciones Geográficas (IG) son herramientas utilizadas para agregar valor y para proteger y reconocer la calidad diferencial de un producto como consecuencia de la influencia ambiental y territorial. El objetivo de este trabajo consistió en determinar los perfiles físicoquímicos, melisopalinológicos y de azúcares, además de marcadores químicos de mieles monoflorales de Citrus limon producidas en Tucumán-Argentina, para el desarrollo de la IG "Miel de azahar de limón de Tucumán". Se analizaron muestras cosechadas entre agosto y octubre de 2016, 2017 y 2018. Se estableció que además de Citrus limon, la especie de polen más frecuente fue la del tipo Salix. Esta miel es de color muy claro (≤ 30 mm Pfund), ligeramente ácida (≤ 20 meq.kg^{-1}), con valores de humedad de 15 a 19 g/100 g, baja

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actividad diastásica (≤ 27 unidades Gothe), HMF (≤ 3,0 mg.kg^{-1}) y conductividad eléctrica (≤ 0,32 mS.cm^{-1}). El color se correlaciona inversamente con la cantidad de polen de Citrus limon. El perfil de azúcares mostró mayor contenido de fructosa que glucosa, sacarosa y maltosa, y una tendencia intermedia a la cristalización. La hesperidina se detectó y cuantificó fácilmente en todas las muestras. El contenido de metil antranilato fue mucho menor que la referencia internacional para mieles de cítricos y además, no se detectó en algunas muestras. Por lo tanto, debería ser un indicador complementario y la hesperidina podría usarse en su lugar como un marcador químico de autenticidad.

Palabras clave: *miel de azahar de limón, diferenciación, valor agregado, indicación geográfica, metil antranilato, genuinidad.*

INTRODUCTION

Argentina is the world's leading producer and processor of lemon (*Citrus limon*), and Tucumán has the highest production index (40,000 hectares in 2016 with 1,350,840 tons) (MDP, 2016-2017). This context has allowed beekeepers to obtain a monofloral honey of great acceptance among consumers. The traditional method to establish the botanical origin of honeys is the melissopalynological analysis, which identifies and quantifies pollen types. In citrus honeys, problems arise because the moment of maximum nectar production does not coincide with the maturation of the anthers (Ferreres et al., 1994) and therefore this type of pollen is under-represented (Escriche *et al*., 2011). In Argentina, the regulation establishes that monofloral citrus honeys must contain a minimum of 10% of this pollen type (SAGPyA Resolution 274/95). Additional sensory analysis and/or the determination of specific chemical markers such as methyl anthranilate (MA) and hesperetin are commercially used to ensure the authenticity of this type of honeys (Bonvehí and Coll, 1995; Ferreres *et al*., 1994).

Figure 1. Sampling points of *Citrus limon* monofloral honey from Tucumán. Original, made for this paper.

Although these aspects and methods make it possible to differentiate honey, there are others that make it more valuable and outstanding like physicochemical, melissopalynological, sensory and biological characteristics linked to geographical origin, climate and soil, together with the practices and techniques used for production. In this sense, Geographical Indications (GI) are used to protect and recognize the differentiated quality of a product as a consequence of its relation with the territory.

Therefore, the objective of this work was to determine physicochemical, melissopalynological and sugar profiles plus chemical markers of *Citrus limon* monofloral honey produced in Tucumán as a contribution to the development of the Geographical Indication "*Lemon blossom honey from Tucumán*".

MATERIALS AND METHODS

Honey samples

The 24 samples of *Citrus limon* monofloral honeys used were harvested by local beekeepers (figure 1) between August and October 2016 to 2018 in Tucumán, Argentina. Each sample was divided into 2 sub samples and were stored at -20°C until used: one for the melissopalynological analysis and the other for the rest of analysis. Each sample was analyzed in triplicate.

Chemical products and standards

All standards were chemical grade of the highest purity. Sucrose, D-(+)-Maltose monohydrate, D-(+)-Trehalose dihydrate and D-(+)-Melezitose hydrate, Methyl anthranilate (MA) and Hesperidin were purchased from Sigma-Aldrich. D-Fructose was obtained from Crescent Chemical Co and D-Glucose from Chem service. Acetonitrile, acetic acid and water were HPLC grade. The other reagents and solvents were of analytical grade.

Qualitative melissopalynological analysis

Honey samples were processed according to the methodology proposed by Louveaux *et al*. (1978) and were later acetolized (Erdtman, 1960). At least 600 grains of pollen were counted to calculate the percentages of each pollen type identified up to the botanical family level.

Physicochemical analysis

Equipment used: Refractometer-Abbé type Brixometer (AO-American Optical-Model 10460); Honey color analyzer (Hanna C221), pHmeter (Altronix), Conductimeter: (Consort C830), UV-Vis spectrophotometer: HP 8452 A.

Moisture was determined indirectly by refractometry (Bogdanov, 2002). Color was established with a honey color analyzer. Hydroxymethylfurfural (HMF) was determined by UV spectrophotometry-White method. (Method 980.23 A.O.A.C, 1998). Electrical conductivity (EC) was read using a digital conductometer. (Bogdanov, 2002). The pH and free acidity (FA) was found by potentiometry and titrimetry. (Pereira *et al*., 2013) (Method 962.19 A.O.A.C, 1998). Total phenols (TP) were registered by spectrophotometry, using the Folin-Ciocalteu reagent (Singleton et al., 1999). It is expressed in milligrams of equivalent gallic acid per gram of honey (mg $GAE.g^{-1}$). The Diastatic activity (DA) was analyzed by the method described by Bogdanov (2002). The Reducing Sugars (RS), Total Sugars (TS) and Apparent Sucrose (AS) are measured using the Fehling-Causse-Bonnans reagent. (IRAM 15934, 1995).

Sugar profile by HPLC

Ten samples of *Citrus limon* monofloral honey from Tucumán randomly selected were analyzed by HPLC. The sample was prepared by dissolving 2.0 g of honey in 5 mL of HPLC-grade ultrapure water, placed in a 10 mL graduated flask, clarified by adding 250 µL of Carrez I and 250 µL of Carrez II solution and brought to volume with ultra-pure water. It was then filtered through Whatman No. 4 paper and subsequently through nylon 0.45 µm pore filters.

HPLC analysis was performed by using a Waters 1525 Binary Pumps system with a Waters 1500 Series Column Heater and a Refractive Index Detector (Waters 2414). A Polyamine II column (250 mm X 4.6mm, -YMC HPLC Column) was used. The mobile phase consisted of acetonitrile: water (8: 2, v/v) and a running flow of 1.0 mL/min. The system was maintained at 35°C. Identification of fructose, glucose, sucrose, maltose, trehalose and melezitose was made by comparison of retention times with commercial standards. For quantification, calibration curves were made for each sugar, using different concentrations of the standard.

Chemical markers of botanical origin: Methyl anthranilate and Hesperidin

A group of samples similar to those for sugars profile was analysed using the method proposed by Nozal *et al*. (2001) with modifications. 2.5 grams of honey were dissolved in 10 mL of

Pollen type	Family	Minimum (%)	Maximum (%)	Acronym
Acacia	Fabaceae	0.0	3.5	acac
Allophylus edulis	Sapindaceae	0.0	7.0	allo
Apiaceae	Apiaceae	0.0	4.9	apia
Asteraceae	Asteraceae	0.0	11.6	aste
Bignoniaceae	Bignoniaceae	0.0	15.7	bign
Boraginaceae	Boraginaceae	0.0	4.3	bora
Citrus	Rutaceae	11.1	56.3	citr
Eucalyptus	Myrtaceae	0.0	7.8	euca
Lamiaceae	Lamiaceae	0.0	9.1	lami
Malvaceae	Malvaceae	0.0	3.1	malv
Myrtaceae	Myrtaceae	0.0	11.7	myrt
Parapiptadenia excelsa	Mimosaceae	0.0	3.8	para
Rapistrum rugosum	Brassicaceae	0.0	9.0	rapi
Rosaceae	Rosaceae	0.0	3.2	rosa
Salix	Salicaceae	12.2	63.6	salx
Schinus sp.	Anacardiaceae	0.0	6.4	schi
Solanaceae	Solanaceae	0.0	13.5	sola
Celtis +	Celtidaceae			
Fraxinus +	Oleaceae			
Junglans +	Juglandaceae			
Urticaceae-Moraceae +	Urticaceae-Moraceae			

Table 1. Pollen types in *Citrus limon* monofloral honey from Tucumán. Original, made for this paper.
+ Pollen of plants with anemophilous pollination or without nectar.

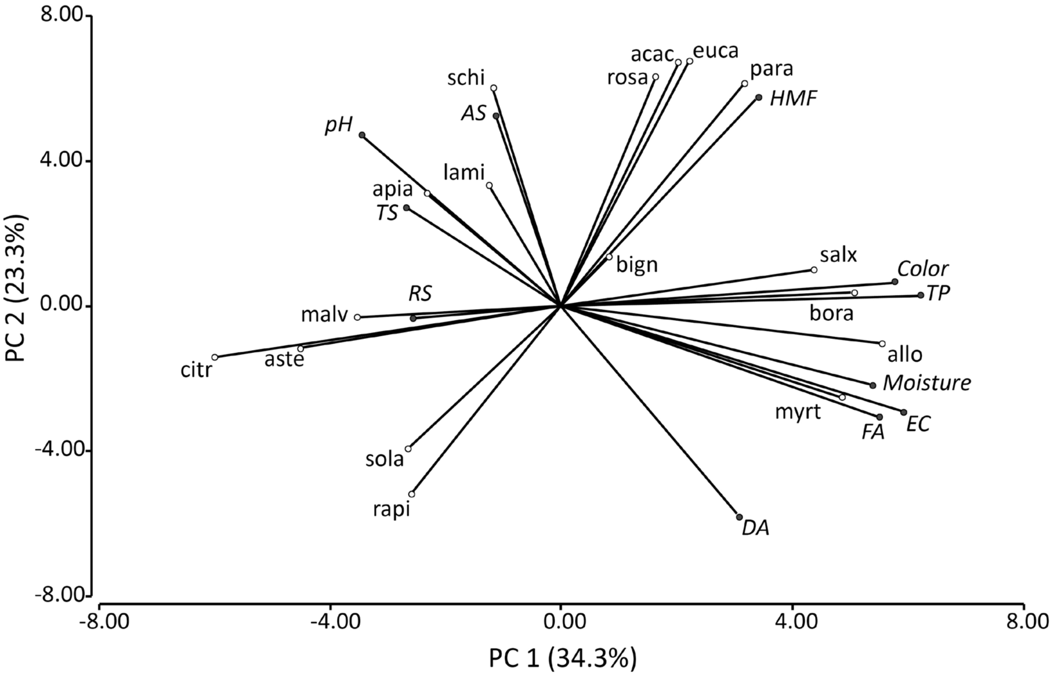

Figure 2. PCA. Relationship between physicochemical parameters and pollen types. Original, made for this paper.

1M sulfuric acid in a 25 mL graduated flask, 500 µL of Carrez I solution and 500 µL of Carrez II were added and completed at volume with 1M sulfuric acid. The mixture was passed through a Nº 4 Whatman paper and then through a sep-pack solid phase extraction cartridge (Waters) previously activated with 1 mL of 0.5% sulfuric acid and 1 mL of methanol. Later, it was eluted with 1 mL of HPLC grade methanol and filtered through 0.45 µm pore nylon membrane.

The HPLC analysis was performed by using a Waters 1525 Binary Pumps system with a Waters 2998 photodiode array detector (PDA). A YMC - C18 column (250 mm x 4.6mm, i.d. 5 µm) at 32°C, a binary gradient solvent system consisting of solvent A (1% acetic acid: acetonitrile) (97:3) and solvent B (acetonitrile:water) (1:1) (starting with 100% A (0-20 min), 100 to 30% (20-21 min), 30% (21-40 min), 30 to 0% (40-41 min), 0% (41-46 min), 0 to 100% (46-48 min), 100% (48-62 min) and a flow rate of 1.0 mL/min were used for separation. PDA acquisitions were performed from 200 to 500 nm, and the chromatograms were integrated at 290 nm for hesperidin and 330 nm for MA. Identification of individual compounds was made by comparison of retention times and UV spectral data with commercial standards. Calibration curves were made using different concentrations of the standard for quantification.

Statistical analysis

The results present the average of the determinations. ANOVA and then the Tukey test were used for the comparison of means. A value of P<0.05 was considered significant. Principal component analysis (PCA) was carried out in an exploratory mode to establish the relationship between physicochemical parameters and pollen types. The Pearson correlation coefficient was calculated. A value of P<0.05 was considered significant and P<0.01, very significant. The pollen types corresponding to anemophilous species were not considered for the analysis because they do not correspond to nectariferous resources (Louveaux *et al*., 1978). The Infostat software version 2018 (Di Rienzo *et al*., 2018) was used.

RESULTS AND DISCUSSION

Melissopalynological analysis

The pollen content of *Citrus limon* in all samples was higher than 10% and honeys were considered monoflorals. The mean content ± standard deviation was 31.1 ± 17.3%, widely varying between 11.1 and 56.3%. At least, between 7 and 18 nectariferous pollen types were identified, but only those whose percentage was higher than or equal to 3% were quantified, since they represented between 87 and 100% of the total pollen present (table 1). For the same type of honey, between 14 and 19 pollen types for sample were reported (Gurini *et al*., 2009), while in *Citrus* honey from Italy and Spain were found 124 and 188 types of pollen respectively (Corvucci *et al*., 2015).

Other pollen species frequently present were *Salix* type (Family: Salicaceae) in 100% of the samples, Asteraceae in 73% and Solanaceae, *Rapistrum rugosum* in 55%. Also, *Celtis, Fraxinus, Juglans* and Urticaceae-Moraceae were identified but they correspond to plants of anemophilous pollination and only provide information about the apiary environment. Previously, Gurini *et al*. (2009) reported the presence of *Cercidium* and *Heliotropium*, but without specifying collection sites.

	Year			
Parameter	**2016**	**2017**	**2018**	**Mean**
Moisture	15.7±0.8[a]	16.1±0.5[a]	17.2±0.6[b]	16.6±0.9
[g/100 g]	(15.0-16.9)	(15.5-16.8)	(16.2-18.2)	(15.0-18.2)
Color	19±9[a]	20±7[a]	20±8[a]	20±8
[mmPfund]	(7-28)	(11-28)	(5-29)	(5-29)
pH	3.96±0.10[a]	3.97±0.03[a]	3.86±0.09[a]	3.91±0.10
	(3.82-4.07)	(3.94-4.04)	(3.76-4.10)	(3.76-4.10)
FA	14,0±2,8[a]	13.8±2.0[a]	15.3±2.2[a]	14.6±2.3
[meq.kg^{-1}]	(10.4-16.9)	(11.0-16.0)	(11.7-19.3)	(10.4-19.3)
HMF	1,3±0,3[a]	1.4±0.4[a]	0.9±0.4[a]	1.1±0.5
[mg.kg^{-1}]	(0.9-1.4)	(1.1-2.1)	(0.3-1.6)	(0.3-2.1)
EC	0.21±0.06[a]	0.20±0.01[a]	0.24±0.05[a]	0.22±0.05
[mS.cm^{-1}]	(0.14-0.28)	(0.19-0.22)	(0.17-0.32)	(0.14-0.32)
TP	0.24±0.04[a]	0.19±0.01[ab]	0.22±0.03[b]	0.22±0.04
[mg GAE.g^{-1}]	(0.18-0.29)	(0.17-0.21)	(0.13-0.27)	(0.13-0.29)
DA	16.1±2.4[a]	16.5±2.3[a]	15.5±5.8[a]	15.9±4.5
[Gothe units]	(12.8-18.1)	(13.0-19.4)	(9.6-26.9)	(9.6-26.9)
RS	75.79±1.54[a]	76.49±1.70[a]	76.16±1.04[a]	76.20±1.30
[g/100 g]	(74.50-78.03)	(73.62-78.95)	(74.47-77.87)	(73.62-78.95)
TS	79.19±1.09[a]	78.91±1.99[a]	80.46±1.63[a]	79.79±1.77
[g/100 g]	(78.20-80.38)	(74.91-81.08)	(77.47-84.00)	(74.91-84.00)
AS	3.23±0.85[a]	2.30±0.92[ab]	4.08±1.07[b]	3.42±1.24
[g/100 g]	(2.23-4.24)	(1.23-3.90)	(2.49-6.19)	(1.23-6.19)

Table 2. Physicochemical parameters of *Citrus limon* monofloral honey from Tucumán. Original, made for this paper.
Mean values ± standard deviation, *(minimum - maximum)*. Mean values with a common letter in the same row are not significantly different (Tukey test, P>0.05).

Physicochemical analysis

The results of the parameters determined in 3 consecutive harvests are indicated in table 2. In all cases they meet the requirements of the Argentine Food Code for honey. The color varied between 5 and 29 mm Pfund, equivalent to Water White and White respectively (USDA classification). The range of variation was higher than that reported for citrus honeys from Tucumán (Isla *et al*., 2011), possibly due to the greater number of samples analyzed in this study.

The values obtained for FA, less than 40 meq.kg^{-1}, show that the honeys analyzed were fresh and with no fermentative processes, as also suggested by the values of diastatic activity comparable to those reported for orange monofloral honeys from Spain (Bonvehí and Coll, 1995).

The low values of EC are in agreement with those reported by Isla *et al*. (2011) and those proposed for citrus honey from Italy (Gardini *et al*., 2013). This would indicate that the mineral content in *Citrus limon* monofloral honey is low, since the values of FA are also low. Reducing sugars and apparent sucrose contents were higher than those reported by Isla *et al*. (2011), possibly due to the greater freshness of the honeys analyzed in this work, a fact confirmed by the lower values of HMF obtained. Phenolic compounds, secondary metabolites of plants, are transported to honey through the nectar. The mean value of total phenols obtained was 0.22 mg GAE.g^{-1} of honey, consistent with those reported for honey from northwestern Argentina (Isla *et al*., 2011; Salomón *et al*., 2014).

Relationship between physicochemical parameters and pollen types

The influence of pollen types on the physicochemical parameters and on each other was determined by means of a

	Moisture	Color	pH	FA	HMF	EC	TP	DA	RS	TS
Color	0.638*									
pH	-0.611*									
FA	0.755+	0.620*	-0.843+							
EC	0.800+	0.776+	-0.734+	0.936+						
TP	0.707*	0.862+	-0.590+	0.875+		0.897+				
DA	0.603*			0.799+		0.788+				
TS									0.824+	
AS										0.606*
acac					0.673*					
allo	0.752+	0.670*	-0.751*	0.942+		0.905+	0.922+			
aste					-0.666*					
bora	0.749+	0.624*		0.715*		0.626*	0.775+	0.313*		
citr		-0.767+		-0.669*		-0.702*	-0.831+			
euca					0.800+					
myrt	0.702*	0.694*				0.704*				-0.749+
para					0.762+					
rapi					-0.712*				0.663*	
rosa					0.644*					
salx		0.615*								

Table 3. Pearson correlation coefficients. Original, made for this paper.
Correlation: (*) significant (P<0.05), (+) very significant (P<0.01).

PCA to reduce dimensionality. Hence, the problem of studying the interaction between 28 variables (11 physicochemical parameters and 17 pollen types) could be carried out through 9 principal components (PC) with which 99% of the total variability was explained. With the first two, 58% of the variability is explained and the parameters studied can be represented graphically, as shown in figure 2. The angle between the vectors can be interpreted as the correlation between them. Values lower or higher than 90 degrees indicate positive or negative relationships (direct or inverse). The correlation coefficients are presented in table 3, where only those that were significant and very significant are indicated. Tables 4 and 5 show the effects that pollen content variations would cause on the physicochemical parameters. A moisture increase would cause a greater dissociation of chemical species that increase FA and EC while those honeys of more intense color would present greater EC, TP and lower *Citrus limon* pollen content.

Profile of sugars

The typical chromatogram is shown in figure 3, while the average, minimum and maximum contents of each of the sugars studied are presented in table 6. Results show consistency with those of reducing sugars and apparent sucrose. The concentrations of both monosaccharides were higher than those reported for monofloral orange honeys from Spain, while sucrose and maltose were lower compared to honeys from Spain and Greece (Bonvehí and Coll, 1995; Lazaridou *et al*., 2004). Trehalose and melezitose were determined in small quantities, lower than the other sugars and in general higher than those reported for orange honeys from Spain and Greece (Bonvehí and Coll, 1995; Lazaridou *et al*., 2004).

Honey may be considered as a concentrated solution of sugars that can often be oversaturated in relation to its glucose content, which causes it to crystallize under certain conditions, causing marketing problems since it is separated into a solid and a liquid phase. In order to predict the behavior of honey in relation to crystallization, some indicators have been proposed. One of the most reliable is based on the Glucose/Moisture relationship. If the value obtained is lower than 1.70, honey will not crystallize, but it will quickly do so if it is higher than 2.16 (Bhandari *et al*., 1999). In *Citrus limon* monofloral honey from Tucumán, a value of 1.95 was obtained, which would indicate an intermediate tendency towards crystallization. Gurini *et al*. (2009) classify them as "incipient" crystallization honeys.

Increase in parameter	Effect
Moisture	Greater free acidity and electrical conductivity.
Color	Greater electrical conductivity and total phenols.
pH	Lower free acidity, electrical conductivity and total phenols.
Free acidity	Lower pH. Higher electrical conductivity, total phenols and diastatic activity.
Electrical conductivity	Greater color (darker), free acidity, diastatic activity and total phenols.
Total phenols	Greater color (darker), free acidity and electrical conductivity.

Table 4. Relations between the physicochemical parameters of *Citrus limon* monofloral honey from Tucumán.

Increase in% pollen	Effect on physicochemical parameters
Citrus limon	Lower color (lighter), free acidity, electrical conductivity and total phenols.
Salix type	Greater color (darker).
Allophylus edulis	Greater moisture, free acidity, electrical conductivity, total phenols.
Boraginaceae	Greater moisture and total phenols.
Eucalyptus *Parapiptadenia excelsa*	Greater content of HMF.
Myrtaceae	Greater color (darker), moisture and electrical conductivity. Lower content of total sugars.
Rapistrum rugosum	Lower content of HMF. Greater amount of reducing sugars.
Apiaceae, Bignoniaceae, Lamiaceae, *Schinus* sp., Solanaceae.	No significant effects.

Table 5. Influence of pollen types on the physicochemical parameters of *Citrus limon* monofloral honey from Tucumán.

Sugar	Mean±SD	Minimum	Maximum
	(g/100 g)		
Fructose	37.4±2.6	34.6	40.8
Glucose	33.1±4.7	23.7	38.2
Sucrose	4.0±0.8	2.9	4.8
Maltose monohydrate	1.9±1.0	0.3	3.0
Trehalose dihydrate	0.2±0.1	0.1	0.4
Melezitose trihydrate	0.8±0.3	0.4	1.2

Table 6. Content of sugars in *Citrus limon* monofloral honey from Tucumán. Original, made for this paper.
SD: standard deviation.

Chemical markers of botanical origin

Methyl anthranilate is an important component of the aroma of citrus honeys. European buyers have commercially established the minimum value at 2.0 mg.kg^{-1}, but sometimes this value is not achieved by genuine monofloral citrus honeys (Gardini *et al*., 2013; Juan-Borrás *et al*., 2015) and thus, MA could be used as an additional descriptive element (Juan-Borrás *et al*., 2015; Sesta *et al*., 2008).

Hesperetin has also been proposed as a chemical marker since it is not volatile and is found in the nectar of citrus blossoms, but in the form of its hesperidin glycoside (Ferreres *et al*., 1994). In previous works, hesperidin and hesperetin have been reported in *Citrus limon* honeys from Tucumán in a ratio (hesperidin:hesperetin) between 2:1 and 26:1 (Isla *et al*., 2011), and that makes hesperidin better for quantification.

Figure 4 shows typical chromatograms of honey and commercial standards. For MA a variation range of 0.10 to 0.32 mg.kg^{-1} was obtained with an average value of 0.18 ± 0.08 mg.kg^{-1} (mean ± standard deviation) (LOD: 0.07 mg.kg^{-1}). MA was not detected in two samples, a fact also reported for citrus honeys from Morocco and Spain (Karabagias *et al*., 2017). These results indicate that the *Citrus limon* monofloral honeys from Tucumán have a low and sometimes undetectable content of methyl anthranilate.

Hesperidin variation and mean value ± standard deviation were 0.20 to 4.42 mg.kg^{-1} and 2.40 ± 1.41 mg.kg^{-1} respectively (LOD: 0.2 mg.kg^{-1}). They are lower than those reported for the same type of honeys from Tucumán (Isla *et al*., 2011; Salomón *et al*., 2011), and can be used to establish the minimum values.

CONCLUSIONS

In *Citrus limon* monofloral honeys from Tucumán, the typical nectariferous pollen species present were *Citrus limon*, *Salix* type, Asteraceae, Solanaceae and *Rapistrum rugosum*. The *Salix* type was present in all samples, then could be used as a geographic origin marker.

Physicochemical parameters indicated that this honey is very light in color (≤ 30 mm fund), slightly acidic (≤ 20 meq. kg^{-1}), with moisture values from 15 to 19 g/100g, low diastatic activity (≤ 27 Gothe units), HMF (≤ 3.0 mg.kg^{-1}) and electrical conductivity (≤ 0.32 mS.cm^{-1}). Color is strongly and directly correlated with EC and TP content and inversely with *Citrus limon* pollen amount. The increase of *Salix* type pollen would result in darker honeys and a higher content of TP.

The sugar profile indicated that the fructose content was higher than glucose, sucrose and maltose, with an intermediate tendency towards crystallization.

Hesperidin was easily detected and quantified in all samples and could thus be used as an indicator of the authenticity (≥ 0.2 mg.kg^{-1}). Since MA was not detected in some samples and the maximum content was lower than 2.0 mg.kg^{-1}, should be considered as a complementary indicator (≥ 0.1 mg.kg^{-1}). These results provide fundamental information to

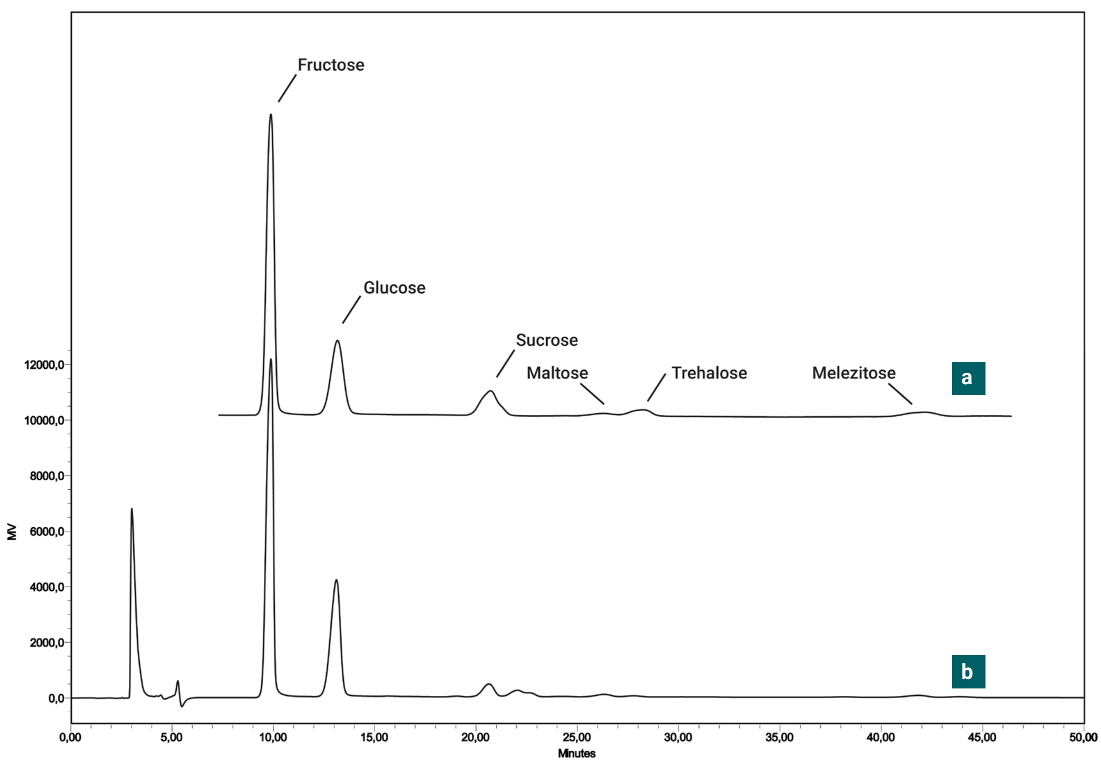

Figure 3. Sugar profile of *Citrus limon* monofloral honey from Tucumán. Chromatograms (a) mixture of sugar standards (b) honey. Original, made for this paper.

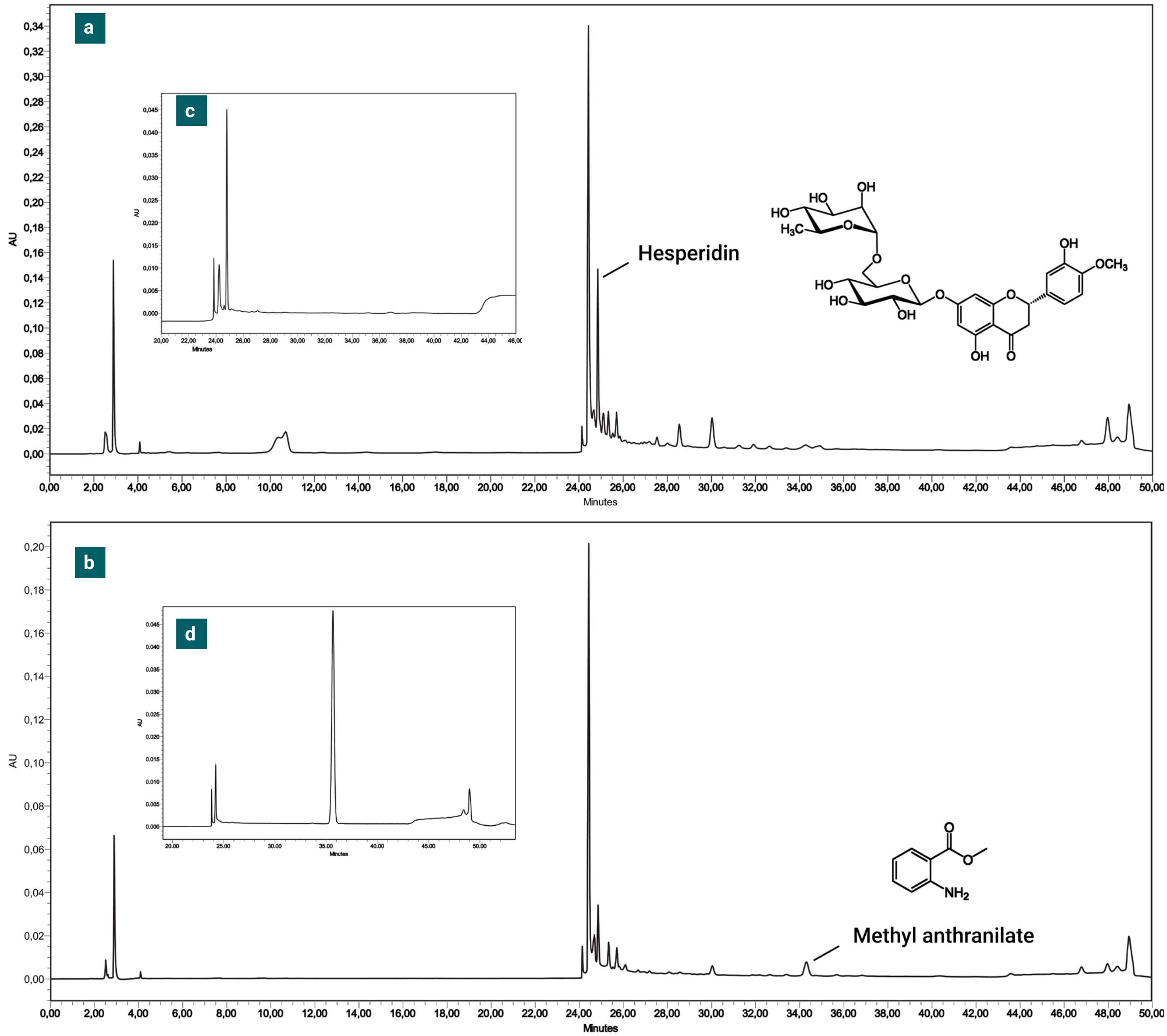

Figure 4. Chromatograms of *Citrus limon* monofloral honey from Tucumán. (a) extracted at 290 nm, indicating hesperidin peak, b) extracted at 330 nm, indicating methyl anthranilate peak, c) standard of hesperidin, d) standard of methyl anthranilate. Original, made for this paper.

establish reference values that allow *Citrus limon* monofloral honeys produced in Tucumán to use the GI seal "*Lemon blossom honey from Tucumán*".

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Conflicts of Interest

The authors declare no conflict of interest.

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