

Colour, antioxidant capacity, phenolic and flavonoid content of honey from the Humid Chaco Region, Argentina

Color, capacidad antioxidante, contenido de fenoles y flavonoides en mieles de la Región del Chaco Húmedo, Argentina

Cabrera M¹, M Perez², L Gallez^{3,4}, A Andrada³, G Balbarrey³

Abstract. Our objective was to correlate colour with total phenolic compounds, total flavonoids and antioxidant capacity of honey from the Humid Chaco region. With this purpose, 19 representative samples were selected from the 2009-2012 year period. Pollen analysis showed a predominance of native species of different landscapes such as forests and wetlands. Of the total number of samples, eleven were mixed or multifloral and eight samples were dominated by different native nectariferous woody species. The colour of honey, according to the Pfund scale, ranged from extra light amber to dark amber. Total phenolic content ranged from 40.18 to 118.82 mg GAE/100 g honey. Flavonoid content varied between 6.94 and 67.76 mg QE/100 g honey, and antioxidant capacity, evaluated by the DPPH radical method, between 17.36% and 56.53%. Extra light and light amber honeys had lower levels of total phenolic compounds, total flavonoids, and antioxidant capacity than amber and dark honeys. The correlation observed between colour and flavonoids ($r=0.78$) was higher than that between colour and total phenols ($r=0.53$). A direct relationship between phenolic content and antioxidant capacity ($r=0.91$) was found. The studied honeys, as compared to honeys from other regions, were rich in flavonoids and show a noticeable antioxidant activity. It is important to take into account these features for human health.

Keywords: Honey; Colour; Phenolic content; Flavonoids; Antioxidant capacity; Humid Chaco region; Native flora.

Resumen. Nuestro objetivo fue correlacionar el color con los componentes fenólicos totales, flavonoides totales y capacidad antioxidante de mieles de la región del Chaco Húmedo. Con este propósito, se seleccionaron 19 muestras representativas durante el período 2009-2012. El análisis del polen mostró una predominancia de especies nativas provenientes de diferentes paisajes como bosques y humedales. Del número total de muestras, once fueron mixtas o multiflorales y ocho muestras estuvieron dominadas por diferentes especies de leñosas nativas nectaríferas. El color de la miel, de acuerdo a la escala Pfund, varió desde ámbar extra claro a ámbar oscuro. El contenido fenólico total varió desde 40,18 a 118,82 mg GAE/100 g de miel. El contenido de flavonoides varió entre 6,94 y 67,76 mg QE/100 g de miel, y la capacidad antioxidante, evaluada por el método del radical DPPH, entre 17,36% y 56,53%. Las mieles color ámbar extra claro y ámbar claro tuvieron menores niveles de compuestos fenólicos totales, flavonoides totales, y capacidad antioxidante que las mieles color ámbar y oscuras. La correlación observada entre color y flavonoides ($r=0,78$) fue más alta que aquella entre color y fenoles totales ($r=0,53$). Se observó una relación directa entre el contenido de fenoles y la capacidad antioxidante ($r=0,91$). Las mieles estudiadas, comparadas con las mieles de otras regiones, fueron ricas en flavonoides y mostraron una notable capacidad antioxidante. Es importante tener en cuenta estas características para la salud humana.

Palabras clave: Miel; Color; Contenido fenólico; Flavonoides; Capacidad antioxidante; Región del Chaco Húmedo; Flora nativa.

¹Facultad de Humanidades. Universidad Nacional de Formosa. Formosa, Argentina.

²INQUISUR, Dpto. de Química. Universidad Nacional del Sur (UNS)-Conicet, Bahía Blanca, Argentina.

³Dpto. Agronomía, Universidad Nacional del Sur (UNS), Bahía Blanca - Argentina.

⁴CIC (Comisión de Investigaciones Científicas, Provincia de Buenos Aires, Argentina)

Address correspondence to: Mg. Liliana Gallez, Fax, +54-291-4595127, Phone, +54-291-4595102, e-mail: lgallez@uns.edu.ar

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INTRODUCTION

Honey produced by *Apis mellifera* L. bees exhibits a characteristic colour, aroma and texture according to the plant nectar from which it derives and to the specific environmental conditions. Colour is a sensory attribute of particular importance in the typification of honey and together with consistency influences consumer preference (Aubert & Gonnet, 1983). Honey colour is the first trait taken into account when grading honey for commercial purposes, and therefore it reflects on the price of bulk honey (Gallez, 2007). Raw honey colour depends on the botanical and geographical origin of the product (Terrab et al., 2003a,b). Some authors reported a relationship between honey colour and mineral composition (Balbarrey et al., 2012; González Miret et al., 2005).

From the chemical point of view honey, is a concentrated solution of reducing sugars and lesser amounts of other carbohydrates, proteins, amino acids, organic acids, enzymes, vitamins, and minerals. It has also been found to contain pigments such as carotenoids, volatile components, and polyphenols (White & Doner, 1980; Crane, 1990). Phenolic compounds are directly involved in the colour of honey, mainly through flavonoids (Amiot et al., 1989). Plant nectar is a source of phenolic compounds. The type and concentration of phenolic compounds are the main determinants of the bioactive properties of honey (Märghitaş et al., 2009). These compounds are considered to reduce the risk of oxidative damage in cells and numerous studies show their role as antioxidants (Aljadi & Kamaruddin, 2004; Baltrusaitytė et al., 2007; Bertoneclicj et al., 2007). Therefore, honey phenolic compounds confer to this product possible protective effects against various diseases such as heart disease, cancer, atherosclerosis, infection, and inflammation (Martinez-Florez et al., 2002; Jaganathan & Mandal, 2009).

There are very few studies characterizing the honey from the Humid Chaco region in terms of its physico-chemical attributes (Ciappini et al., 2009; Cabrera et al., 2011; Salgado & Maidana, 2014). This region shows a great diversity of native species of melliferous interest and a low volume of honey production (Cabrera et al., 2013).

The aim of the present study was to establish the relationships among colour, antioxidant capacity, and phenolic and flavonoid content for contributing to the characterization of honeys from the native flora of the Humid Chaco region.

MATERIALS AND METHODS

Honey samples. The study area is located between 26° 30' and 25° 05' S, and 57° 39' and 60° W along the Tropic of Capricorn, i.e., in the subtropical region of northern Argentina (Fig. 1). It belongs to the Humid Chaco region (Morello et al., 2012). Nineteen centrifuged honey samples were obtained from the apiaries dispersed throughout the districts of Formosa ($n=5$), Laishí ($n=1$), Pilcomayo ($n=3$), Pilagás ($n=2$), Pi-

rané ($n=4$), and Patiño ($n=4$). The samples were collected during the 2009-2012 spring-summer beekeeping seasons. All honey collections were refrigerated at 5°C (± 2 °C) in airtight plastic containers until further analysis.

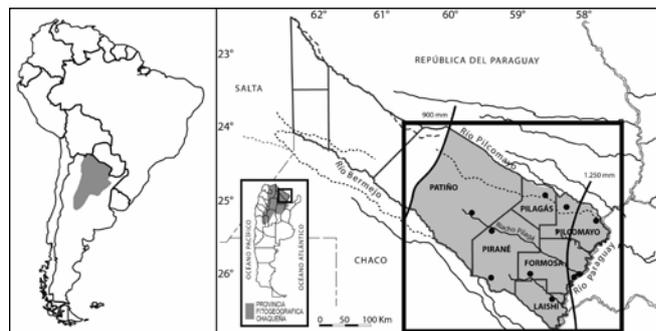


Fig. 1. Geographical location of the sampling area. The dots on the map show the apiaries from where the honey samples were taken.

Fig. 1. Localización geográfica del área de muestreo. El mapa muestra los apiarios donde se obtuvieron las muestras de miel.

Pollen. The qualitative palynological analysis was performed according to Louveaux et al., (1978) considering the following pollen frequency classes: dominant pollen (>45%), secondary pollen (16-45%), important minor pollen (3-15%) and pollen trace (<3%). The morphological types were identified by consulting a pollen database of the regional flora and specialized literature on the subject.

Physico-chemical analysis

Colour. The honey samples were liquefied and centrifuged for 5 min at 3000 rpm to eliminate bubbles. Six measurements for each sample were performed on a Pfund colour grader (Koehler Instrument Company Inc. New York) and a corrected average obtained. The honey colour was expressed in mm Pfund and named in accordance with the standard nomenclature.

Determination of total phenolic content. Total phenolic content was determined according to the method described by Marghitaş et al. (2009). Each honey sample was dissolved in water to obtain a 10% solution. Five hundred microlitres of the aqueous solution were mixed with 2.5 mL of Folin-Ciocalteu reagent (0.2 N), and after 5 minutes two ml of a solution of Na₂CO₃ (75 g/L) were added. All samples were kept at room temperature for 2 h, and their absorbance was measured at 760 nm in a Metrolab RC 325 spectrophotometer. The amount of total phenolic compounds was determined by a calibration curve, using dilutions of a stock solution of gallic acid (0.25 mg/mL) in methanol (70%). The linearity obtained was R²=0.993. The results were expressed in mg gallic acid equivalents (GAE)/100 g honey, as the mean of three replications.

Determination of total flavonoid content. The total flavonoid content of honey samples was determined by the method described by Marghitaş et al. (2009). One millilitre of a honey aqueous solution (0.6 mg/mL) was mixed with 0.3 mL of

NaNO₂ solution (5%), and after 5 minutes 0.3 mL of AlCl₃ solution (10%) were added. After 6 min, the samples were neutralized with 2 mL of NaOH (1 M) and the absorbance was read at 510 nm in a Metrolab RC 325 spectrophotometer. Different concentrations of quercetin in ethanol (80%) were used for the calibration curve. The linearity obtained was R²=0.9981. The results were expressed in mg quercetin equivalents (QE)/100 g honey, as the mean of three replications.

Antioxidant capacity. The scavenging activity of honey for the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH radical) was used to evaluate the antioxidant capacity of samples, according to the method of Meda et al. (2005) with some modifications. DPPH solutions have an intense violet colour and show a strong absorption band at 517 nm. The method is based on a reduction of colour solution in presence of antioxidant compounds; thus, the less the absorbance at 517 nm, the greater the antioxidant capacity of the sample. The honey samples were diluted with distilled water (25 mg/mL), and 0.75 mL of honey solution was mixed with 1.5 mL of DPPH methanolic solution (0.02 mg/mL). In parallel, a control solution with 0.75 mL of methanol and 1.5 mL of DPPH was prepared. To eliminate the interference of colour of the honey solution, a blank with 0.75 mL of the honey solution and 1.5 mL of methanol was measured. The mixtures were shaken vigorously and kept in the dark for 15 minutes at room temperature. Their absorbances were measured at 517 nm in a Metrolab RC 325 spectrophotometer. The antioxidant capacity of honey samples was calculated as:

$$\text{Antioxidant capacity (\%)} = \frac{[A_{\text{Control}} - (A_{\text{Sample}} - A_{\text{Blanc sample}})]}{A_{\text{Control}}} \times 100$$

where A_{Control} is the absorbance of the control (containing all reagents except the honey solution), A_{Sample} is the absorbance of the honey solution with DPPH, and $A_{\text{Blanc sample}}$ is the absorbance of the honey solution with methanol.

Statistical analysis. The obtained data were subjected to an analysis of variance (ANOVA), measurement comparison, and regression analysis. The comparison between two sample groups was performed using the Student's t-test. Multivariate techniques such as the Principal Component Analysis (PCA) using the 2014 version of the InfoStat software were also carried out in order to summarize the information. Bivariate linear analysis was carried out and correlation coefficients (r) are shown.

RESULTS

Pollen analysis. Of the total number of samples, eleven were mixed or multifloral and eight samples were dominated by different native nectariferous woody species: *Schinopsis balansae* Engl. (Anacardiaceae), *Astronium balansae* Engl. (Anacardiaceae), *Copernicia alba* Morong. (Arecaceae), *Geoffroea decorticans* (Gillies ex Hook. & Arn.) Burkart (Fabaceae), *Eu-*

genia uniflora L. (Myrtaceae), *Ziziphus mistol* Griseb. (Rhamnaceae), and *Bulnesia sarmientoi* Lorentz ex Griseb. (Zygophyllaceae) (Table 1). The pollinic description presented in the table refers only to the main native and naturalized species identified in each honey sample.

Colour. The average colour of the honey samples was 93.32 ± 30.62 mm Pfund and ranged from extra light amber to dark amber. The lightest sample scored 40.67 mm Pfund and the darkest one was the only one that surpassed 140 mm (Table 1). In the particular case of the darkest honey, in order to get a score to correlate with the other parameters, the scale was extended and the value obtained was 150.50 mm (sample 983). Six samples were identified as light (extra light amber-ELA and light amber-LA), seven as amber (A), and six as dark (D).

Total phenolic content, total flavonoid content and antioxidant capacity. Phenolic compounds or polyphenols constitute one of the most numerous and widely distributed groups of substances in the plant kingdom. Notably, flavonoids and simple phenolic derivatives were the most common polyphenols.

Table 2 shows the results of total phenols, antioxidant capacity and total flavonoids of the analysed honey. Total phenolic content, total flavonoids content, and antioxidant capacity of the honey samples from the Humid Chaco region, grouped by colour are shown in Figure 2. The first group involved ELA and LA ranges. This group showed 40.18 and 47.31 mg GAE/100g honey as minimum and maximum values for phenolic compounds respectively, whereas flavonoids ranged from 6.94 to 14.39 mg QE/100g and antioxidant capacity from 17.36 to 27.09%. Within the group named A, the range of phenolic compounds was 46.35-118.82 mg GAE/100g, that of flavonoids 9.38-37.47 mg QE/100g and that of antioxidant capacity 24.76-60.95%. The third group, named D showed the following ranges: phenolic compounds 52.06-79.06 mg GAE/100g, flavonoids 15.95-67.76 mg QE/100g and antioxidant capacity 23.43-56.53%. In all three variables, values were significantly higher ($t \leq 0.05$) for the honeys above 85 mm Pfund (group ELA-LA) in comparison to honeys below 85 mm Pfund (groups A and D).

Correlations. Plots of the first two components clearly indicated that darker honeys had higher flavonoid content than lighter honeys, and that antioxidant capacity was strictly related to total phenolic content (Fig. 3). Bivariate linear analysis showed a high association between colour and flavonoid content ($r=0.78$) and between antioxidant capacity and phenolic content ($r=0.91$). Colour was less correlated with total phenols ($r=0.53$) and with antioxidant capacity ($r=0.51$) than with flavonoids. The correlation coefficients between flavonoid content and antioxidant capacity ($r=0.45$; $P<0.01$) and that between flavonoid and total phenolic ($r=0.48$; $P<0.01$) were lower than those mentioned above. The darkest sample, atypically high in flavonoid content, reduced both coefficients.

Table 1. Colour and pollen types of honey samples from the Humid Chaco region. Percentage of pollen type present in the sample is shown in brackets.

Tabla 1. Color y tipos polínicos de las muestras de miel de la Región del Chaco Húmedo. El porcentaje de tipos polínicos presentes en las muestras está señalado entre paréntesis.

Sample	Colour range	Colour (mm Pfund)	Frequency Polinic
			DP (>45%), SP (16-45%), MP (3-15%) and TP (<3%)
923	Extra Light Amber	40.67	<i>Eugenia uniflora</i> (38), <i>Clematis montevidensis</i> (14), <i>Parkinsonia aculeata</i> (13)
853	Extra Light Amber	47.36	<i>Ziziphus mistol</i> (47), <i>Prosopis</i> sp. (24), <i>Vicia macrograminea</i> (2), <i>Cappari cordis tweediana</i> (2)
920	Light Amber	54.07	<i>Senecio grisebachii</i> (24), <i>Prosopis</i> sp. (28), <i>*Echium plantagineum</i> (8)
861	Light Amber	59.53	<i>Prosopis</i> sp. (24), <i>Vicia macrograminea</i> (8), <i>Eryngium elegans</i> (4)
922	Light Amber	60.20	<i>Eugenia uniflora</i> (45), <i>Prosopis</i> sp. (11), <i>Cappari cordis tweediana</i> (10), <i>Clematis montevidensis</i> (9)
873	Light Amber	77.10	<i>Ziziphus mistol</i> (32), <i>Prosopis</i> sp. (13), <i>Acacia aroma</i> (7)
958	Amber	85.06	<i>Geoffroea decorticans</i> (52), <i>Acicarpa tribuloides</i> (19), <i>Cecropia pachystachya</i> (3), <i>Heimia salicifolia</i> (1)
940	Amber	88.46	<i>Schinopsis balansae</i> (68), <i>Ziziphus mistol</i> (6), <i>Prosopis</i> sp. (4), <i>Scoparia montevidensis</i> (3)
910	Amber	92.46	<i>Schinopsis balansae</i> (49), <i>Copernicia alba</i> (23), <i>Solidago chilensis</i> (5), Tipo <i>Baccharis-Eupatorium</i> (5)
933	Amber	97.13	<i>Prosopis</i> sp. (35), <i>Anisocapparis speciosa</i> (8), <i>Maytenus vitis-idaea</i> (4)
966	Amber	97.60	<i>Copernicia alba</i> (64), <i>Astronium balansae</i> (7), <i>Sapium baematospermum</i> (3), <i>*Melilotus</i> sp. (5)
975	Amber	104.10	<i>Astronium balansae</i> (52), <i>Caesalpinia paraguariensis</i> (12), <i>Salix humboldtiana</i> (8), <i>Pisonia zapallo</i> (4)
973	Amber	110.00	<i>Copernicia alba</i> (36), <i>Tessaria integrifolia</i> (23), <i>Agalinis communis</i> (4)
954	Dark	115.63	<i>Astronium balansae</i> (29), <i>Mimosa</i> sp. (9), Tipo <i>Baccharis-Eupatorium</i> (1), <i>Celtis</i> sp. (3)
987	Dark	119.47	<i>Eugenia uniflora</i> (46), <i>Copernicia alba</i> (22), <i>Schinopsis balansae</i> (6), <i>Sagittaria montevidensis</i> (1)
990	Dark	120.13	<i>Bulnesia sarmientoi</i> (59), <i>Tessaria integrifolia</i> (6), <i>Opuntia</i> sp. (9), <i>Pisonia zapallo</i> (1)
967	Dark	123.96	<i>Copernicia alba</i> (75), <i>Sagittaria montevidensis</i> (3), <i>*Cirsium vulgare</i> (2), <i>Agalinis communis</i> (4)
974	Dark	129.66	<i>Schinopsis balansae</i> (46), Tipo <i>Baccharis-Eupatorium</i> (35), <i>*Helianthus</i> sp. (1), <i>Salix humboldtiana</i> (1)
983	Dark	>140	<i>Bulnesia sarmientoi</i> (29), <i>*Helianthus</i> sp. (16), <i>Senecio grisebachii</i> (5)

Note: DP: dominant pollen; SP: secondary pollen; MP: important minor pollen; TP: trace pollen.* Introduced species.

Nota: PD, polen dominante; PS, polen secundario; PM, polen de menor importancia; PT, polen en traza.* Especies introducidas.

Table 2. Mean, Range and Standard Deviation (SD) for the Total phenolics, Antioxidant capacity and Total flavonoids.

Tabla 2. Medias, rango, desvío estandar (DS) de Fenoles totales, Capacidad antioxidante y Flavonoides totales.

Sample	Colour	Total phenolics	Antioxidant capacity	Flavonoids
	(mm Pfund)	(mg eq AG/100 g of honey)	DPPH(0.025)	(mg QE/100 g of honey)
923	40.67	45.44	17.36	9.11
853	47.37	45.31	20.52	7.77
920	54.07	45.62	27.09	14.39
861	59.53	40.97	17.48	9.80
922	60.20	47.31	27.04	12.69
873	77.10	40.18	18.86	6.94
958	85.07	47.28	24.76	9.38
940	88.47	95.93	56.25	19.16
910	92.47	76.39	42.11	17.18
933	97.13	118.82	60.95	24.39
966	97.60	46.35	25.34	18.61
975	104.10	66.84	29.33	13.53
973	110.00	73.00	47.35	37.47
954	115.63	67.70	36.10	15.95
987	119.47	69.92	43.36	34.79
990	120.13	52.06	23.43	24.17
967	123.97	75.54	45.93	23.28
974	129.67	79.06	56.53	43.68
983	>140	77.64	30.17	67.76
Media	90.15	63.76	34.21	21.58
DS	28.10	21.08	14.09	15.23
Minimum	40.67	40.18	17.36	6.94
Maximun	>140	118.82	60.95	67.76

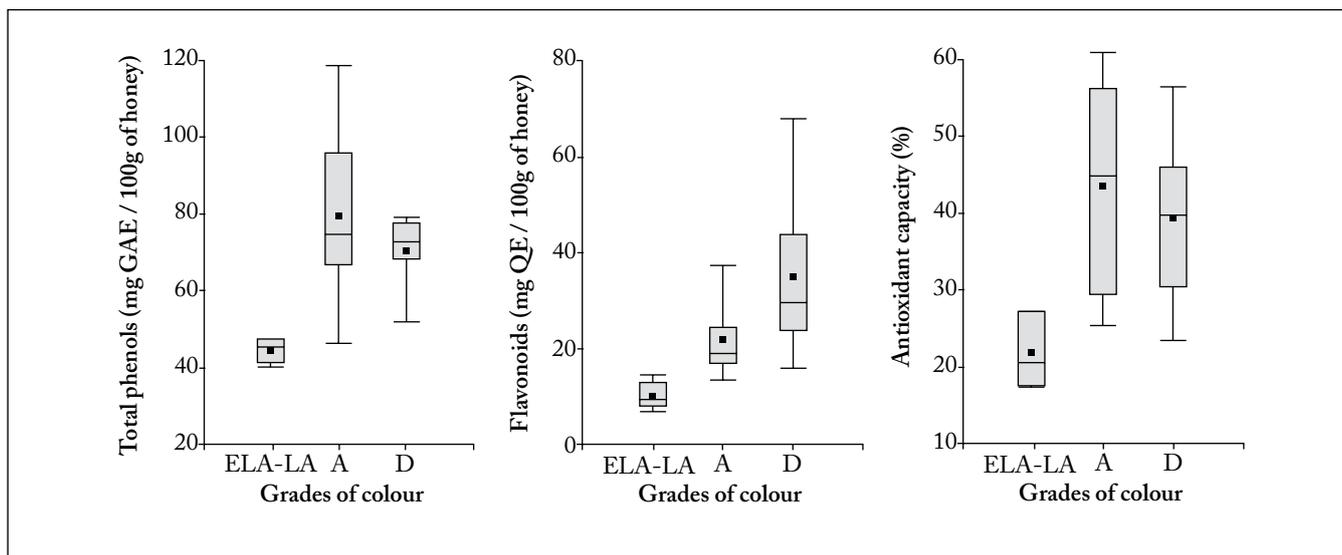


Fig. 2. Descriptive statistics of flavonoids, total phenols and antioxidant capacity within the three colour ranges. Colour grades: Extra Light Amber - Light Amber (ELA-LA) n= 6; Amber (A) n= 7; Dark (D) n= 6.

Fig. 2. Estadística descriptiva de flavonoides, fenoles totales y capacidad antioxidante en los tres rangos de color. Escalas de color: Ámbar extra claro-Ámbar claro (ELA-LA) n= 6; Ámbar (A) n= 7; Oscuro (D) n= 6.

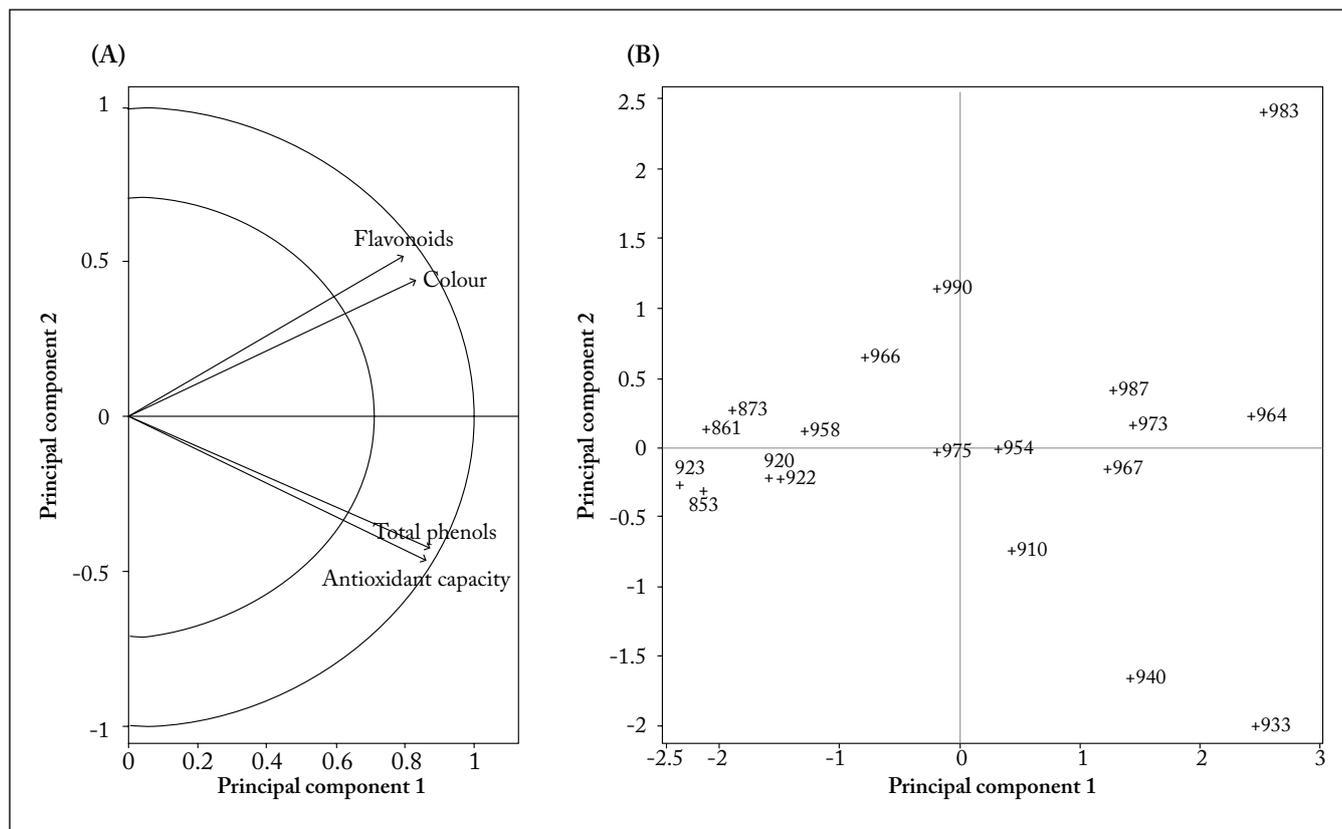


Fig. 3. Principal components analysis of the studied variables. . Reconstruction percentages were 70.88 % and 21.54 % for the first and second components, respectively. (A) G-plot; vectors between concentric circles have more than 70% representation. (B) H-plot.

Fig. 3. Análisis de componentes principales de las variables estudiadas. Los porcentajes de reconstrucción fueron 21.54% y 70.88% para el primer y segundo componente, respectivamente. (A) G-plot; vectores entre círculos concéntricos tienen más del 70% de representación. (B) H-plot.

DISCUSSION

Honeys from the Humid Chaco region are characterized by the dominance of the native flora and a great diversity of pollen types (Cabrera et al., 2011). These results are in accordance with the melliferous flora reported by Cabrera et al. (2013) and by Salgado et al. (2014).

The wide colour range observed could be attributed to the great diversity of the nectariferous flora and to environmental conditions (Gallez, 2006). The colour of honey from the Humid Chaco region was also investigated by other authors. Maidana et al. (2010) and Salgado et al. (2014) found that most of the samples were ELA and LA. The average colour of our samples was higher than those above mentioned.

Although studies on honey composition started long ago (White & Doner, 1980; Crane, 1990; Baldi Coronel, 2010), the analysis of honey phenolic compounds has increased due to the recent interest in natural antioxidants.

The flavonoid concentration of our honeys differed from those reported by other authors (Iurlina et al., 2009; Isla et al., 2011; Ciappini et al., 2013), who found lower levels in dark honeys from other regions of Argentina. As compared with honeys from other world regions, our groups A and D showed considerably higher flavonoid concentrations (Vit et al., 2008; Perna et al., 2013; D'Oliveira Sant'Ana et al., 2014).

In this regard, phenolic content of our samples was within the same range than those reported for northeastern Argentine honeys by Isla et al. (2011). Values of our honeys belonging to group A were similar to those obtained by Vit et al. (2008) and D'Oliveira Sant'Ana et al. (2014), and higher than those reported by Perna et al. (2013) and Ferreira et al. (2009).

Numerous techniques are available to evaluate the antioxidant activity of simple compounds and complex products such as honey (Alam et al., 2013). Samples from the Humid Chaco region, evaluated by the DPPH radical method, showed a noticeable antioxidant activity. Many authors also analysed honey samples from different regions by using this radical (Bertoncelj et al., 2007; Perna et al., 2013; Pontis et al., 2014). However, it is often difficult to compare results due to differences in the way they are expressed, and in the adopted experimental methods.

These findings agree with the analyses undertaken on honeys from other regions of Argentina and from other countries, where a strong positive correlation was found between colour and flavonoid content (Alvarez-Suarez et al., 2010; Isla et al., 2011; A-Rahaman et al., 2013; Ciappini et al., 2013; D'Oliveira Sant'Ana et al., 2014; Pontis et al., 2014). Other authors observed a high correlation between phenolic content and antioxidant capacity (Al-Mamary et al., 2002; Wilczyńska, 2010; Tornuk et al., 2013).

Several studies show that dark honeys have higher values of phenolic content and antioxidant capacity than lighter honeys (Bertoncelj et al., 2007; Ferreira et al., 2009; Alvarez-Suarez et al., 2010; Perna et al., 2013).

Honeys from the Humid Chaco region were characterized by their amber colour, ranging from extra light to dark, and showed a predominance of the typical native plant species of the forests and wetlands. The sample colour intensity was better related to the flavonoid content than to total phenolics. Therefore, the amber colour intensity observed in our honeys can be attributed mostly to the content of flavonoids. The studied honeys, as compared to honeys from other regions, were rich in flavonoids and showed a noticeable antioxidant activity; these are interesting features to take into account in human health.

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