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Physicochemical characterisation of honey produced in the Chaco province (Argentina)

Caracterización físico-química de las mieles producidas en la provincia del Chaco (Argentina)

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ABSTRACT

The aim of this study was to analyze the physico-chemical properties of honey produced in the province of Chaco, as a contribution to the characterization of these honeys. Color, moisture, electrical conductivity, total acidity and pH in a total of 189 honey samples were analyzed. Diastase content was determined in 64 samples and hydroxymethylfurfural activity was tested in 16 samples. Samples were extracted by centrifuging during three consecutive beekeeping season: 2005- 2006, 2006- 2007 and 2007-2008. The physicochemical parameters were found to be within acceptable ranges (color: 22-150 mm Pfund, moisture: 15.4-20%, total acidity: 8-68 meq kg^{-1}, pH: 3.2-6.4, EC: 0.189-1.347 mS cm^{-1}, diastase: 10-30% and HMF: 2.3 - 24.5). Physicochemical properties studied adjust to normal parameters and the obtained values meet all major international specifications for commerce. Positive significant relationships were found between color and conductivity, moisture and acidity, conductivity with acidity and pH, and moisture with acidity. Negative significant relationships were found between pH with moisture and acidity. ANOVA shows significant differences in color, electrical conductivity, moisture, and pH between honeys produced in agricultural areas where crops predominate and honeys produced in less disrupted areas where native forests prevail.

RESUMEN

El objetivo del presente estudio fue analizar las propiedades físico-químicas de las mieles producidas en la provincia del Chaco, como aporte a la caracterización de las mismas. Fueron analizados el color, humedad, conductividad eléctrica, acidez total y pH en 189 muestras de miel. La actividad diastásica se determinó en 64 muestras y HMF en 16 muestras. Las muestras se extrajeron por centrifugación durante tres temporadas apícolas: 2005-2006, 2006-2007 y 2007-2008. Los parámetros fisicoquímicos están dentro de los rangos aceptables (color: 22-150 mm Pfund, humedad: 15,4-20%, acidez total: 8-68 meq kg^{-1}, pH: 3,2-6,4, CE: 0,189- 1,347 $mScm^{1}$, actividad diastásica: 10-30 %, hidroximetilfulfural: 2,3- 24,5). Las propiedades fisicoquímicas estudiadas se ajustan a los parámetros normales y los valores obtenidos reúnen las especificaciones internacionales para su comercialización. Se observaron relaciones positivas significativas entre el color y la conductividad, la humedad y la acidez, conductividad, con acidez y pH, humedad y acidez. Relaciones negativas significativas fueron observadas entre pH con la humedad y la acidez. Con ANOVA se observaron diferencias significativas en el color, conductividad eléctrica, humedad y pH entre mieles producidas en áreas agrícolas y mieles producidas en las zonas menos perturbadas donde predominan los bosques nativos.

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Keywords

Apis mellifera • Argentina • Chaco province • honey • physicochemical analysis

Palabras clave

Apis mellifera • Argentina • provincia del Chaco • miel • análisis fisicoquímico

INTRODUCTION

Honey is a highly concentrated water solution consisting mainly of two kinds of simple sugars, dextrose and levulose, and little amounts of at least 22 other more complex sugars. These sugars are responsible for the honey's main physical characteristics and behavior; nevertheless many other substances found in honey (*e. g.*, flavonoid content, pigments, acids, and minerals) are responsible for differences between types of honey (35).

Honey is the natural sweet substance produced by honey bees from the nectar of plants or from secretions of living parts of plants or excretions of plant sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in the honey comb to ripen and mature (13).

Consequently, some honey physicochemical characteristics, such as color, moisture, flavor, taste, aroma, pH, electrical conductivity, mineral content, and acidity, are associated with the botanic origin of the collected substances (26). However, there are some other honey characteristics, such as hydroxymethylfurfural (HMF), cleaning, purity, etc., that are linked to the manufacturing process.

Argentina is an important honey producer and international exporter (especially to Germany and USA), with 95% of the total production sold loose (80%), as homogenized bulk honey (15%), and fractionated (0.4%) (Source: Ministerio de Agricultura, Ganadería y Pesca de la Nación Argentina. MinAgri. www. minagri.gob ar). The country's central region is the most productive area. Honeys come from lands with Leguminosae (12) or Brassicaceae plants grown as forage, and their color is light and up to 60 mm Pfund (14, 31). Recently, the northeastern region of Argentina has undergone an important increase in beekeeping activity, with the Chaco as the most productive province in that region. Most of the honeys produced in this region derive from wild forest vegetation and are generally darker than those produced in the central region of Argentina, exceeding the 60 mm Pfund.

Argentinean honeys meet all major international specifications of demanding trades (2, 11). A study of the microbiological and physicochemical characteristics of Argentinean honeys from the central region was carried out by (15). Recent studies tend to associate physicochemical characteristics with pollen properties (16, 23, 24).

In the Chaco province, beekeeping is an emerging activity. Physicochemical studies of honeys are few. However, melissopalynological and sensory studies are reported (10, 28, 29, 30).

Integrated studies of pollen composition, physicochemical and sensory properties of honey are important to its characterization, and add value to the product.

Objective

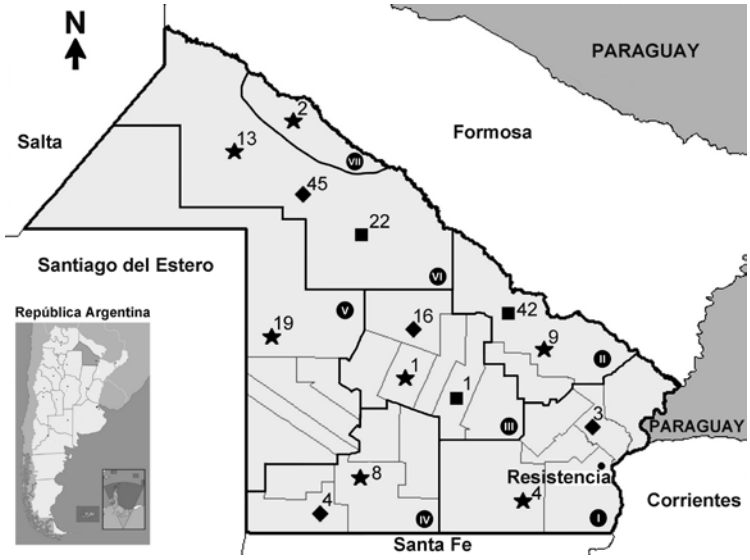
- The aim of this study was to determine the physicochemical properties of honey produced in the province of Chaco as a contribution to its characterization.

MATERIALS AND METHODS

Honey samples

Chaco province is divided arbitrarily into seven beekeeping regions; each of them has honey extraction rooms that are permitted by SENASA (Servicio Nacional de Sanidad Animal - National Animal Health Service). These beekeeping regions were grouped into three zones according to their level of human disturbance. Regions I and II form an area with submeridional plains, where there are palm trees and flood plains (Zone 1). Regions III, IV, and V form an important area with agricultural purposes (Zone 2). Finally, regions VI and VII form an area that is an only slightly disturbed (Zone 3), also known as "El Impenetrable" because of its xerophytic vegetation.

A total of 189 honey samples, extracted by centrifuging, were obtained and analyzed during three beekeeping periods: 2005-2006 (56 samples), 2006-2007 (68 samples), and 2007-2008 (65 samples). The geographic distribution of samples is shown in figure 1.



★ 2005-2006 (56 samples), ◆ 2006-2007 (68 samples) and ■ 2007-2008 (65 samples).
★ 2005-2006 (56 muestras), ◆ 2006-2007 (68 muestras) and ■ 2007-2008 (65 muestras).

Figure 1. Number of analyzed Chaco honey samples per zone and beekeeping season.
Figura 1. Número de muestras de miel del Chaco analizadas por zona y temporada apícola.

The amount of fluid honey obtained for each sample was approximately 500 g and were drawn from 330 kg drums. Each sample was labeled with a tag including the name of the beekeeper, the RENAPA (Registro Nacional de Productores Apícolas) number, the place of origin and the date of harvest.

The physicochemical parameters considered to determine the commercial quality of honey were analyzed: color, moisture, total acidity, pH, electrical conductivity, diastase activity, and hydroxymethylfurfural (table 1). Processing and determination followed (22). Studies were carried out in the CEDIA (Centro de Investigaciones Apícolas - Bee Research Centre) laboratory at the Universidad Nacional de Santiago del Estero (UNSE).

Table 1. Internationals physicochemical parameter of honey.
Table 1. Parámetros fisicoquímicos internacionales de la miel.

Parameter	Unit	Comision Codex Alimentarius FAO - WHO - Alinorm 01/25 (2001)	
		Min	Max
Color	mm Pfund	0	> to 114
Water content	%	-	20
Free acidity	meq kg^{-1}	-	40
pH	-	-	-
Electrical conductivity	dS m^{-1}	0.8 (honeydew)	0.8 (flower honey)
Diastase	Gothe sacale	8	-
HMF	mg kg^{-1}	-	40

Physicochemical analysis

The main physicochemical parameters important to determine honey's quality were analyzed. HMF values and diastase activity, the latter are usually analyzed to determine freshness of samples.

The color of honey is due to pigments such as carotene and xanthophylls; honey also contains polyphenols of the flavones type. Within honey's legislation, color is the only property that is subject to accurate coding. The reference unit is the Pfund scale (17). In this study, measurements were made with the HANNA colorimeter (C 221 Honey Color Analyzer, Hungary, Europe). The following scale is used to determine honey colors according to the Pfund scale in mm (table 2).

Table 2. Color scale of honey according to the Código Alimentario Argentino (CAA).
Tabla 2. Escala de color de la miel según el Código Alimentario Argentino (CAA).

Commercial color scale (CAA)	Equivalence in PFUND scale
Water white	1-8 mm
Extra white	8-16.5 mm
White	16.5-34 mm
Extra light amber	34-50 mm
Light amber	50-85 mm
Amber	85-114 mm
Dark amber	> 114 mm

Moisture is a quality parameter important for shelf life of honey and can be artificially altered during honey processing. Moisture was determined with an Bausch & Lomb analogic refractometer type Abbé - 3L.- USA at 20°C (4), and the value of moisture was obtained using the Chataway table revised by (33).

Total acidity was determined by titration with 0.1 N NaOH, which means that an acid is neutralized by hydroxide in the presence of an internal indicator, phenolphthalein. All honeys have an acidic reaction coming from the action of enzymes. They have free organic acids or combined in lactones. Gluconic acid is the most abundant organic acid in honey (between 70% and 80%). Acidity is expressed in meq kg^{-1}, and it can range between 10 and 60 meq kg^{-1} depending on its origin, although (9, 27) tolerates up to 50 meq kg^{-1}.

The pH reflects the buffer action by mineral constituents in honey against organic acids. All honeys are acidic with normal pH values ranging from 3.5 to 5.5 due to the presence of organic acids that contribute to the honey flavor and stability against microbial contamination and deterioration (35). The pH of each sample was determined in a solution of double-distilled water with a Ph Conductivity Meter-Direct Benchtop Metter (pH/mV/°C) Cole-Parmer (Singapur, Asia).

In honey, electrical conductivity is linked to and enabled by mineral content (1). This parameter is commonly used to differentiate honeydew from blossom honey, except for chestnut honey (6). Electrical conductivity was measured using volumetric method (3) in a solution containing 20% of honey in double-distilled water and a Dist Conductivity TDS Meters Hanna Instruments (Mauritius, Africa). The obtained values are expressed in mS.

The hydroxymethylfurfural (HMF) content of honey after processing and/or blending should not exceed 40 mg kg^{-1} (9, 26), its content was determined using Winkler's spectrophotometric method with a UV-Visible Spectrophotometer CAM SPEC M330 (United Kingdom).

The enzymatic capacity to hydrolyze starch together with a resulting colored starch-iodine complex is used to quantify diastase activity. Most of the international regulations require a diastase index superior to the number 8 on the Gothe scale, however, a minimum of 3 is allowed for those honeys that have a naturally low index, provided that the HMF does not exceed 15 mg kg^{-1}. Several countries argue that this parameter is not valid as a quality factor due to its variability in fresh honeys from different floral origin (22).

Statistical analysis

Data were descriptively analyzed using the STATISTICA software (31), the degree of association between them was assessed by Pearson's correlation coefficient. Differences in physicochemical parameters of honeys from different areas were analyzed by ANOVA.

RESULTS AND DISCUSSIONS

Physicochemical and descriptive statistical analysis

Results from the physicochemical statistical analyses of all analyzed variables are summarized (table 3). Physicochemical characteristics correspond to normal parameters and are within the requirements of the CAA and international marketing parameters (table 1, page 194).

Table 3. Descriptive statistics analyses of physicochemical parameters of Chaco honeys.
Tabla 3. Estadística descriptiva de los análisis de los parámetros fisicoquímicos de las mieles del Chaco.

Parameter	N	Mean	Range		SD	CV
			Min	Max		
Color	189	67	22	150	22.99	34.1
Water content	189	18.0	15.4	21.0	0.97	5.4
Total acidity	189	24	8	68	8.31	35.4
pH	189	4.1	3.2	6.4	0.58	14.3
Electrical conductivity	189	0.668	0.189	1.347	0.21	32.4
Diastase	64	25	10	30	5.57	22.4
HMF	16	9.7	2.3	24.5	6.97	71.4

N = analyzed samples. Min = Minimum value. Max = maximum value. SD = standard deviation. CV = coefficient of variation.
N = muestras analizadas. Min = valor mínimo. Max = valor máximo. SD = desviación estándar. CV = coeficiente de variación.

Color and electrical conductivity

Color is the most important commercial criterion. Chaco honeys show a mean of 67 mm Pfund (light amber) with a minimum value of 22 mm Pfund (white amber) and a maximum value of 150 mm Pfund (dark amber). Frequently dark colored honeys are associated with a very aromatic product, while a light colored honey suggests more subtle aromas; although this phenomenon is generally accurate, it is not an absolute rule. Sugars melanization phenomenon which occurs during honey's aging or heating process intensifies its color (18).

Electrical conductivity (EC) in analyzed honeys had a mean of 0.668 mS, and ranged 0.189-1.347 mS. Note the high values obtained for blossom honeys, due to the fact that a EC > 1.2 mS cm^{-1} is registered for honeydew honey and below 0.8 mS cm^{-1} for blossom honeys (13). According to blossom honeys they should have less, honeydew honey over 0.8 mS cm^{-1}. Nonetheless there are exceptions registered in some blossom honeys, such as monofloral honeys of *Arbutus*, *Banksia*, *Erica*, *Eucalyptus*, *Leptospermum*, *Melaleuca* and *Tilia* (5).

The correlation analysis showed significant positive relationships between color and EC ($r = 0.513$, $p = 0.000$; figure 2, page 197). According to Gómez Pajuelo (1995), mineral content has an influence on honey's color; generally, lighter honeys show a lower EC than darker honeys in which EC is higher due to a higher mineral content.

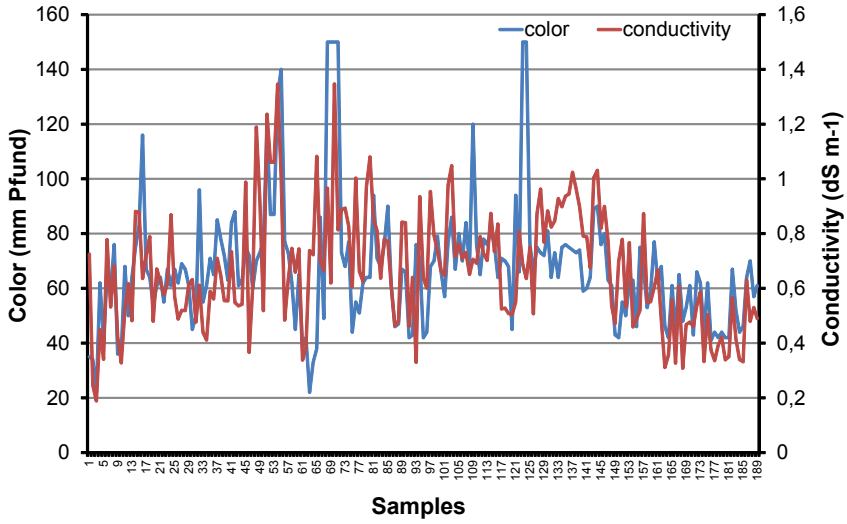


Figure 2. Behavior of two positively correlated variables, color and electrical conductivity in Chaco honeys.
Figura 2. Comportamiento de dos variables correlacionadas positivamente, el color y la conductividad eléctrica en las mieles del Chaco.

Honey color also showed significant positive correlation with moisture ($r = 0.204$, $p = 0.005$) and total acidity ($r = 0.468$, $p = 0.000$). Electrical conductivity showed significant positive relationship with total acidity ($r = 0.160$, $p = 0.028$) and pH ($r = 0.473$, $p = 0.000$).

Based on the coefficient of variation (CV), color (CV = 34.1) and EC (CV = 32.4) are the most variable properties of Chaco honey (table 3, page 196).

Color and EC are the physicochemical properties of Chaco honey that appear to best contribute to identify geographic origin since they could be directly linked to their botanical origin. The positive correlation observed between color and EC is due to a higher mineral content in dark honeys (17). Mineral content in honey is strictly related with its nutritional power.

Moisture, pH, and total acidity

Standard honeys consist 18% of water, and when moisture levels surpass or are below this value, the quality of honey is affected. Mean moisture content in Chaco honeys was 18.0%, with values ranging from 15.4% to 20.0%. However, four samples showed higher values (20.2, 20.4, 20.6 and 21%) (figure 3, page 198). According to Maidana (2004), if moisture content is higher than 18%, honey can ferment because its sugars concentration is not enough to prevent the growth of yeasts present in it. Conversely, if moisture content is below 15% the viscosity increases hindering the extraction work and it also crystallizes in a hard mass, little appreciated by consumers, because it is difficult to extract and use. The best crystallization conditions occur when

the water content is close to 17.5%. Honeys that are too wet tend to crystallize and separate into two layers: a solid layer formed by the crystallized phase and a liquid layer consisting of the liquid.

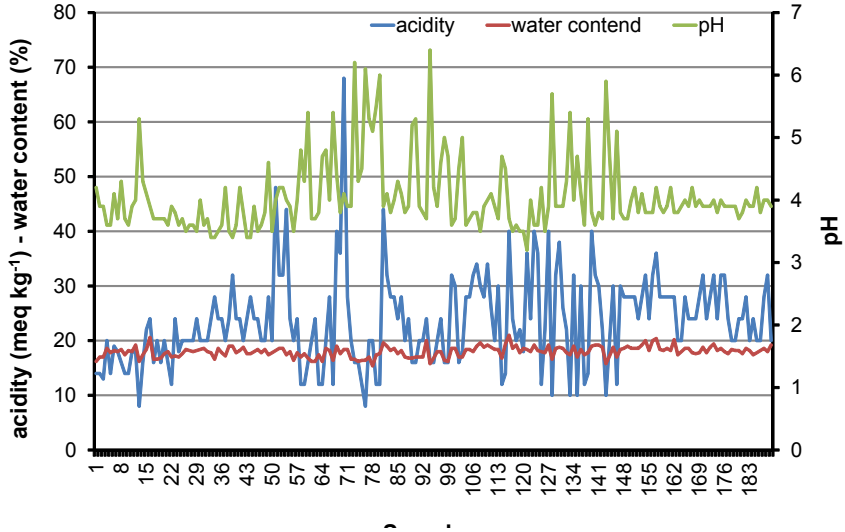


Figure 3. Behavior of three variables negatively correlated: acidity, moinsture and pH.
Figura 3. Comportamiento de tres variables correlacionadas negativamente: acidez, humedad y pH.

The analyzed honeys show normal mean and range values for both total acidity and pH (table 3, page 196).

While moisture and acidity were significantly positively correlated ($r = 0.578$, $p = 0.000$), moisture was significantly negatively correlated with pH ($r = -0.457$, $p = 0.000$) and pH was significantly negatively correlated with total acidity (-0.405, $p = 0.000$). Total acidity (CV = 35.4) is more variable than moisture (CV = 5.4) and pH (CV = 14.3).

HMF and diastase activity

Analyzed honeys show normal values for both variables (table 3, page 196), which indicates that honeys are new, and extracted and stored correctly.

HMF indicates freshness and that the honey was not heated. Before testing diastase activity and color it is advisable to verify that HMF content does not exceed 15 mg kg^{-1} (6). HMF is one of the compounds formed due to degradation of sugary products, and its formation is directly related to heat alterations (21) and to production of unpleasant flavors and odors. This relationship makes HMF one of the most used food quality parameters, especially in honey. Properties of sugary products are sensitive to heat and can deteriorate during storage (22).

Diastase of honey results from proteins of the hypopharyngeal glands that are added to the nectar by the worker bees (34). These glands may vary their activity and also depend on the colony's state of development (25). When artificial feeding begins or the queen bee starts spring oviposition, the hypopharyngeal glands activate starting to synthesize enzymatic proteins (7, 8). During larvae feeding period (5 to 20 d), nurse bees have fully developed hypopharyngeal glands and high protein synthesis activity (7). In winter there is no oviposition; yet glands are completely developed (hypertrophic), but their protein synthesis is low (22). Factors such as age, the role of bees in the hive, juvenile hormone, food, and kinds of pollen strongly influence the enzymatic secretion of the glands and, therefore, indirectly, also the final content of diastase in honey (14, 19, 20).

Honey characterization by zones

ANOVA indicates a trend for lighter honeys with less EC towards southern zones of the province (Zones 1 and 2), and darker honeys with higher EC towards the northern zone of the province (Zone 3).

In Zone 1, mean color of honeys is 54 mm Pfund, in Zone 2 is 64 mm Pfund, and in Zone 3 it is 79 mm Pfund. The mean EC is 0.505 mS in Zone 1, 0.609 mS in Zone 2, and 0.810 mS in Zone 3 (figure 3, page 197).

In Zones 1 and 2, honeys range from extra light amber to light amber and EC values are considerably below 0.6 mS, while honeys in Zone 3 range from amber to dark and EC values are above 0.6. Zone 2 includes honeys with the lowest color and EC values of the province (22 mm Pfund and 0.189 mS, respectively), whereas Zone 3 includes honeys with the highest values (150 mm Pfund and 1.347 mS, respectively) (figure 4).

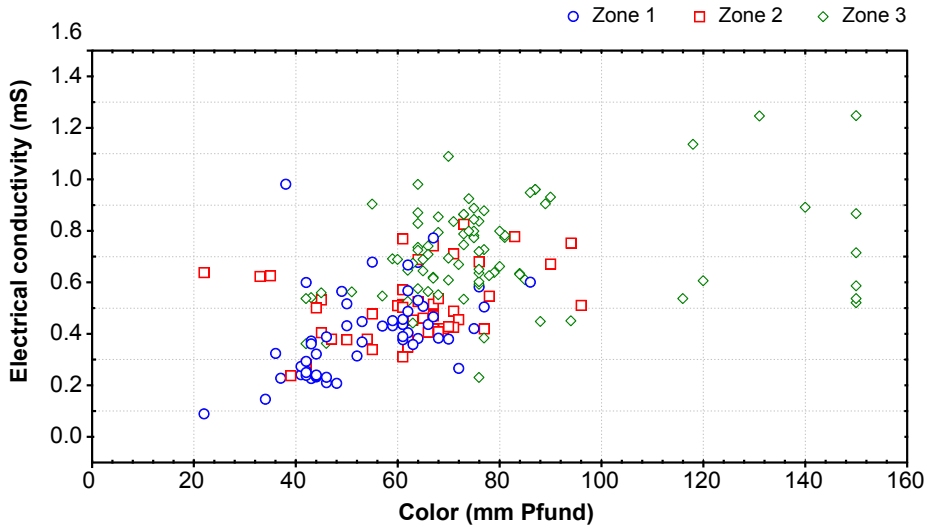


Figure 4. Graphic representation of honey's electrical conductivity (mS) and color (mm Pfund) according to the zone of sample origin.

Figura 4. Representación gráfica de la conductividad eléctrica de la miel (mS) y color (mm Pfund) de acuerdo con la zona de origen de la muestra.

The other characteristics studied are similar among the three Zones with following mean values: Water content (Zone 1 = 18.2%, Zone 2 = 17.8%, Zone 3 = 17.8%), total acidity (Zone 1 = 23, Zone 2 = 21, Zone 3 = 24), and pH (Zone 1 = 3.9, Zone 2 = 3.7, Zone 3 = 4.2).

CONCLUSIONS

The physicochemical characteristics of all Chaco honey samples analyzed in this study can be considered to be within normal parameters and constitute a quality product within national and international parameters. Total acidity, HMF values and diastase activity are good indicators of freshness of Chaco honeys. Water content met international parameters and was below 20%, except one sample with 21%. Honey color ranges from light amber, amber, to dark amber with EC values ranging from medium to high.

In Chaco, darker honeys with higher EC were produced in the North (Zone 3), where native forests still prevail and tree species are the main source of nectar. These honeys are also called "forest honeys". Lighter honeys with lower EC values were produced in the agricultural areas of the province (Zone 2), where a few crop plants are widely cultivated. Honeys known as "forest honeys" have a higher nutritional value compared to lighter honeys from meadows and with an exotic origin, which are produced in other regions of the country.

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