

Physicochemical characteristics and quality analysis of commercial honeys of *Apis mellifera* produced by artisanal beekeepers in Corrientes (Argentina)

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ABSTRACT

Artisanal honeys of *Apis mellifera* produced in Corrientes province (Argentina) for local and international markets, were characterized on the basis of their chemical and physical properties. One hundred sixty (160) honey samples from harvests for three years were analyzed for quality according to the national regulatory norms (Argentine Food Code, CAA) and international standards (GMC Mercosur; Codex Alimentarius, FAO). The ranges and mean obtained values of the analysis of free acidity, pH, moisture content, electrical conductivity, hydroxymethylfurfural (HMF), proline, reducing sugars, apparent sucrose, diastase activity, color, insoluble solids and ash were examined. The values obtained for the properties of most of the honey analyzed were in agreement with international specifications for blossom honeys of a good quality.

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KEYWORDS

Honey;
Physicochemical properties;
Quality;
Corrientes;
Argentina.

INTRODUCTION

Honey, a natural sweet substance produced by bees mainly from the nectar of plants, is an important food and is subject of great scientific and commercial interest. Current norms^[1,3] classify honey according to their origin; distinguish between blossom honey or nectar honey and honeydew honey, describing the characteristics or known properties of each type of honey. Honey consists essentially of carbohydrates, predominantly fructose (30-45 %) and glucose (24-40 %), and water. It also contains other substances such as organic

acids, amino acids, enzymes and flavonoids (one of the largest family of secondary metabolites of the plants), as well as mineral components, together with solid particles, like pollen and traces of wax.

Since primitive times honey has been used as food and at the present time consumption by the people and its commercialization probabilities are dependent on to its quality, especially of factors such as color, flavor, fragrance and consistence. All these features are related with its sanitary conditions, chemical composition, physical properties and microbiological characteristics, which are correlated with the floral origin, the

production regions of the honey, seasonal variations in climatic conditions as well as the extraction and storage conditions. So the composition and quality of different types of commercial honeys vary in its compositions, quality and cost.

In the world market Argentina is a one the largest producer and exporter of honey together with China and Mexico. In 2000 Argentina were the third largest producer and the second exporter of honey in the world^[12] and in 2002 became the first exporter the honey of world. Actually Argentina is a member of Southern Common Market^[17] and the quality of its commercial honey production should meet norms the Argentine Food Code and GMC Mercosur^[17]. These norms are concordant with that of Codex Alimentarius (FAO).

The Corrientes province at the North-East of Argentina (27°15' and 30° 43' South latitude and 57° 47' and 58°12' West longitude) has an area of 88,890 km² and their climatic conditions are the characteristics of a subtropical region without dry season. The rains are irregular and vary between 1,100 and 1,900 mm/year. This province includes wide rural areas where there are a variety of native plants with different flowering periods and several nature reserves rich in biodiversity, as well as significant planted forests areas, eucalyptus and citrus mainly^[8]. Beekeeping is practiced by small and medium producers; some beekeepers are members of cooperatives that provide access to equipment for the extraction and facilities to the honey storage. In recent years there has been a significant increase in honey production due increasing demand and interest by consumers, good climatic conditions and governmental action, with the introduction of modern technologies. The production is destined for local trade and for export as non specific honey without floral origin. According to Department of Beekeeping (Ministry of Production, Corrientes Government) the artisanal beekeepers are distributed in six honey production areas, called basins 1, 2, 3, 4, 5 and 6 (Figure 1). However little information is available about the honey produced in this province, except for studies on pollen content of honeys published by Salgado & Pire^[19,20], indicating honeys monofloral mainly from native species (*Astronium balansae*, *Eryngium* spp. and *Salix* ssp.) together Citrus ssp. and honeys of mixed floral origin from *Eu-*

calyptus, *Astronium balansae*, *Sapium haematospermum* and *Eryngium* mainly, and a few honeydew indicators. Thus the botanical origin of the honeys is floral and bees use nectar of exotic and native species as a source^[8]. Agreement between the National University of Northeast (UNNE) and the Government of Corrientes has allowed the creation of Analysis Laboratory of Bee Products (LABAPI) to assist producers in controlling quality of honey and other bee products and conduct research to improve and strengthen the development of beekeeping in the region. This study is part of investigations efforts to know physicochemical quality of honey produced in Corrientes province as well as to find out whether they meet national and international standards of honey specifications.



Figure 1 : Corrientes (Argentina): áreas of honey production

Usually, the parameters examined to evaluate the quality and authenticity of honey include the measurement of pH, free acidity, moisture content, HMF, color, proline content, diastase activity, insoluble solids and ash. Another group of chemical parameters less frequently analyzed includes the profiles of sugar, amino acids, proteins and flavonoids, and also volatile compounds, phenolic acids, ¹³C/¹²C ratio and determination of mineral ionic content, employing techniques such as High Performance Liquid Chromatography, Gas

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Chromatography-Mass Spectrometry, Ion Chromatography, Mid-infrared, Pyrolysis-mass Spectroscopy and Raman Spectroscopy^[3,10]. According to different authors the use of multiparameter studies and principal component analysis, associated with chemometrics, yields satisfactory results for honey classification^[3,9,10,18,23]. However, according to Bogdano & Martin^[4], these methods have been used for research purposes and its use for routine control of honey is unlikely due to the drawback of using sophisticated instruments.

Melissopalynology (pollen identification) is also employed as standard method for determination the floral origin of honey but this technique have some disadvantages and the interpretation of pollen analysis requires specialized professional with satisfactory knowledge of pollen morphology to achieve reliable results^[11,22].

Thus in this study were analyzed the parameters to evaluate the honey samples authenticity and quality, such as pH, free acidity, moisture content, HMF, color, proline content, diastase activity, insoluble solids and ash.

EXPERIMENTAL

Samples

This study was conducted with 160 fresh artisanal honey samples collected in 2008, 2009 and 2010 during two harvest seasons each year. The samples were provided by local beekeepers who have certified their regions of origin but only some of them the likely floral origin. The samples were stored in plastic bottles or glass with screw cap at room temperature until analyzed.

Physicochemical parameters

The physicochemical properties were analyzed using the following methods.

pH measurement was performed potentiometrically at 20°C in a 10% (w/v) solution of honey in distilled water^[15] using a HANNA pH-meter HI 98129.

Free acidity was measured by titrimetric method to pH 8.50 by adding 0.05 M NaOH solution^[15]. Results were expressed as meq/Kg.

Water content (moisture) was determined measuring the refractive index at 20 °C in an Abbe type refractometer and then using the Wedmore table to con-

vert the measurement to percent moisture^[1,15].

The electric conductivity of a 20% (w/v) honey solution in distilled water has been measured using a ORION 3 STAR conductimeter.

The content of apparent reducing sugars as well as the apparent sucrose content were measured by the Fehling-Causse-Bonnans method^[15].

Diastase (amylase) catalyzes the transformation of starch to maltose. Diastase activity (DA) was measured using a buffered solution of soluble starch and honey, which was incubated in thermostatic bath. The endpoint was determined photometrically (AOAC 958.09). The diastase activity is expressed in Gothe degrees (°G)

Hydroxymethylfurfural (HMF) was determined after clarifying honey samples with Carrez reagents and the addition of sodium bisulfate^[1,15]; the absorbance was determined at 284 and 336 nm.

Proline was determined by a colorimetric method based on the reaction of the amino acid with ninhydrin in an acidic medium, measuring the absorbance of the product of reaction at 520 nm.

To measure the color according to mm Pfund has been used a HANNA C221 Honey Color Analyzer.

The content of insoluble matter was determined by noting the increase in weight of a porous crucible after filtration of a known amount of honey dissolved in water^[15] and results were expressed as g/100 g of honey.

The ash percentage was determined by sample calcinations at 550 ± 50 °C in a muffle furnace until a constant weight was achieved^[15].

Statistical analysis

Analysis of each parameter was carried out in duplicate. To find out the mean and standard deviation values of all the tests performed statistical tests were applied.

RESULTS AND DISCUSSION

TABLES 1 and 2 show the means, standard deviations and ranges of the results obtained from the analysis of physicochemical parameters of the honey samples. Histograms of the pH, moisture, free acidity, reducing sugars, sucrose apparent and HMF obtained results are shown in Figure 2(a)-(f).

In honeys the pH is an important factor because it

influences characteristics such as texture, color and stability. All honeys are acidic with a pH-value generally among 3.5 and 5.5^[3]. The samples analyzed have a range 3.67- 5.52 units (TABLE 1) with a mean value of 4.61 (SD 0.40). The histogram for these results corresponds to a variable with a normal distribution with higher frequency values among 4.1 and 5.2 units (Figure 2(a)). The values for 2008 and 2010 do not differ greatly (TABLE 2). According to Bogdanov et al.^[2] pH-values among 4.5 and 6.5 correspond to honeydew honeys. Higher pH values (>4.5) obtained in some analyzed samples could indicate the presence of honeydew honey or at least a mixed honey, depending on typical native flora from each region and their climatic conditions.

TABLE 1: Physicochemical characteristics of corrientes honeys

pH	(n=135)	Moisture content (%)	(n=159)
Máximo	5.52	Máximo	22.6
Mínimo	3.67	Mínimo	15.0
Mean (SD)	4.61(0.40)	Mean (SD)	17.3 (1.19)
Free acidity meq/Kg)	(n=160)	Reducing sugars (g/100g)	(n=99)
Máximo	46.24	Máximo	82,9
Mínimo	11.54	Mínimo	66,4
Mean (SD)	25.02 (7.70)	Mean (SD)	76.83 (3.88)
Sucrose apparent (g/100g)	(n=158)	HMF (mg/kg)	(n=152)
Máximo	8.93	Máximo	86.5
Mínimo	0.30	Mínimo	0.15
Mean (SD)	1.65 (1.24)	Mean (SD)	7.30 (10.43)
Diastase activity (°Gothe)	(n=159)	Proline (mg/kg)	(n=158)
Máximo	45.41	Máximo	1462
Mínimo	3.28	Mínimo	163
Mean (SD)	15.19 (6.72)	Mean (SD)	700.7 (284.0)
Color (mm Pfund)	(n=157)	Conductivity (mS/cm)	(n=159)
Máximo	150	Máximo	1.178
Mínimo	29	Mínimo	0.160
Mean (SD)	71.9 (21.3)	Mean (SD)	0.625 (0.21)
Ash (%)	(n=98)	Insoluble solids (%)	(n=27)
Máximo	0.64	Máximo	0.496
Mínimo	0.06	Mínimo	0.000
Mean (SD)	0.30 (0.09)	Mean (SD)	0.023 (0.005)

The water content is very important for the stability of honey and dependent on climatic conditions, season

of harvest and maturity degree. Moisture content in natural honeys can be as low as 13% or reach 23%^[4]. The high water content favors the fermentation process with a loss of honey quality; this risk is usually not a problem for the honey with water content lower than 18%. In the analyzed samples the water content was lower than 23% w/w (TABLE 1). Although 22.6% of samples exceeded the limit of 18% established by the national legislation (CAA, 2010), most of honeys had water content between 15.5 to 19.5% (Figure 2(b)), indicating optimum harvesting and a good degree of maturity. Only three samples exceeded the quality standard the 20% allowed by Codex Alimentarius.

TABLE 2 : Annual results of physicochemical parameters of honeys analyzed

	2008	2009	2010
pH	(n = 100)		(n = 35)
Range	5.4 - 3.75	-	5.52 - 3.67
Mean (SD)	4,57 (0.37)		4.71 (0.46)
Moisture content (%)	(n = 100)	(n = 24)	(n = 35)
Range	20.2 - 15.2	18.7 - 15.5	22.6 - 15.0
Mean (SD)	17.10 (0.97)	16.60 (0.77)	18.40 (1.54)
Free acidity (meq/Kg)	(n = 100)	(n = 24)	(n = 36)
Range	46.24 - 11.54	36.08 - 15.65	40.0 - 14.55
Mean (SD)	24.31 (7.86)	24.88 (5.83)	27.06 (8.16)
Sugars reducing (g/100g)	(n = 99)	(n=24)	(n = 35)
Range	82,94 - 66.40	80,49 - 72.00	79,41 - 65.88
Mean (SD)	76.83 (3.88)	76,21 (2.33)	75.12 (2.85)
Sucrose apparent (g/100g)	(n = 99)	(n = 24)	(n = 35)
Range	4,10 - 0.30	5,30 - 0.67	8,93 - 0.36
Mean (SD)	1.36 (0.98)	2.29 (1.21)	2.04 (1.64)
HMF (mg/Kg)	(n = 92)	(n = 24)	(n = 36)
Range	86.5 - 0.15	28.14 - 1.00	82.00 - 1.00
Mean (SD)	6.49 (9.71)	9.58 (6.85)	7.85 (13.71)
Diastase activity (°Gothe)	(n = 99)	(n = 24)	(n = 36)
Range	45.41 - 3.28	25,57 - 4.97	42.0 - 7.0
Mean (SD)	14.47 (6.58)	13.92 (5.30)	18.00 (7.34)
Proline (mg/kg)	(n = 100)	(n = 23)	(n = 35)
Range	1462 - 300	1235 - 351	1344 - 163
Mean (SD)	752.5 (266.3)	734.4 (264.8)	530.6 (290.8)
Color (mm Pfund)	(n = 100)	(n = 23)	(n = 34)
Range	150-29	114 - 30	122-29
Mean (SD)	74.7 (20.4)	62.1 (20.4)	70.4 (22.9)
Conductivity (mS/cm)	(n = 100)	(n = 24)	(n = 35)
Range	1.178 - 0.289	0.672 - 0.215	0.865 - 0.160

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	2008	2009	2010
Mean (SD)	0.724 (0.169)	0.446 (0.119)	0.464 (0.162)
Ash (g/100g)	(n = 98)		
Range	0.64-0.06	-	-
Mean (SD)	0.30 (0.09)		
Insoluble solids (g/100g)	(n = 27)		
Range	0.496 - 0.00	-	-
Mean (SD)	0.023 (0.095)		

The free acidity, due to the presence of organic acids, particularly gluconic acid, ranged between 11.54 and 46.24 meq/kg (mean 25.02, SD 7.70) (TABLE 1). The histogram for results obtained shows a distribution slightly skewed in favor of the lower ranges of acidity (Figure 2(c)). All samples collected in 2008 had values lower than 50 meq/kg and only 5% were greater than 40 meq/kg, while none of the samples collected during 2009 and 2010 (TABLE 2) exceeded the limit allowed by national (40 meq/kg) and international regulations (50 meq/kg), indicating the absence of undesirable fermentations. Differences of free acidity could be due a different floral origin of honeys and climatic condition of the geographical regions or are related with variation in the harvest season.

The values of "apparent reducing sugar content" correspond roughly to the sum of the main sugars of honey, fructose and glucose and some minor reducing disaccharides, mainly maltose^[5]. All the analyzed honeys had a content of reducing sugars higher than 65% fixed as minimum for floral honey in norms of National Code and Mercosur rules and above Codex minimum (not less than 60 g/100 g). The sugars content ranged 66.4 to 82.9 g/100 g honey (mean 76.83) (TABLE 1) with an asymmetric frequency distribution toward to the higher values (Figure 2(d)).

Apparent sucrose content is calculated as the difference in apparent reducing sugars before and after hydrolysis of sucrose. Most of the analyzed honey samples (98%) had sucrose content lower than 5 % and the frequency histogram shows a skewed distribution to lower values (Figure 2(e)); only four samples had sucrose content higher than 5.0%, one of these above 8%, while the annuals average values were lower than 2.5% (TABLE 2). Codex Alimentarius standards set as limit for the content of sucrose, in general, no more than 5 g/100 g^[4]. Moreover CAA standards and

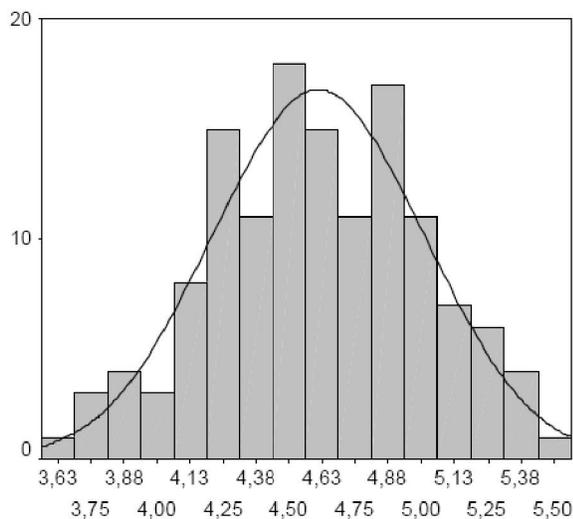
Mercosur indicate a maximum of 8% for the honey of flowers and 10% of honeydew honey. Thus the values obtained indicate a floral origin and a good quality of the honey samples analyzed.

The HMF content is a widely accepted indicator of the freshness of the honey. The maximum of HMF for floral honeys is the same for rules of National Food Code and Mercosur (40 mg/kg), while according Bogdanov et al.^[5] the Codex Alimentarius proposal is to change the limit of 40 mg/kg for a maximum of 60 mg/kg. The proposal is based on the experience that HMF increases on honey storage in warm climate countries. The analyzed honeys had generally low HMF values (TABLE 1), with a mean 7.30 mg/kg (SD 10.43), and only two samples showed HMF content above 40 mg/kg (Figure 2(f)).

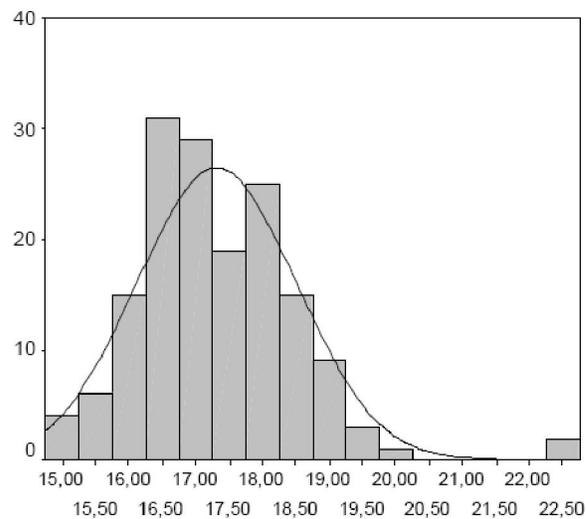
The diastase activity (DA) also is a quality factor and an indicator of the freshness of the honey as it is influenced by storage and heating of the honey; however some honeys have a naturally low diastase activity^[5]. The CAA and Mercosur standards indicate that the honey must have a minimum of diastase activity of 8 on the Gothe scale, but in the case of honeys with low enzymatic activity containing less than 15 mg/kg of HMF is accepted at least 3 °Gothé for the DA (GMC Mercosur). These values are consistent with Codex standards. In the analyzed honeys the range of values of DA was wide (TABLE 1) with the largest number of samples between 4.0 and 22.0 °Gothé. However 11.25% of the samples had less than 8 °Gothé, and more studies are needed to determine whether the low activity of diastase of these honeys is due to its botanical origin or perhaps could be accounted for a inadequate processing.

Although in honey can be found different amino acids, proline is the most abundant and is considered the most important. The proline content is commonly used as a criterion of maturity or in some cases of the honey adulteration. Minimum value of 180 mg/kg is accepted for genuine honey, however there is considerable variation depending on the honey type^[5]. In this study, the proline content of 99.4 % of samples was above 180 mg/kg and 13.9 % were higher than 1,000 mg/kg (TABLE 1) with predominance of samples from 250 to 850 mg/kg.

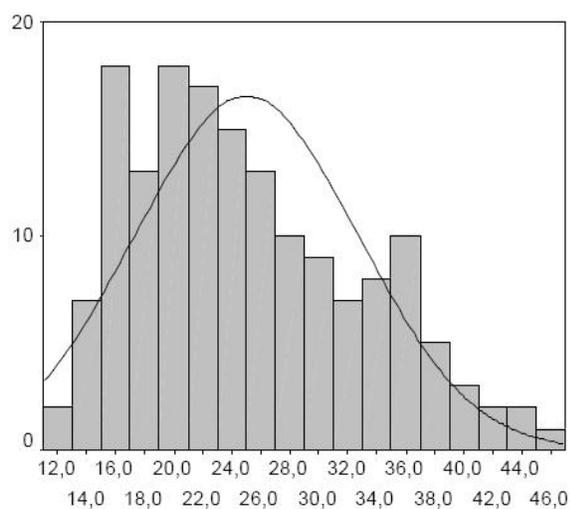
Color has always been an important factor for the



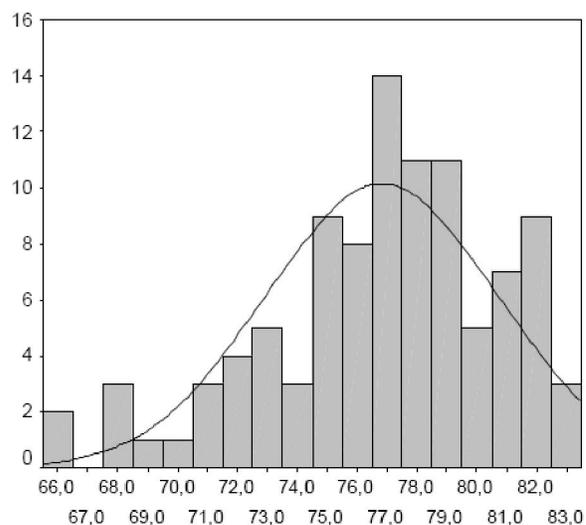
(a) pH



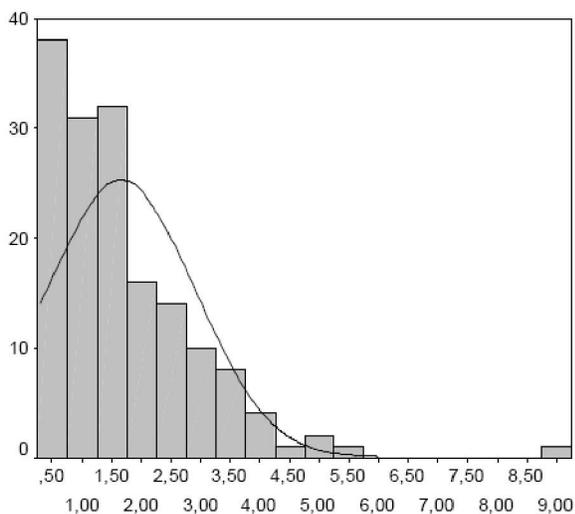
(b) Moisture (%)



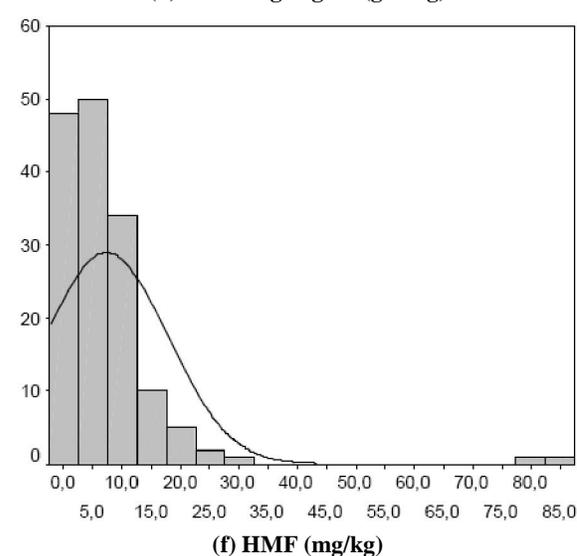
(c) Free acidity (meq/kg)



(d) Reducing sugars (g/100g)



(e) Sucrose apparent (g/100g)



(f) HMF (mg/kg)

Figure 2 : Frequency distribution of physicochemical parameters values of honeys analyzed

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presentation of honey and in itself is a useful indicator and a first step in the process of recognition of honey type^[4]. Noteworthy that color range are generally characteristic for each honey of floral type. In this study the color of the samples analyzed was from white to dark amber (TABLE 1) coinciding with the color characteristics of honeys, almost colorless to dark amber according CAA, Mercosur and Codex alimentarius. The greatest number of samples had values below 114 mmPfund, corresponding to honeys extra light amber colored (12.5%), light amber (63.8%) and amber (15.6%), as shown in Figure 3. The range of values the first year was the wider (TABLE 2) and included the highest value recorded, but only 3.8 % of the total samples were dark amber.

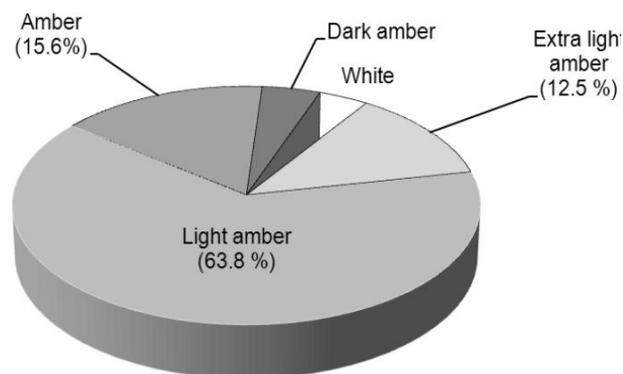


Figure 3 : Color of honey samples analyzed (n = 160)

Electrical conductivity of honeys varies with botanical and geographical origin, and at present it is the most useful quality parameter for the classification of unifloral honeys^[3]. In general floral honeys have conductivity values below than 0.8 mS/cm, while honeydew honey has not less than 0.8 mS/cm^[4,13]. The conductivity of the analyzed honeys ranged from 0.160 to 1.178 mS/cm (mean 0.625) (TABLE 1). The first year 23% of samples had values over 0.8 mS/cm but in the last year only two samples showed value above 0.8 mS/cm (TABLE 2). The values obtained in the set of samples correspond with mostly of the honeys with conductivity lower than 0.8 mS/cm; this suggests that most honeys collected for this study were of floral origin.

A quality criterion of particular importance is the ash content because is indicative the concentration of minerals in honey and is related with their botanical origin. The mineral content affects the color of honey and

is a possible indicator of environmental pollution and also serves as an indicator of the geographical origin of honey. Values below 0.64% show a floral origin since the blossom honeys generally have a lower ash content ($\leq 0.6\%$) than honeydew honeys ($\leq 1.2\%$). The ash content in 99.9% of honey samples analyzed in 2008 was lower than 0.64% (TABLE 1). The maximum values allowed by the quality standard according to Codex Alimentarius (Draft CL 1998/12-S) are 0.6 g/100 g in general and 1.2 g/100 g for the honeydew or blends of blossom honey^[5], while for standards the CAA and Mercosur the values are 0.6% and 1.0% for the honey of flowers and honeydew, respectively. Therefore, these samples showed ashes percentages in agreement with the international standards for quality honey the floral origin.

The measurement of insoluble matter is an important means for detecting impurities in honey in quantities exceeding the allowed values. The insoluble solids content of 27 honey samples analyzed during the first year (TABLE 1) was lower than 0.5%, while 96.3% the analyzed honeys had values less than 0.1%, in agreement with national and international standards which set as maximum 0.1% of solids insoluble in water. Only one sample had a content exceeding 0.1%, indicating that it could be a pressed honey.

CONCLUSIONS

This study has allowed assessing and confirming the good quality of the honey that is produced in Corrientes province at North-East Argentina and also establishing a database of its properties. The physicochemical characteristics of most samples correspond to honeys of floral origin and results obtained were in agreement with regulatory limits established by the Argentine Food Code (CAA) and international standards (GMC Mercosur; Codex Alimentarius FAO) for honey quality.

Honeys analyzed presents high reducing sugars content (more than 65 %) and very low values of sucrose. The HMF content and the free acidity values were mostly low and moisture content less than 20 % indicating honey freshness and good conservation. These results are very important for commercialization of honeys in the national and international markets, because

indicate a product that offers good quality. The comparative analysis of results, particularly the higher pH-values, indicates that of differences could be on account of various factors like different sources of origin (honeydew or nectar), the floral source and geographical origin or perhaps could be accounted for an inadequate processing. Further studies are needed in order to improve the specificity and accuracy of the characterization of the honeys from each region (basins) as well as to know its botanical origin and their seasonal variability. Also it is advisable to standardize the beekeeping practices at all the different areas to contribute to maintain or to improve honey quality.

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