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Physicochemical Characterization and Sugar Profile of Argentinian Honeys from the Phytogeographic Provinces Paranaense and Pampeana in Misiones

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Abstract

The characterization of honeys from the Biomes and vegetation units of the Phytogeographical Provinces (PP) located in the Province of Misiones (Argentina) was performed by analysis of sugar profile and physicochemical parameters (Free Acidity, pH, Electrical Conductivity (EC), colour and contents of moisture, ash, total soluble solids and hydroxymethylfurfural) used for quality control of honey. The honeys analysed were authentic and traceable, and met the specifications of the national and/or international standards for the evaluated parameters, denoting blossom origin and confirming high quality, maturity and freshness. The influence of biome and Vegetation Units (VU) on physical and chemical parameters of the studied honeys was defined regarding both flora and pedoclimatic conditions. Honeys from VU-27 presented significantly higher contents of fructose, ash and total soluble solids, pH and EC and lower moisture content than those from VU-3. To the authors' best knowledge, sugar profiles, free acidity and contents of ash and total soluble solids in honeys from the different PPs, vegetation units and Biomes of Misiones are reported for the first time. This study contributes to the classification of honeys from Misiones, helping to increase added value and access new markets.

Keywords: Honey; Saccharides; Physicochemical parameters; Geographical origin; Traceability; Quality

Introduction

Honey is the natural sweet substance produced by Apis mellifera bees from nectar and/or honeydew (exudates of plants or plant-sucking insects) later transformed by combining with specific substances of their own and final dehydration ripening and storage in honeycombs [1,2]. Physicochemical properties of honey depend on the type of nectar, pedoclimatic conditions (climate and soil composition), beekeeping practices, processing, storage and transportation conditions during commercialization [3,4]. Honey characterization from different botanical and/or geographical origins is highly relevant to the honey market since every region may present particular quality characteristics determining commercial value [5]. Physicochemical parameters of honey, such as free acidity, colour, pH, electrical conductivity, moisture, and contents of

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sugars, hydroxymethylfurfural, proline, minerals and ash, in addition to diastase and invertase activities are used to assess honey quality and safety. These parameters are assessed using harmonized methods provided by the International Honey Commission (IHC), the Codex Alimentarius Commission, the European Council and Mercosur [6] which have also been used to define the geographical and botanical origin of honey [5,7,8] and detect honey adulteration [9]. Argentina is the third largest global honey producer after China and the United States, and first in the southern hemisphere, achieving 70% of production [10]. Honey from the provinces in the northeast of Argentina, i.e Formosa, Chaco, Corrientes and Misiones, represents 27% of the total national production [10]. Each of these provinces presents a particular climate and vegetation, obtaining different types of honey with unique characteristics. Misiones is located in the country's extreme northeast, near the Tropic of Capricorn, under a wet subtropical climate with warm and humid summers, mild winters, and abundant, constant and regular rainfall. This climate leads to evergreen vegetation and high biodiversity [11,12]. Misiones includes two Phytogeographic Provinces (PP) and two Vegetation Units (VU) [13,14], i.e. VU-3 in the PP Paranaense, and VU-27 in the PP Pampeana; and seven Biomes in the PP Paranaense, named Planaltense, Palo Rosa, Lauraceae, Tree Ferns, Urunday, Paraná River and Uruguay River, and one Biome in PP Pampeana, called Fields [14]. Consequently, quality control of produced honey is a multi-factorial approach linked to the botanical and geographical origins and beekeeping practices. Classifying honeys according to production area would guarantee quality and traceability, correct labelling, and ultimately produce certified honeys with designation of origin, increasing commercial value [1,15]. These issues are of great interest to producers, consumers, the food industry and regulatory authorities [1]. This study aims to characterise the Argentinian honeys produced in the different PP and Biomes of Misiones, according to their sugar profile and physicochemical quality parameters, for the first time to the authors' knowledge.

Materials and Methods

Honey Samples

One hundred and twenty-one authentic and traceable honey samples of Apis mellifera were collected during four harvest seasons (2013-2014, n=35; 2014-2015, n=29; 2015-2016, n=27; 2016-2017, n=30) from apiaries of the main honey producing areas in the province of Misiones. The apiaries are located within the areas corresponding to the vegetation units VU-3 (semi-deciduous forest) of the PP Paranaense and VU-27 (savannah of Aristida jubata with Acacia and palm trees) of the PP Pampeana [13,83]. The related environments include the following types of Biomes: Planaltense (A), Palo Rosa (B), Lauraceae (C), Tree Ferns (D), Urunday (E) and Fields (F) (Figure 1 and Table S1) [11,14]. The samples (about 1kg of raw honey each) and farming information were provided directly by beekeepers and/or the honey-producing cooperatives. Farming information consisted of harvest date and conditions, declared botanical origin, apiary location (GPS), agricultural system, colony treatments, etc. The honeys were harvested between November and May and all samples were stored in screw-capped plastic containers at 4ºC in the dark until analysis.

Reagents and Solvents

The analytical standards 5-Hydroxymethyl-2-Furaldehyde (HMF), fructose, glucose, sucrose, erlose, maltose, trehalose and maltotriose were provided by Sigma-Aldrich (Darmstadt, Germany), as well as the HPLC-grade solvents methanol and acetonitrile. Sodium hydroxide, potassium acid phthalate, phenolphthalein, absolute ethanol, and the sugar standards of turanose, melezitose and raffinose were supplied by Supelco (Bellefonte, PA, USA). All chemicals and reagents used were of analytical quality grade. Water of HPLC-grade was used in all solutions and dilutions.

Determination of Physicochemical Parameters in Honey

Physicochemical parameters moisture, free acidity, pH, EC and colour, were measured in the honey samples using the official methods of Instituto Argentino de Normalización y Certificación (IRAM) adopted from IHC. Three replicates were analysed for each sample. Honey moisture and total soluble solid content in degree Brix (ºBrix) were determined according to IRAM standard 15931 (1994), using an Abbé refractometer 5 (Bellingham & Stanley Ltd, Longfield Road, Tunbridge Wells, United Kingdom). The EC was determined in a solution of honey at 20% (w/v) at 20±2ºC according to IRAM standard 15945 (1997) using an Adwa AD31 conductometer (Adwa Instruments, Inc., Szeged, Hungary). The ash content in honey was calculated from the EC measurements as described previously [6]. Honey-free acidity was determined by titration according to IRAM standard 15933 (1994). The pH was determined in a solution of honey at 10% (w/v) according to IRAM standard 15938 (1995) using a HI 2020-02 HANNA pH-meter (Hanna Instruments Inc., Woonsocket, Rhode Island, USA). Honey colour measurements were performed according to IRAM standard 15941-2 (1997) using HI 96785C HANNA colourimeter (Hanna Instruments Inc., Woonsocket, Rhode Island, USA). Crystallized honey was melted at 55±2ºC in thermostatic bath until complete dissolution and elimination of air bubbles, as indicated in the IRAM standard protocol. Colour was expressed in the Pfund-scale.

Determination of sugars in honey

Sugar content in the honey samples was determined according to IHC [16] on an Agilent Series 1100 HPLC system equipped with a binary pump, a thermostatted autosampler, a thermostatted column compartment and a Refractive Index Detector (RID), connected to an Agilent ChemStation software. A reversed-phase Zorbax NH^2 (250mm×4.6mm i.d, 5µm) column was used. Injection volume was 5µL. Mobile phase was acetonitrile–water (83:17, v/v). Chromatographic separation was carried out in isocratic conditions at a flow rate of 0.65 $mL·min^{-1}$ and 35 ºC. Saccharide identification in the HPLC chromatograms of the samples was achieved by comparison with retention times of available standards. Saccharides quantitation was performed by reporting the measured integration areas in the calibration equation of the corresponding standards.

HMF Determination in Honey: The HMF content in honey was determined according to the IRAM standard 15937-3 (2008) on an Agilent Series 1100 HPLC system equipped with a binary pump, a thermostatted autosampler, a thermostatted column compartment and a UV detector, connected to an Agilent ChemStation software. A reversed-phase Waters Symmetry C18 (250mm×4.6mm i.d, 5µm) column was used. The injection volume was 20µL, the mobile phase was water–methanol (95:5, v/v) and chromatographic separation was carried out in isocratic conditions at a flow rate of 0.7$mL·min^{-1}$ and 25ºC. HMF chromatographic peak was monitored and quantified at 280nm. HMF identification was performed by comparison with standard retention time, and quantitation, by reporting the measured integration areas in the calibration equation of the standard.

Data Analysis

For each honey sample, the mean and standard deviation of the three replicates were calculated for individual sugar concentration and quality parameters, indicating the relative standard deviation (n=3) at 5% or below and confirming methodological repeatability. Samples were grouped according to the vegetation units (VU-3 and VU-27) and Biomes (A-F). The dataset made up of the mean values of the physical and chemical parameters measured on the honey samples was analysed by statistical procedures, such as analysis of variance (ANOVA), the Fisher test, Least Significant Difference Test (LSD) and box and whiskers plots. Regarding the box and whiskers plots, the symmetry of data distribution, mean, median, minimum, maximum, outliers and extreme values were evaluated according to the vegetation units and Biomes. Outliers or extreme values that strayed too far from the dataset were not considered in the final data analysis results for honey characterisation. Bivariate correlations were studied by Pearson's correlation and linear regression. The significance was calculated for $p<0.05$. Data analysis was performed using the statistical software packages SPSS Statistic 17 (SPSS Inc., Chicago, IL, USA, 1993-2007) and Statistica 7.0 (StatSoft Inc., Tulsa, OK, USA, 1984-2004).

Results and Discussion

Honeys from the two vegetation units of the PPs and the different Biomes present in Misiones were characterised by sugar composition and physicochemical quality parameters, namely moisture, free acidity, pH, electrical conductivity, colour and the contents of ash, total soluble solids and HMF (Tables 1-4 and Figures S1-S5). These honeys, analysed during several seasons, displayed characteristic sugar profiles and physicochemical parameters, which are described in the next sections. The different vegetation units and Biomes were linked to the composition and quality parameter values measured in the different honeys. These parameters exhibited great variability, likely due to the different botanical species flowering throughout the honey production season. However, significant differences were only found between certain seasons and physicochemical parameters (data not shown), as had been already observed [17,18].

Sugar Profiles of Honeys

Major sugars: The amounts of fructose and glucose and F+G in honeys from Misiones varied in the range of 33-41 % fructose, 23-37 % glucose and 57-77% F+G (Table 1 and Figure S1). The Codex Alimentarius standard (Codex STAN 12-1981 Rev. 2, 2001) was fulfilled by most of the honeys for all harvests, indicating that they were genuine blossom honeys. The exceptions were three samples from Biome E and one from Biome D with F+G values between 57 and 60%, close to the limit, and whose blossom origin was confirmed by other physicochemical parameters (EC, ash content and pH). All samples contained higher amounts of fructose than glucose, supporting that almost all types of honey present greater contents of fructose than glucose [19].

The variability of the contents of fructose (33-40 %), glucose (23-37 %), F+G (57-77 %) and F/G (0.96-1.46) was larger in honeys from VU-3 than in honeys from VU-27 (35-41 % fructose, 28-35 % glucose and 63-74 % F+G and F/G 1.1-1.3) (Table 1 and Figure S1). This is since VU-3 covers a larger geographical area and includes five different Biomes (A-E); whereas VU-7, has only one Biome (F). The variability observed can be explained

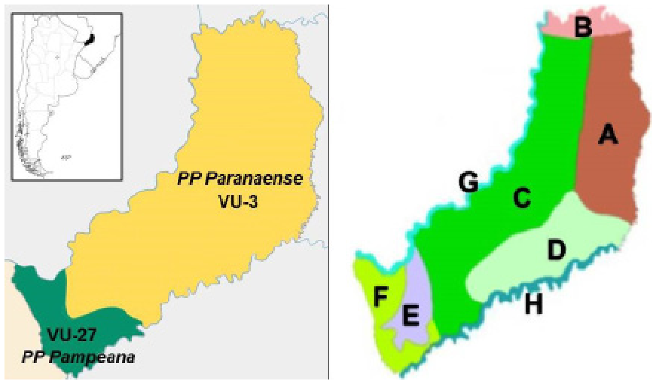

Figure 1: Honey samples were collected from the Biomes Planaltense (A), Palo Rosa (B), Lauraceae (C), Tree Ferns (D), Urunday (E) and Fields (F) in the Vegetation Units (VU) of the Phytogeographic Provinces (PP) Paranaense (VU-3) and Pampeana (VU-27) of Misiones (Argentina).

Table 1: Sugar composition of honeys from the Argentinian province of Misiones and their Vegetation Units (VU) of the Phytogeographical Provinces (PP).

Physicochemical Parameter		Misiones	PP Paranaense VU-3	PP Pampeana VU-27
Fructose	n	120	100	20
(g/100 g honey)	Mean	36.4	36.2^{a}	37.3^{b}
	SD	1.5	1.5	1.4
	Min	33.1	33.1	35.3
	Max	40.8	39.8	40.8
	Median	36.3	36	37.2
Glucose	n	120	100	20
(g/100 g honey)	Mean	30.5	30.4^{a}	30.9^{a}
	SD	2.9	3	2.3
	Min	22.9	22.9	27.6
	Max	39	39	35
	Median	30.3	30.2	30.8
F+G	n	120	100	20
(g/100 g honey)	Mean	66.9	66.6^{a}	68.2^{a}
	SD	3.8	3.9	3.5
	Min	57.2	57.2	63
	Max	76.5	76.5	74.3
	Median	66.7	66.5	67.5
F/G ratio	n	120	100	20
	Mean	1.2	1.20^{a}	1.208^{a}
	SD	0.1	0.11	0.062
	Min	0.96	0.96	1.074
	Max	1.51	1.51	1.303
	Median	1.2	1.19	1.219
Sucrose	n	120	100	20
(g/100 g honey)	Mean	0.16	0.14^{a}	0.22^{a}
	SD	0.16	0.16	0.18
	Min	n.d.	n.d.	n.d.
	Max	0.5	0.5	0.5
	Median	0.2	n.d.	0.3
Maltose	n	62	53	9
(g/100 g honey)	Mean	2.3	2.2^{a}	2.5^{a}
	SD	1.6	1.6	1.6
	Min	n.d.	n.d.	0.7
	Max	6.3	6.3	4.4
	Median	2.4	2.4	2.4
Turanose	n	62	53	9
(g/100 g honey)	Mean	2.26	2.25^{a}	2.28^{a}
	SD	0.46	0.46	0.49
	Min	1.2	1.2	1.6
	Max	3.1	3.1	2.7
	Median	2.3	2.3	2.6
Erlose	n	62	53	9
(g/100 g honey)	Mean	0.21	0.19^{a}	0.32^{a}
	SD	0.43	0.45	0.26
	Min	n.d.	n.d.	n.d.
	Max	2	2	0.6
	Median	n.d.	n.d.	0.4

Abbreviations: n: Number of Samples; SD: Standard Deviation; Min: Minimum; Max: Maximum; nd: Not Detected; F+G: Total Content of Fructose and Glucose; F/G ratio: Fructose/Glucose Ratio. Different letters within each row indicate significant differences according to Fisher's test ($p<0.05$).

by the differences in the vegetation of each Biome, since in blossom honeys the major sugars come from the nectar of the plant species that the bees visit [20]. Only the fructose content of the studied honeys showed significant differences between vegetation units. The mean value for VU-27 honeys (37%) was higher than for VU-3 honeys (36%). Significant differences were also found in the contents of fructose between Biomes, except for Biomes A, C, D, and E and Biomes C,B and F (Table 3 and Figure S3). Honeys from Biomes A, D and E presented an average fructose content of 36%; those from Biome C, 36.5%; and those from Biomes B and F, 37%. The fructose amounts of honeys from Biomes A,C,D, and E were similar to those from south of the Argentinian province of Córdoba (36%, n=56); and from Biomes B and F, to honeys from the north of Córdoba (37%, n=19) [5].

The average fructose content of honeys from Misiones (36%) was lower than those reported for the Argentinian provinces of Buenos Aires (39%, n=322 [18]; 43%, n=24 [15]), Catamarca (38%, n=68) and La Rioja (39%, n=44) [18]. The mean glucose content of honeys from Biome E (28.3%) was significantly lower than that of honeys from the other Biomes (D, 31.4%; F, 30.9%; C, 30.8%; B, 30.6%; A, 30.2%) (Table 3 and Figure S3), but was comparable to that contained in honeys from the north of Córdoba (29%, n=19) [5]. Honeys from Misiones exhibited a lower average glucose concentration (31%) than that found in honeys from Buenos Aires (33%, n=315 [18]; 33%, n=24 [15]), La Rioja (33%, n=42) and Catamarca (32%, n=69) [18]; and similar to honeys from the south of Córdoba (31%, n=56) [5].

Significant differences were observed in the average F+G between honeys from Biome E(64%) and the other Biomes (B and F, 68%; C and D, 67%), except for Biome A(66%) (Table 3 and Figure S3). The mean F+G in honeys from Misiones (67%) was lower than in those from the Argentinian province of Corrientes (75-78%, n=141) [1]; and similar to the values reported for honeys from the Argentinian province of La Pampa (68%, n=38) [21]. The average F+G of the honeys from PP Pampeana in Misiones (VU-27, 68%) was lower than that reported for eucalyptus (71%, n=28) and clover honeys (72%, n=53) from this PP in Buenos Aires [22], which include different vegetation units. The sucrose content in honeys varies as a result of the invertase activity during the ripening process of honey [23]. The presence of sucrose in honey can provide information about its adulteration and its botanical origin. Unaltered honey should present less than 5% sucrose according to the Codex Alimentarius standard (Codex STAN 12-1981 Rev. 2, 2001). Higher contents of sucrose can be due to the addition of exogenous sugars or the early harvesting of honey [24]. The sucrose contents in honeys from Misiones (≤0.50%) confirmed that all of them were authentic mature honeys harvested at the proper time, and not subjected to fraudulent practices (Table 1). The average amount of sucrose found in honeys from Misiones (0.16 %) was lower than those reported for honeys from Corrientes (1.3-2.1%, n=141) [1], La Pampa (4.1%, n=38), Buenos Aires (0.18%, n=324 [18]; 1.0-1.6%, n=24 [15]) and Catamarca (0.24%, n=66) [18]. The F/G ratio indicates the ability of honey to crystallize; thus honey seems to remain liquid when its F/G ratio is high and vice versa. Moreover, honey crystallization seemed to be slower when the F/G ratio exceeds 1.3, and faster when the ratio is below 1.0. However, the F/G ratio-based crystallization remained not demonstrated because honey contains other sugars and insoluble substances affecting the crystallization process [25, 26]. Indeed, the F/G in honeys from Misiones ranged between 0.96–1.46 (Table 1), and were found to be either crystallized or uncrystallised at r.t. before analysis. Bentabol Manzanares et al. (2011) reported an average F/G of around 1.2 for blossom honey and around 1.3 for honeydew honey [27]. In this regard, the mean F/G values were 1.15 for honeys from Biome D, 1.19 for Biome A, 1.2 for Biome C, 1.21 for Biome F, and 1.2 for Biome B, inferring their blossom origin. Honeys from Biome E exhibited the highest variability of F/G, as well as the highest average F/G ratio (1.3), being significantly different from those of the honeys from other Biomes. The F/G of honeys from Biome E seems to be a characteristic conferred to honeys by this particular Biome, since other physicochemical parameters (EC, ash content and pH) measured confirmed the blossom origin of these honeys.

Minor Sugars: Minor sugars were determined in the honeys harvested in 2014 and 2015 (Tables 1 and 3). The contents varied between 1.2-3.1% turanose, nd-6.3% maltose, and nd-1.4% erlose. Trehalose, melezitose, raffinose and maltotriose were not detected in most samples. No significant differences were observed between the average minor sugar contents of honeys from the two vegetation units (Table 1), and between the mean maltose and erlose amounts of honeys from the different Biomes (Table 3). However, the average concentration of turanose was significantly different in honeys from Biome D (2.1%) and those from Biomes A (2.4%) and E (2.7%); and in honeys from Biome B (2.1%) and E. Honeys from Biome E exhibited the highest turanose mean value; honeys from Biomes E, F, and A, the highest medians; and honeys from Biome B, the lowest median. The mean concentration of turanose (2.3%) in the honeys from Misiones was double that reported for honeys from other Argentinian phytogeographic regions, while the average amounts of maltose (2.3%) were similar [28]. The average contents of maltose (2.3%) and turanose in the studied honeys were higher than those of honeys from Buenos Aires (1.7% and 1.8% respectively, n=240 [18]), and the mean amount of turanose was also higher than that of honeys from Catamarca (2.0%, n=4) [18].

Physicochemical Quality Parameters of Honeys

Moisture: The moisture content of the honeys studied was within the range of 15.2-22.8% (Table 2); 18% of the honeys were higher than the limit established by the Codex standard. Honey is hygroscopic, i.e. it can absorb or lose water depending on environmental conditions (wet or dry respectively) [25]. The subtropical climate of Misiones without a dry season with high temperatures (16-26ºC on average) and abundant rainfall throughout the year (annual mean of 1970mm) [12,29] is likely to be the reason for the higher moisture values found in honeys from this province. The influence of rainfall on honey water content was previously reported for honeys from Córdoba; thus honeys from the southern region of the province, which receives more annual precipitation, presented the highest moisture values [5]. This was also observed in honeys from the Tabasco region (Mexico) [2] and West Bank (Palestine) [30]. The median moisture observed for honeys from Misiones (18.8%) was similar to those reported before for this province (18.4 %, n=13) [31]. Honeys from VU-3 displayed significantly higher average moisture (19%) than those from VU-27 (18%); the latter being similar to those from Corrientes (17-18 %, n=141) [1,35] (Table 2 and Figure S2). Significant differences in the mean water contents were observed between honey from Biome F (17.8%) and the other Biomes (18.8-19.7%), except for Biome E (18.7%) (Table 4 and Figure S4), which is explained by the fact that Biomes F and E are next to each other with similar climatic and pedoclimatic characteristics. Honey from Biomes

Table 2: Physicochemical parameters of honeys from the Argentinian province of Misiones and their Vegetation Units (VU) of the Phytogeographical Provinces (PP).

Physicochemical Parameter		Misiones	PP Paranaense VU-3	PP Pampeana VU-27
Moisture	n	121	101	20
(%)	Mean	18.8	19.0^{a}	17.8^{b}
	SD	1.3	1.2	1.2
	Min	15.2	16.8	15.2
	Max	22.8	22.8	19.8
	Median	18.8	18.8	17.8
Free acidity	n	121	101	20
(meq/kg honey)	Mean	41	41^{a}	37.1^{a}
	SD	10	10	9.7
	Min	17	17	18.5
	Max	66	66	51.7
	Median	41	42	37.5
pH	n	115	97	18
	Mean	3.87	3.84^{a}	4.02^{b}
	SD	0.31	0.32	0.24
	Min	3.18	3.18	3.55
	Max	4.93	4.93	4.54
	Median	3.87	3.83	4.04
EC	n	116	96	20
(µS/cm)	Mean	542	525^{a}	626^{b}
	SD	131	132	92
	Min	275	275	326
	Max	790	790	758
	Median	551	511	630
Ash content	n	120	100	20
(mg/100 g honey)	Mean	317	308^{a}	360^{b}
	SD	80	81	53
	Min	158	158	187
	Max	476	476	435
	Median	318	298	362
Colour	n	121	101	20
(mm Pfund)	Mean	87	87^{a}	86^{a}
	SD	22	23	19
	Min	41	41	52
	Max	150	150	121
	Median	82	82	83
Total soluble	n	118	100	18
content (ºBrix)	Mean	79.5	79.4^{a}	80.21^{b}
	SD	1	1.1	0.68
	Min	76.9	76.9	79.01
	Max	81.7	81.7	81.46
	Median	79.6	79.5	80.31
HMF	n	53	42	11
(mg/kg honey)	Mean	13.7	13.8^{a}	13^{a}
	SD	8.9	8.2	12
	Min	1	2	1
	Max	34	34	31
	Median	12	12	11

Abbreviations: See Table 1; EC: Electrical Conductivity; HMF: 5-Hydroxymethyl-2-Furaldehyde. Different letters within each row indicate significant differences according to Fisher's test (p<0.05).

Table 3: Sugar composition of honeys from the Biomes located in the Argentinian province of Misiones.

Physicochemical Parameter		Biomes *Planaltense* (A)	*Palo Rosa* (B)	*Lauraceae* (C)	*Tree Ferns* (D)	*Urunday* (E)	*Fields* (F)
Fructose	n	18	11	19	37	15	20
(g/100 g honey)	Mean	35.6^{a}	37.4^{b}	36.5^{ab}	36.0^{a}	36.1^{a}	37.3^{b}
	SD	1.7	1.2	1.2	1.5	1.2	1.4
	Min	33.1	35.8	34.8	33.3	34.3	35.3
	Max	38.7	39.6	38.6	38.9	38.3	40.8
	Median	35.1	37.4	36.3	35.8	35.6	37.2
Glucose	n	18	11	19	37	15	20
(g/100 g honey)	Mean	30.2^{a}	30.6^{a}	30.8^{a}	31.4^{a}	27.8^{b}	30.9^{a}
	SD	2.8	2.3	3.3	2.5	3	2.3
	Min	26.7	25.6	27	25.3	22.9	27.6
	Max	37.4	33.6	39	36.3	34.2	35
	Median	29.3	31.2	30.1	31.7	28.3	30.8
F+G	n	18	11	19	37	15	20
(g/100 g honey)	Mean	65.8^{ac}	68.0^{ab}	67.3^{ab}	67.4^{ab}	63.8^{c}	68.2^{b}
	SD	4.1	2.7	4.1	3.4	4	3.5
	Min	61.8	64.3	62	59.7	57.2	63
	Max	75.8	72.2	76.5	74.3	72.5	74.3
	Median	64.7	68.5	67.4	68	64.1	67.5
F/G ratio	n	18	11	19	37	15	20
	Mean	1.186^{ab}	1.23^{a}	1.19^{ab}	1.151^{b}	1.31^{c}	1.208^{a}
	SD	0.078	0.11	0.1	0.091	0.11	0.062
	Min	1.03	1.14	0.96	0.963	1.12	1.11
	Max	1.315	1.28	1.31	1.36	1.51	1.303
	Median	1.168	1.19	1.21	1.14	1.28	1.219
Sucrose	n	18	11	19	36	16	20
(g/100 g honey)	Mean	0.15^{a}	0.19^{a}	0.14^{a}	0.13^{a}	0.15^{a}	0.22^{a}
	SD	0.16	0.19	0.16	0.15	0.15	0.18
	Min	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Max	0.4	0.4	0.4	0.4	0.5	0.5
	Median	0.1	0.3	n.d.	n.d.	0.16	0.3
Maltose	n	9	5	9	22	8	9
(g/100 g honey)	Mean	2.5^{a}	2.4^{a}	2.5^{a}	2.1^{a}	1.9^{a}	2.5^{a}
	SD	1.8	1.4	1.6	1.3	2.4	1.6
	Min	0	0.9	0.5	0	0	0.7
	Max	4.4	3.9	5.2	4	6.3	4.4
	Median	3.4	2.8	2.7	2.4	1.2	2.4
Turanose	n	9	5	9	22	8	9
(g/100 g honey)	Mean	2.43^{ac}	2.08^{ab}	2.30^{abc}	2.06^{b}	2.65^{c}	2.28^{abc}
	SD	0.3	0.69	0.4	0.4	0.38	0.49
	Min	2.1	1.3	1.8	1.2	1.9	1.6
	Max	2.8	3.1	2.9	2.9	3	2.7
	Median	2.5	1.8	2.2	2.1	2.75	2.6
Erlose	n	9	5	9	22	8	9
(g/100 g honey)	Mean	0.10^{a}	0.56^{a}	0.08^{a}	0.15^{a}	0.30^{a}	0.32^{a}
	SD	0.15	0.77	0.16	0.44	0.64	0.26
	Min	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Max	0.3	1.4	n.d.	n.d.	0.6	0.6
	Median	n.d.	n.d.	n.d.	n.d.	n.d.	0.4

Abbreviations: See Table 1. Different letters within each row indicate significant differences according to Fisher's test (p<0.05).

B and A displayed the highest median values, i.e. 19.8 and 19.2 % respectively, in concordance with the higher humidity of the rainforest in the north of Misiones. In contrast, honeys from Biome F exhibited the lowest average value (17.8%) comparable to those reported for honeys from Buenos Aires (17.4%, n=292) [18], and about one thousand honey samples from all over the world (17.9%) [32]. The average humidity contained in the honeys analysed (18.8%) was higher than that reported for honeys from Buenos Aires (17. %, n=292 [18]; 17.0%, n=24 [15]), Catamarca (16.9%, n=62) and La Rioja (16.5%, n=44) [18], La Pampa (16.%, n=38) [21], multifloral honeys from Argentina (17.0%, n= 16) [33], Italy (17.4%, n=40) [34], and Spain (18.1%, n=40) [35].

Free Acidity: The free acidity in honeys from Misiones ranged from 17–66 meq/kg honey (Table 2), and is slightly related to its moisture. The correlation coefficient observed for these two parameters in the present study was 0.28 at p<0.05. Seventeen percent of the honeys exceeded the Codex limit, but only five samples from Biome D were also above the established limit for the free acidity parameter by the Codex standard. No significant differences were observed between the mean free acidities of honey from VU-3 and VU-27 (41 and 37 meq/kg honey respectively) (Table 2 and Figure S2). The mean free acidities of honey from Biomes C and D (45.1 and 46.3 meq/kg honey respectively) were significantly higher than those from Biomes A,B,E, and F (34.5, 35.5, 37 and 37.1 meq/

kg honey respectively) (Table 4 and Figure S4). Honeys from Misiones presented an average free acidity (41 meq/kg honey) which was higher than that for honeys from other Argentinian provinces, such as Corrientes (21.5-30.4 meq/kg honey, n=141) [1, 35], Jujuy (20.1-26.1 meq/kg honey, n=58) [36], Córdoba (20.4-22.8 meq/kg honey, n=75) [5], Catamarca (26.0 meq/kg honey, n=39 [37]; 31 meq/kg honey, n=60 [18]), La Rioja (24.5 meq/kg honey, n=43 [18]), La Pampa (30.2 meq/kg honey, n=38) [21] and Buenos Aires (17.3-21.3 meq/kg honey, n=144 [38]; 20.2 meq/kg honey, n=30 [39]; 17.0 meq/kg honey, n=24 [15]; and 23.8 meq/kg honey, n=324 [18]).

pH: The pH of most of the honeys ranged between pH 3.2 and 4.3 (Table 2 and Figure S2), confirming their blossom origin. Only two samples displayed pHs of 4.8 and 4.9, but according to their EC and ash content, they were blossom honeys. Honey pH is inversely proportional to its moisture. The correlation coefficient observed for these two parameters in the present study was -0.57 at $p < 0.05$. Honeys from VU-3 (pH 3.8) and VU-27 (pH 4.0) showed significantly different average pHs (Table 2 and Figure S2), probably due to the different botanical species growing in these VUs. Significant differences were also observed between the mean pHs regarding Biomes (pH 4.0 for Biomes A, E and F, pH 3.8 for Biomes C and D, and pH 3.7 for Biome B) (Table 4 and Figure S4). Average pH of honey from Biomes A, E and F was close to that reported for honeys from Chaco (pH 4.1, n=189) [40] and Catamarca (pH 4.1, n=61) [18]; and the mean pH of honeys from Biome B, to that of honeys from Buenos Aires (pH 3.6, n=292) [18]. Honeys from Biomes A (pH 3.3-4.9) and E (pH 3.3-4.8) presented the largest variability. The pH ranges coincided with those reported for honeys from the northeast region of Argentina (pH 3.4-4.9, n=50) [31,34]. The average pH of honeys from Misiones (pH 3.9) is lower than that observed in honeys from La Rioja (pH 4.3, n=44) [18] and Corrientes (pH 4.6, n=141) [1]. The median pH of the analysed honeys (pH 3.87) was slightly higher than that reported before (pH 3.75, n=13) [31].

Table 4: Physicochemical parameters of honeys from the Biomes located in the Argentinian province of Misiones.

Physicochemical Parameter		Biomes					
		***Planaltense* (A)**	***Palo Rosa* (B)**	***Lauraceae* (C)**	***Tree Ferns* (D)**	***Urunday* (E)**	***Fields* (F)**
Moisture (%)	**n**	18	11	19	37	16	20
	Mean	19.1^{ab}	19.65^{a}	18.8^{ab}	19.1^{ab}	18.7^{bc}	17.8^{c}
	SD	1.3	0.94	1	1.3	1.3	1.2
	Min	16.8	18.8	16.8	17	16.8	16.6
	Max	21	21	21	22	21.4	19.4
	Median	19.2	19.8	18.6	18.8	18.4	17.8
Free acidity (meq/kg honey)	**n**	18	11	19	37	16	20
	Mean	34.5^{a}	35.5^{a}	45.1^{b}	46.3^{b}	37^{a}	37.1^{a}
	SD	6.2	5	9.6	9.4	13	9.7
	Min	23.4	26.8	27.6	28.3	17	18.5
	Max	44.5	42.3	59.9	61.8	57	51.7
	Median	34.6	37.5	46.7	46.7	37	37.5
pH	**n**	18	10	19	36	14	18
	Mean	3.97^{ab}	3.71^{c}	3.80^{bcd}	3.77^{c}	3.97^{ad}	4.02^{a}
	SD	0.43	0.18	0.25	0.26	0.39	0.24
	Min	3.31	3.47	3.21	3.32	3.34	3.55
	Max	4.93	3.95	4.17	4.15	4.78	4.34
	Median	4.01	3.74	3.87	3.79	4.05	4.04
EC (µS/cm)	**n**	17	11	18	37	13	20
	Mean	523^{ac}	355^{d}	556^{a}	578^{ab}	474^{c}	626^{b}
	SD	121	67	114	117	125	92
	Min	399	307	315	359	275	549
	Max	763	359	748	790	680	758
	Median	463	325	536	577	466	630
Ash content (mg/100 g honey)	**n**	17	11	19	37	16	20
	Mean	301^{a}	204^{c}	327^{ab}	332^{ab}	310^{a}	360^{b}
	SD	69	39	72	67	103	53
	Min	229	176	181	206	158	315
	Max	438	206	465	454	476	435
	Median	266	187	309	331	281	362
Colour (mm Pfund)	**n**	18	11	19	37	16	20
	Mean	78^{a}	103^{b}	83^{a}	86^{a}	92^{ab}	86^{a}
	SD	17	21	16	22	31	19
	Min	41	69	55	54	56	52
	Max	95	133	97	108	150	121
	Median	80	106	79	82	79	83
Total soluble content (ºBrix)	**n**	18	11	19	36	16	18
	Mean	79.2^{ab}	78.72^{b}	79.60^{a}	79.53^{a}	79.7^{a}	80.21^{c}
	SD	1.2	0.79	0.97	0.89	1.3	0.68
	Min	77.4	77.96	77.38	77.87	76.9	79.01
	Max	81.7	79.46	81.26	81.01	81.3	81.46
	Median	79	78.51	79.71	79.61	80.3	80.31
HMF (mg/kg honey)	**n**	9	6	8	13	6	11
	Mean	19.4^{a}	8.3^{a}	15.6^{a}	13.1^{a}	10.2^{a}	13.3^{a}
	SD	4.9	6.3	8.1	9.5	7.1	11.5
	Min	12	3	8	2	3	1
	Max	27	17	33	34	22	31
	Median	20	6	12	11	9	11

Abbreviations: See Tables 1 and 2. Different letters within each row indicate significant differences according to Fisher's test ($p<0.05$).

Electrical conductivity: The EC value measured for the honey samples ranged from 275 to 790µS/cm (Table 2), confirming their blossom origin. The EC mean values of honeys from VU-3 (525µS/cm) and VU-27 (626µS/cm) were significantly different, the variability of the latter being considerably lower (Table 2 and Fig S2), as expected since these honeys belonged to only one Biome. The average EC of honey harvested in Biome B (355 µS/cm) differed significantly from honeys from all the other Biomes (F, 626µS/cm; D, 578µS/cm; C, 55µS/cm; A, 523µS/cm; E, 474µS/cm); honeys of Biome F from the others Biomes except for Biome D; and honeys of Biome E from the others Biomes except for Biome A (Table 4 and Fig S4). These observations reflect the influence of the botanical species and pedoclimatic characteristics of each Biome on the honey EC. The median EC of honeys from Misiones (550µS/cm) was similar to that reported for this province before (551µS/cm; n=13) [31]. The median EC of honeys from Biome E (466µS/cm) and A (463µS/cm) were comparable to that of honeys from Formosa (430µS/cm, n=10) [31]. The mean EC of honeys from Biome D and C were similar to that observed for honeys from Corrientes (573µS/cm; n=29) [1]. Honeys from Misiones (542µS/cm) exhibited lower average EC than honeys from Chaco (668µS/cm, n=189) [40], and from all over the world (640µS/cm) [32]; and higher than those from Buenos Aires (295µS/cm, n=326), Catamarca (434µS/cm, n=59) and La Rioja (501µS/cm, n=39) [18].

Ash content: All honeys studied exhibited ash contents below 600mg/100 g of honey, indicating blossom origin (Table 2). The average ash content of honeys from VU-3 (308mg/100g honey) and VU-27 (360mg/100g honey) was significantly different, the variability of the former being considerably higher due to the different Biomes included in VU-3 (Table 2 and Figure S2). Honey harvested in Biome B (204mg/100g honey) exhibited a significantly lower mean ash content with respect to all the other Biomes (A, 301mg/100g honey; E, 310mg/100g honey; C, 327mg/100g honey; D, 332mg/100g honey; and F, 360mg/100g honey); and honeys from Biome F displayed a significantly higher average value than those from Biomes B, A, and E (Table 4 and Figure S4). The ash content variability of honeys from Biomes C (181-465mg/100g honey), D (206-454mg/100g honey) and E (158-476 mg/100g honey) were large and quite similar among these Biomes. Honeys from Misiones presented an average ash content (317mg/100g honey) which was considerably higher than that of honeys from other Argentinian provinces such as La Pampa (110mg/100g honey, n=38) [21], Chubut (110mg/100g honey, n=62) [41], Buenos Aires (170mg/100g honey, n=326 [18]; 59-148mg/100g honey, n=144 [38]), and Catamarca (260mg/100g honey, n=39 [37]; 281mg/100g honey, n=67 [18]); but similar to that measured in Spanish honeys (320mg/100g honey, n=25) [42].

Colour: Most of the studied honeys displayed colours ranging from extra light amber (41mm Pfund) to dark (125.0 mm Pfund), with the average colour value being in the amber grade (87mm Pfund) (Table 2 and Figure S2). Only the average colour of honeys from Biome B (103mm Pfund) was significantly higher with respect to honeys from Biomes A (78mm Pfund), C (83mm Pfund), D (86mm Pfund) and F (86mm Pfund) (Table 4 and Figure S4). The median colour values of honeys from all Biomes were similar (79-83mm Pfund) and in the light amber grade, except for honeys from Biome B (106mm Pfund) in the amber grade. Taking into account the range of colour, honeys from Biomes B, E and F presented colours in the range from light amber to dark; from Biomes C and D, from light amber to amber; and from Biome A, from extra light amber to amber; which could be explained by the particular phenolic profiles of the botanical species characteristic of each Biome. The colour range of honeys from Biome E (56-150mm Pfund, n=16) was similar to that reported previously for honeys from Misiones (55-150 mm Pfund, n=13) [31]. Dark honeys were also found in Corrientes (29-150mm Pfund, n=141) [1] and Chaco (range: 22–150mm Pfund; n=189) [40], but in these provinces also lighter honeys than those from Misiones were produced. The colour of honeys from Misiones (41-125mm Pfund) were darker than those from Buenos Aires (18-54mm Pfund, n=321 [18]; 1-100mm Pfund, n=44 [38]), and Chubut (5-114mm Pfund, n=62) [41].

Total soluble solid content: Honey typically contains about 83 ºBrix (ºBx) [34,42] and is inversely proportional to its moisture content. The correlation coefficient observed for these two parameters in the present study was -0.94 at $p<0.05$ in agreement with previous studies [18,34,42]. The total soluble solid content of the honeys from Misiones ranged between 76.9 and 81.7 ºBx (Table 2), presenting a lower average ºBrix value (79.5 ºBx) than those reported for honeys from Buenos Aires (80.5 °Bx, n=325), Catamarca (80.8 ºBx, n=70) and La Rioja (81.7 ºBx, n=44) [18]. This could be explained by the wet subtropical climate of Misiones, which favours higher moisture contents in honey, resulting in lower °Brix values [43]. The mean ºBrix values of honeys from VU-3 (79.4 °Bx) were significantly lower compared to those of honeys from VU-27 (80.2 °Bx), and exhibited higher variability (Table 2 and Figure S2). Honeys from Biomes F (80.2ºBx) and B (78.7ºBx) contained significantly different average amounts of total soluble solids between each other and with respect to the other Biomes (A, 79.2 ºBx; D, 79.5 ºBx; C, 79.6 ºBx; and E, 79.7 ºBx) (Table 4 and Figure S4).

HMF content: The HMF value was determined for the honeys collected in 2015 and 2017 harvests, ranging between 1-34 mg HMF/kg honey (Table 2), complying with the HMF content limits established by international standards. This was indicative of good quality, fresh and unprocessed honeys, and suggested good practices by beekeepers. The average HMF content of honeys from Misiones (13.7mg HMF/kg honey) was close to that reported for honeys from Catamarca and La Rioja (11.3 (n=27) and 9.8 (n=21)mg HMF/kg honey respectively) [18], and higher than that for honeys from Buenos Aires (4.2 mg HMF/kg honey, n=126) [18]. The median HMF content of honeys analysed (12.0mg HMF/kg honey) was close to that for honeys from Corrientes (11mg HMF/kg honey, n=16), but higher than the data previously reported for honeys from this province (6mg HMF/kg honey); and lower than that for honeys from Formosa (33mg HMF/kg honey, n=10) and Chaco (28mg HMF/kg honey, n=10) [31]. Climate can influence the HMF content [5]. In tropical regions, where honeys are exposed to high temperatures for long periods, sugar decomposition pathways that lead to the formation of HMF are favoured, and therefore, these honeys can present higher HMF amounts [44]. Therefore, the higher HMF contents observed in honeys from Misiones were explained by its characteristic wet subtropical climate and higher moisture content [13]. No significant differences were observed between the mean HMF contents of honeys from the different vegetation units and Biomes (Tables 2 and 4 and Figure S5).

Most of the honeys analysed complied with national (Codigo Alimentario Argentino Ley 18284, Res. MSyAS Nº 003, 1995) and international regulations established by the Codex Alimentarius Commission (Codex STAN 12-1981 Rev. 2, 2001), EU Council (Council Directive 2001/110/EC, 2001) and Mercosur (Res. Nº 89/99, 1999), regarding the physical and chemical quality parameters studied. Only a small percentage of samples presented moistures or free acidities higher than the established limit. These parameter values could be considered characteristic of honeys from Misiones, associated with the particular botanical species of the Biomes and/or the pedoclimatic conditions of the region. Indeed, it is observed that the moisture content has a certain influence on the free acidity values, i.e. increasing humidity can lead to an increase in free acidity and a decrease in pH. The analytical results disclosed that the honeys from Misiones were high-quality honeys obtained under adequate beekeeping and processing practices. Several parameters, i.e. F+G content, F/G ratio, EC, ash content and pH, indicated the blossom origin of the honeys. The contents of sucrose, water and HMF indicated the good maturity and freshness of the honeys, harvested in the proper time and season. The moisture and free acidity measurements revealed the absence of undesirable fermentation in the honeys. Low EC and contents of HMF and sucrose were indicative of a high control of production, good beekeeping practices and good preservation state of samples.

Conclusions

The results of the present study evidence the influence of the different vegetation units, regarding both the flora and the pedoclimatic conditions, and Biomes on the physical and chemical parameters of the honeys from Misiones. In this sense, honeys from VU-3 and VU-27 presented significantly distinctive quality parameters; the contents of fructose, ash and total soluble solids, pH and EC were higher in honeys from VU-27 whereas their moisture was lower. Honeys from Biome E exhibited characteristic darker colours, higher pH, F/G ratio and amounts of turanose, and lower contents of water, glucose and F+G. Honeys from Biome F were also typified by lower moisture content and higher pH, in addition to higher EC and contents of fructose, total soluble solids and ash. In contrast, honeys from Biome B showed lower EC and ash content, but higher amounts of fructose and total soluble solids, and particularly darker colours. Honeys from Biomes C and D were characterised by higher free acidities; and those from Biome A, by higher pH values. However, none of the physical and chemical parameters measured was completely discriminant among the honeys according to the phytogeographical origin, the vegetation unit or Biome to which it belongs. To the best of the authors' knowledge, data on the sugar profiles, free acidity and content of ash and total soluble solids in honeys from the different PPs, vegetation units and Biomes of Misiones are reported here for the first time.

The relevance of this work lies in the extended knowledge generated through the study of the set of traceable and representative honey samples from the different PPs and Biomes located in the province of Misiones and collected during four harvests. Thus, seasonal variability was considered, which is a requirement to characterise any agricultural food product. The classification of the honeys from each of the studied phytogeographical regions and Biomes will provide them with added value and allow them to access new markets. Furthermore, classified honey has a higher commercial value than standard-quality honey. Indeed, there is currently a growing global demand for differentiated products. In this framework, the importance of having typified honeys is evident, and the contribution of this study to the characterization of honeys from Misiones is noteworthy.

Author Statements

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References

1. Fechner DC, Moresi AL, Ruiz Diaz JD, Pellerano RG, Vazquez FA. Multivariate classification of honeys from Corrientes Argentina according to geographical origin based on physicochemical properties. Food BioSci. 2016; 15: 49-54.
2. Viuda-Martos M, Ruiz-Navajas Y, Zaldivar-Cruz JM, Kuri V, Fernández-López J, et al. Aroma profile and physico-chemical properties of artisanal honey from Tabasco, Mexico. Int J Food Sci Technol. 2010; 45: 1111-1118.
3. Gheldof N, Wang XH, Engeseth NJ. Identification and quantification of antioxidant components of honeys from various floral sources. J Agric Food Chem. 2002; 50: 5870-5877.
4. Silva LR, Gonçalves AC, Nunes AR, Alves G. Authentication of honeys from Caramulo region (Portugal): Pollen spectrum, physicochemical characteristics, mineral content, and phenolic profile. J Food Sci. 2020; 85: 374-385.
5. Baroni MV, Arrua C, Nores ML, Faye P, Diaz MdP, et al. Composition of honey from Cordoba Argentina: Assessment of North/South provenance by chemometrics. Food Chem. 2009; 114: 727-733.
6. Bogdanov S, Lüllmann C, Martin P, von der Ohe W, Russmann H, et al. Honey quality and international regulatory standards: Review by the international honey commission. Bee World. 1999; 80: 61-68.
7. Carvalho CAL, Sodre GS, Fonseca AAO, Alves RMO, Souza BA, et al. Physicochemical characteristics and sensory profile of honey samples from stingless bees Apidae: Meliponinae submitted to a dehumidification process. Anais Acad Bras Cienc. 2009; 81: 143-149.
8. Warui MW, Hansted L, Gikungu M, Mburu J, Kironchi G, et al. Characterization of Kenyan Honeys Based on Their Physicochemical Properties, Botanical and Geographical Origin. Int J Food Sci. 2019; 2019.
9. Sakac MB, Jovanov PT, Maric AZ, Pezo LL, Kevresan ZS, et al. Physicochemical properties and mineral content of honey samples from Vojvodina (Republic of Serbia). Food Chem. 2019; 276: 15-21.
10. Ministerio de Agricultura Ganadería y Pesca de Argentina. Apicultura.
11. Rodriguez ME, Cardozo A, Ruiz Díaz M, Prado D. Los bosques nativos misioneros: estado actual de su conocimiento y perspectivas. Ecología y manejo de los bosques de Argentina. 2005: 3-33.
12. Servicio Meteorológico Nacional Argentina. Atlas Climático Argentina.
13. Oyarzabal M, Clavijo JR, Oakley LJ, Biganzoli F, Tognetti PM, et al. Unidades de vegetación de la Argentina. Ecol Austral. 2018; 28: 40-63.
14. Ministerio de Ecología y Recursos Naturales Renovables - Gobierno de Misiones. Bosques nativos.

15. Silvano MF, Varela MS, Palacio MA, Ruffinengo S, Yamul DK. Physicochemical parameters and sensory properties of honeys from Buenos Aires region. Food Chem. 2014; 152: 500-507.

16. Bogdanov S. Harmonised methods of the International Honey Commission. IHC. 2009: 1-63.

17. Scholz MBS, Quinhone Júnior A, Delamuta BH, Nakamura JM, Baudraz MC, et al. Indication of the geographical origin of honey using its physicochemical characteristics and multivariate analysis. J Food Sci Technol. 2020; 57: 1896-1903.

18. Poliero AA, Aubone I, Amadei Enghelmayer M, Rosso VS, Müller PF, et al. Characterization of Argentinian honeys based on their sugar profiles and quality parameters. Journal of Food Chemistry and Nanotechnology. 2022; 8: 26-37.

19. White JW. Physical characteristics of honey. Crane E, editors. in: Honey: A Comprehensive Survey. London: Heinemann. 1975; 207-239.

20. Escuredo O, Dobre I, Fernández-González M, Seijo MC. Contribution of botanical origin and sugar composition of honeys on the crystallization phenomenon. Food Chem. 2014; 149: 84-90.

21. Cantarelli MA, Pellerano RG, Marchevsky EJ, Camiña JM. Quality of honey from Argentina: Study of chemical composition and trace elements. J Argent Chem Soc. 2008; 961: 33-41.

22. Ciappini M, Vitelleschi M, Calvino A. Chemometrics Classification of Argentine Clover and Eucalyptus Honeys According to Palynological, Physicochemical, and Sensory Properties. Int J Food Prop. 2016; 19: 111-123.

23. Crane E. Honey from honeybees and other insects. Ethol Ecol Evol. 1991; 3: 100-105.

24. Da Silva PM, Gauche C, Gonzaga LV, Costa ACO, Fett R. Honey: Chemical composition, stability and authenticity. Food Chem. 2016; 196: 309-323.

25. Amir Y, Yesli A, Bengana M, Sadoudi R, Amrouche T. Physico-chemical and microbiological assessment of honey from Algeria. Elec J Environ Agric Food Chem. 2010; 9: 1485-1494.

26. Saeed MA, Jayashankar M. Physico-chemical characteristics of some Indian and Yemeni Honey. J Bioener Food Sci. 2020; 7: 2832019.

27. Bentabol Manzanares A, García ZH, Galdon BR, Rodríguez ER, Romero CD. Differentiation of blossom and honeydew honeys using multivariate analysis on the physicochemical parameters and sugar composition. Food Chem. 2011; 126: 664-672.

28. Patrignani M, Ciappini MC, Tananaki C, Fagúndez GA, Thrasyvoulou A, et al. Correlations of sensory parameters with physicochemical characteristics of Argentinean honeys by multivariate statistical techniques. Int J Food Sci Technol. 2017; 53: 1176-1184.

29. Piccolo GA, Andriulo AE, Mary B. Changes in soil organic matter under different land management in Misiones province Argentina. Scientia Agricola. 2008; 65: 290-297.

30. Abdulkhaliq A, Swaileh KM. Physico-chemical properties of multi-floral honey from the West Bank, Palestine. Int J Food Prop. 2017; 20: 447-454.

31. Fechner DC, Hidalgo MJ, Ruiz Díaz JD, Gil RA, Pellerano RG. Geographical origin authentication of honey produced in Argentina. Food BioSci. 2020; 33: 100483.

32. Solayman M, Islam MA, Paul S, Ali Y, Khalil MI, et al. Physicochemical properties, minerals, trace elements, and heavy metals in honey of different origins: a comprehensive review. Food Sci Food Saf. 2016; 15: 219-233.

33. Conti ME, Finoia MG, Fontana L, Mele G, Botre F, Iavicoli I. Characterization of Argentine honeys on the basis of their mineral content and some typical quality parameters. Chem Cent J. 2014; 8: 44.

34. Conti ME, Canepari S, Finoia MG, Mele G, Astolfi ML. Characterization of Italian multifloral honeys on the basis of their mineral content and some typical quality parameters. J Food Compos Anal. 2018; 74: 102-113.

35. Rodríguez-Flores MS, Escuredo O, Seijo-Rodríguez A, Seijo MC. Characterization of the honey produced in heather communities NW Spain. J Apic Res. 2019; 58: 84-91.

36. Rios F, Sanchez AC, Lobo M, Lupo L, Coelho I, et al. A chemometric approach: Characterization of quality and authenticity of artisanal honeys from Argentina. J Chemometr. 2014; 28: 834-843.

37. Vergara-Roig VA, Costa MC, Kivatinitz SC. Relationships among botanical origin, and physicochemical and antioxidant properties of artisanal honeys derived from native flora Catamarca, Argentina. Int Food Res J. 2019; 26: 1459-1467.

38. Malacalza NH, Mouteira MC, Baldi B, Lupano CE. Characterisation of honey from different regions of the province of buenos aires, Argentina. J Apic Res. 2007; 46: 8-14.

39. Fangio MF, Iurlina MO, Fritz R. Characterisation of Argentinean honeys and evaluation of its inhibitory action on Escherichia coli growth. Int J Food Sci Technol. 2010; 45: 520-529.

40. Salgado CR, Maidana JF. Physicochemical characterisation of honey produced in the chaco province (Argentina). Revista Facultad Ciencias Agrarias. 2014; 46: 191-201.

41. Aloisi PV. Determination of quality chemical parameters of honey from Chubut Argentinean Patagonia. Chil J Agric Res. 2010; 70: 640-645.

42. Terrab A, Recamales AF, Hernanz D, Heredia FJ. Characterisation of Spanish thyme honeys by their physicochemical characteristics and mineral contents. Food Chem. 2004; 88: 537-542.

43. Batu A, Aydogmuş RE, Bayrambaş K, Eroglu A, Karakavuk E, et al. Changes in Brix, pH and total antioxidants and polyphenols of various honeys stored in different temperatures. J Food Agric Environ. 2014; 12: 281-285.

44. Fallico B, Arena E, Zappala M. Degradation of 5-hydroxymethylfurfural in honey. J Food Sci. 2008; 73: C625-C631.