Exploring the diversity of Uruguayan native flora honeys

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Abstract

This work proposes the revaluation of honeys from the native flora of Uruguay. The results obtained in the pollen and physicochemical analysis allow us to conclude that it is possible to characterize and distinguish monofloral honeys produced from Uruguayan native flora, and that they also present interesting properties that make them stand out from the rest of the honeys. This could be a starting point for the exploitation of this market of little-known honeys with great nutritional potential.

Keywords: honey, native flora, Uruguay, diversity, valorization

Estudio de la diversidad de las mieles de la flora autóctona uruguaya

Resumen

Este trabajo propone la revalorización de mieles provenientes de la flora nativa de Uruguay. Los resultados obtenidos en el análisis polínico y fisicoquímico permiten concluir que es posible caracterizar y distinguir las mieles monoflorales producidas a partir de flora nativa de Uruguay y que, además, presentan propiedades interesantes que las diferencian del resto de las mieles. Este podría ser un punto de partida para la explotación de este mercado de mieles poco conocidas y con gran potencial nutricional.

Palabras clave: miel, flora nativa, Uruguay, diversidad, valorización

Explorando a diversidade dos méis da flora nativa do Uruguai

Resumo

Este trabalho propõe a revalorização dos méis da flora nativa do Uruguai. Os resultados obtidos nas análises polínicas e físico-químicas permitem concluir que é possível caraterizar e distinguir os méis monoflorais produzidos a partir da flora nativa do Uruguai e que estes também apresentam propriedades interessantes que os destacam do resto dos méis. Este poderia ser um ponto de partida para a exploração deste mercado de méis pouco conhecidos e com grande potencial nutricional.

Palavras-chave: mel, flora nativa, Uruguai, diversidade, valorização

1. Introduction

In Uruguay, honey remains a prominent component among the products earmarked for export. However, the export market has experienced significant annual fluctuations, contributing to considerable uncertainty for honey producers in recent years[1]. Given this situation, there is a need to explore tools that facilitate efforts to achieve a more favorable pricing structure for honey production. Simultaneously, there is an opportunity to promote domestic honey consumption by enhancing the perceived value of our honey. Honey gains heightened value when predominantly sourced from a single plant species (monofloral honeys) or from a specific geographical region, referred to as honeys of specific origin (e.g., those from bushes, meadows)[2][3][4]. The production of monofloral/multifloral honeys depends on various factors, particularly the availability of nectar-producing plants in each region and season. In the case of monofloral honeys, derived from the nectar of a specific plant, their elevated commercial value results from the ability to showcase distinctive chemical and sensory characteristics, including flavor and aroma.

Honey composition depends on the floral origin of the nectar on which the bees feed, as well as on external factors such as climate, environment, and processing conditions[5]. Floral origin can be very varied, which is reflected in final products that differ both in appearance and in sensory perception and composition. From a phytogeographical point of view, the flora of Uruguay is mostly found in the Pampean province[6]. The main ecosystems of Uruguay include natural grasslands, forests, wetlands, and coastal ecosystems.

In recent years, the area of grassland has been significantly reduced due to the increase in forestry production, cultivated pastures and annual crops[7]. One response to the soil deterioration on the part of producers is the planting of certain promising (non-native) legumes to complement pasture[8]. Native forests, on the other hand, occupy a much smaller portion of the territory than grasslands (3.5%), however, they have a high ecological value due to the number of plant species present[9].

In the beekeeping context, this aggregation of ecosystems (bush, grassland) and agroecosystems (afforestation, grasslands improved with legumes) is where apiaries for honey production are installed at different intensities. These ecosystems are neither isolated nor permanent but vary over time and present ecotones that are reflected in the botanical composition of our honeys[10][11][12][13]. In turn, the forest dynamics plays a role of great importance, because it is characterized by early flowering (a low flowering period in December-January followed by a new flowering in February-March. During the low flowering period of the bush, melliferous production is mainly based on grasslands (white clover, lotus) or on other annual (e.g. sunflower) or permanent crops (fruit trees, *Eucalyptus* sp.). In turn, the late flowering of the bush is very useful for the hive to store reserves for the winter[14].

Starting from all such considerations, this study demonstrates that the combination of melissopalynology and physicochemical parameters, as a first approximation to achieve a complete characterization, can be a useful tool for discrimination between different kinds of honeys according to their bee species origin.

2. Materials and Methods

2.1 Honey sampling

Fifteen honey samples were collected from the Southeastern region of Uruguay. Eight samples were harvested between March and April 2016 from Maldonado department, and one obtained from Lavalleja department (**Table 1**). A second group of six samples corresponded to honeys harvested during September and October 2016, being

five obtained from Canelones and one from Rocha (1 sample) departments (**Table 1**). All samples were coded and stored in sterile glass jars, kept at 4°C in the dark until analyzed (no longer than two months).

Table 1. Detail of tested honey samples

Detail of honey location		N° of samples	Sampling time
Villa del Rosario	Lavalleja Department	1	March-April
Aiguá	Maldonado Department	4	
Carapé		1	
Las Cañas, San Carlos		1	
San Carlos		2	
Castillos	Rocha Department	1	September-October
Canelón Chico	Canelones Department	1	
Toledo		2	
Sauce		1	
Santa Lucia		1	

2.2 Melissopalynological analysis

The melissopalynological analysis of the samples was carried out at the Instituto de Ciencias Geológicas, Facultad de Ciencias-Udelar, Montevideo (Uruguay). This analysis was carried out according to the method proposed by Louveaux and others[15].

2.3 Physicochemical analysis

All the physicochemical parameters were determined in triplicate according to the Harmonized Methods of the International Honey Commission-IHC[16] and the Association of Official Analytical Chemists-AOAC[17].

2.3.1 Color and HMF

Color was determined in mm on the Pfund scale according to the US Department of Agriculture classifications (water white: <9, extra white: 9–17, white: 18–34, extra light amber: 35–50, light amber: 51–85, amber: 86–114, dark amber: >114)[18].

Hydroxymethyl furfural (HMF) was measured using the method of White[19] and honey color was determined using a digital photometer (HANNA HI 96785). The limits of acceptance used were those given by Codex Alimentarius Standard (HMF: ≤40 mg/kg)[20].

2.3.2 Diastase activity

This enzyme hydrolyses starch and dextrins resulting in carbohydrates of lower molecular weight. Due to the sensitivity of diastase to heat and storage, its measurement is used as an indicator of honey freshness[21]. According to national and international regulations, honey must have a minimum diastase activity of 8 on the Gothe scale[16][22]. For the determination of diastase activity, the method of Schade and others[23] was used, which is based on the rate of hydrolysis of a 2% starch solution by diastase enzymes in a buffered solution of honey employing an iodine solution as an indicator, using a spectrophotometric measurement at 660 nm. Diastase activity was calculated as DN (diastase number) as follows[24]:

DN = (60min / tx)* (0,10/0,01) * (1,0/2,0) = 300/tx

where tx is the reaction time (min) to reach 0.235 absorbance.

2.3.3 Sugar composition

Sugar analysis was performed according to Bogdanov and Baumann[25]. Briefly, 5 g of honey were mixed with distilled water (40 mL) and transferred to a 100 mL volumetric flask and then mixed with methanol. Next, 10 mL of this dissolution was filtered through a 0.20-μm cellulose acetate membrane prior to HPLC analysis. The determination of the sugars (fructose, glucose, sucrose, maltose and turanose) was performed using a LC 20-10 series Shimadzu chromatograph equipped with a refractive index detector operated at 30 °C and a SUPELCOSIL™ LC-NH2 HPLC Column (5 μm particle size, 250 × 4.6 mm). Acetonitrile (80%) in water was used as the mobile phase (flow rate 1.3 mL/min). The HPLC sample peaks were identified by comparing the retention times obtained from standards. Duplicate injections were performed, and the average peak areas were used for the quantification.

2.3.4 Mineral content

Na, K, Ca and Mg were analysed by atomic absorption spectrometry with acetylene air flame using an Analyst 200 spectrometer (PerkinElmer, Inc., MA, USA). In order to minimize organic matrix effects, a microwave-assisted sample digestion was performed according to Chudzinska and Baralkiewicz[26] before analysis. In brief, the heating program consisted of a 20 min ramp up to 120 °C, holding at that temperature for 20 min, followed by a 20 min ramp from 120 to 170 °C, and remaining 15 min at that temperature.

2.4 Statistical analysis

Principal components analysis (PCA) was performed using the InfoStat software[27].

3. Results

3.1 Melissopalynological analysis

Table 2 shows the results of the palynological analysis of honeys and its typification (monofloral or polyfloral) according to the percentage of pollen observed. Eight of the fifteen honey samples were classified as monofloral, where five of them were from native plants (SMm03, Sbm04, Sbm05, Sbm06 and Sbm09), and three were originated from introduced plants (Lm01, Lm13, Lm14). The remaining seven honey samples were found to be polyfloral. Among the native monofloral honeys the predominant pollen found was of *Scutia buxifolia* in four of them, and one of *Schinus molle*. The corresponding three non-native monoflorals were found to be *Lotus* sp, while the remaining seven polyfloral samples showed a predominance of *Eucalyptus* sp. pollen.

Figure 1 shows the geographic distribution of the honey samples classified according the melissopalynological analysis as presented in **Table 2**.

Table 2. Main pollen types in the evaluated honeys

Sample Code	Typification	Origen floral	
		% main pollen	**% minor pollen**
Lm01	Monofloral	*Lotus sp* (77.6%)	*Ammi sp* (5.6%); *Eucalyptus sp* (5.0%); *Trifolium pratense* (3.1%); *Asterea* (2.4%); *Echium sp* (1.7%); *Trifolium repens* (1.0%); *Scutia buxifolia* (1.0%)
Sbp02	Polyfloral	*Scutia buxifolia* (43.9%); *Schinus molle* (24.4%); *Eucalyptus sp* (12.5%); *Salix sp.* (5.7%); *Lotus sp* (3.8%); *Blepharocalyx tweediei* (2.8%); *Echium sp* (2.1%); *Allophylus edulis* (1.2%)	
SMm03	Monofloral	*Schinus molle* (62.9%)	*Scutia buxifolia* (17.9%); *Blepharocalyx tweediei* (3.8%); *Lotus sp* (3.6%); *Eucalyptus sp* (2.7%); *Baccharis sp* (2.4%); *Trifolium repens* (1.2%); *Struthanthus acuminatus* (1.2%)
Sbm04	Monofloral	*Scutia buxifolia* (52.8%)	*Blepharocalyx tweediei* (30.8%); *Schinus molle* (10.6%); *Eucalyptus sp* (1.8%)
Sbm05	Monofloral	*Scutia buxifolia* (64.2%)	*Schinus molle* (29.1%); *Eucalyptus sp* (1.7%)
Sbm06	Monofloral	*Scutia buxifolia* (51.4%)	*Schinus molle* (44.5%)
Ep07	Polyfloral	*Eucalyptus sp* (41.7%); *Schinus molle* (18.3%); *Scutia buxifolia* (10.3%); *Baccharis sp* (8.3%); *Echium sp* (6.9%); *Blepharocalyx tweediei* (4.7%); *Trifolium repens* (2.8%); *Trifolium pretense* (1.7%); *Acacia sp* (1.6%)	
Ep08	Polyfloral	*Eucalyptus sp* (39.3%); *Scutia buxifolia* (31.6%); *Echium sp* (9.3%); *Blepharocalyx tweediei* (6.7%); *Lotus sp* (5.2%); *Schinus molle* (1.4%); *Baccharis sp* (1.2%)	
Sbm09	Monofloral	*Scutia buxifolia* (55.2%)	*Schinus molle* (27.8%); *Eucalyptus sp* (8.0%); *Blepharocalyx tweediei* (4.2%)
Ep10	Polyfloral	*Eucalyptus sp* (46.7%); *Acacia longifolia* (34.8%); *Lotus sp* (3.9%); *Papilionoidea* (3.0%); *Shinus molle* (2.6%); *Euphorbiaceae* (2.4%); *Baccharis sp* (2.2%)	
Ep11	Polyfloral	*Eucalyptus sp* (71.5%); *Lotus sp* (5.9%); *Trifolium repens* (5.4%); *Trifolium pretense* (5.3%); *Baccharis sp* (4.7%); *Shinus molle* (1.7%)	
Tp12	Polyfloral	*Trifolium repens* (23.2%); *Trifolium pretense* (22.2%); *Lotus sp* (15.5%); *Eucalyptus sp* (13.5%); *Shinus molle* (5.3%); *Ligudtrum vulgare* (2.8%); *Echium sp* (2.3%); *Brassicaceae* (2.2%); *Cynara cardunculuus* (2.0%); *Mentha pulegium* (1.8%); *Ammi visnaga* (1.5%); *Daucus carota* (1.0%)	
Lm13	Monofloral	*Lotus sp* (49.0%)	*Schinus molle* (12.1%); *Trifolium pretense* (10.2%); *Scutia buxifolia* (9.0%); *Eucalyptus sp* (7.0%); *Trifolium repens* (6.0%); *Ammi visnaga* (1.5); *Cynara cardunculuus* (1.5%)
Lm14	Monofloral	*Lotus sp* (61.6%)	*Eucalyptus sp* (15.9%); *Trifolium repens* (6.5%); *Scutia buxifolia* (3.6%); *Schinus molle* (2.9%); *Ammi visnaga* (2.1%); *Trifolium pretense* (1.4%); *Echium sp* (1.3%)
Sbp15	Polyfloral	*Scutia buxifolia* (42.1%); *Echium sp* (22.4%); *Lotus sp* (14.0%); *Schinus molle* (5.9%); *Eucalyptus sp* (5.7%); *Trifolium repens* (3.7%); *Trifolium pretense* (1.7%); *Brassicaceae* (1.3%)	

Lm, *Lotus* sp. Monofloral; Sbp, *Scutia buxifolia* polyfloral; SMm, *Schinus molle* monofloral; Sbm, *Scutia buxifolia* monofloral; Ep, *Eucalyptus* sp. polyfloral; Tp, *Trifolium* polyfloral. The number corresponds to the order of sample entry. Lm01 (Villa del Rosario), Sbp02 (Aiguá), SMm03 (Aiguá), Sbm04 (Aiguá), Sbm05 (Aiguá), Sbm06 (Carapé), Ep07 (Las Cañas, San Carlos), Ep08 (San Carlos), Sbm09 (San Carlos), Ep10 (Castillos), Ep11 (Canelón Chico), Tp12 (Toledo), Lm13 (Toledo), Lm14 (Sauce), Sbp15 (Santa Lucía)
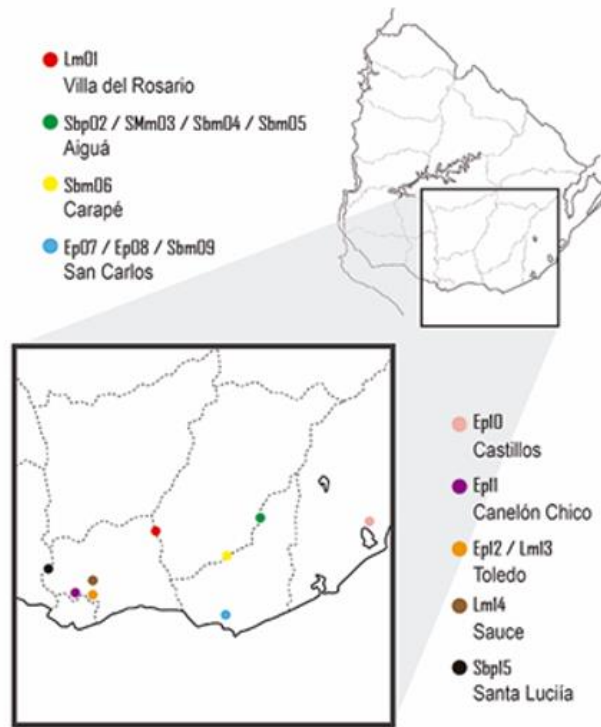
Figure 1. Geographic zones for honeys sampling

3.2 Physicochemical analysis

Table 3 shows the results obtained from the physicochemical analysis of the honey samples.

Table 3. Physicochemical properties of honey samples

Sample Code	pH	Free Acidity (meq/kg)	Moisture (%)	Ash (%)	Diastase activity	HMF (mg/kg)	EC (mS/cm)	*Pfund Color (mm)
Lm01	4.0	21.5	17.6	0.06	48.4	0	0.51	48
Sbp02	5.6	12.0	18.4	0.50	26.6	0	1.32	99
SMm03	4.7	19.0	19.2	0.38	45.2	0	1.06	98
Sbm04	4.9	18.5	18.4	0.46	33.0	0	1.27	112
Sbm05	6.1	7.5	18.8	0.49	32.0	0	1.44	102
Sbm06	5.2	7.0	18.8	0.44	26.4	0	1.17	92
Ep07	5.5	13.5	18.8	0.46	22.7	0	1.38	118
Ep08	4.8	14.5	17.6	0.57	17.5	0	1.72	109
Sbm09	5.8	8.0	17.8	0.45	15.1	0	1.35	92
Ep10	4.2	18.5	15,0	0.20	14.7	0	1.03	71
Ep11	3.6	29.5	17.4	0.19	18.3	15.2	0.63	65
Tp12	3.5	30.0	17.2	0.11	24.8	8.6	0.48	44
Lm13	3.7	24.5	15	0.15	22.7	7.6	0.44	50
Lm14	3.5	36.5	16.8	0.08	24.9	8.5	0.49	60
Sbp15	4.4	17.5	17.4	0.39	n.d.	1.0	1.08	145
min	3.5	7.0	15.0	0.10	14.7	0.0	0.44	44
max	6.1	36.5	19.2	0.60	48.4	15.2	1.72	145

Codex Alimentarius Standard (moisture: ≤ 20%, Pfund color: 0-150 mm; EC: ≤0.8 mS/cm; pH: 3.4-6.1; FA: ≤50 meq/kg; HMF: ≤40 mg/kg; DN: ≥8)[20].

3.2.1 pH and acidity

The pH values ranged from 3.5 to 6.1, which are within the Food Codex standard limits, which establishes the pH limit for honey between 3.4 and 6.1[20]. The pH values are important for honey extraction and storage as it influence its texture, stability, and shelf life[28], being also low enough to inhibit the development of microorganisms[29][30].

Acidity values ranged from 7 to 36.5 meq/kg within the maximum value accepted of 50 meq/kg., while the moisture percentage ranged between 15.0 and 19.2%, a value that is below the maximum limit established of 20% in the Codex standard for honey[20].

3.2.2 HMF

HMF values were all below the maximum allowable limit of 40 mg/kg, indicating that the honeys were not subjected to heat treatment after being harvested. In addition, these low HMF values also agree with the diastase activity values found not less than 8 Schade units[20].

3.2.3 Ash and conductivity

As for the ash percentage, the values obtained were below the maximum limit established (0.6%) in the Uruguayan directives for flower honey[22]. This result is also indicative of the cleanliness of the honey samples and the absence of adulteration with molasses[31].

Conductivity values ranged from 0.44 to 1.70 ms/cm, where only 5 of the 15 honey samples showed values below the maximum limit (0.8 ms/cm) defined by the Codex Alimentarius[20]. Generally, both conductivity and ash content are related to the mineral content in honey[32][33] and so honeys with higher ash percentage presented higher conductivity values. **Figure 2** shows the relationship between ash content and electrical conductivity found for the honeys here studied.
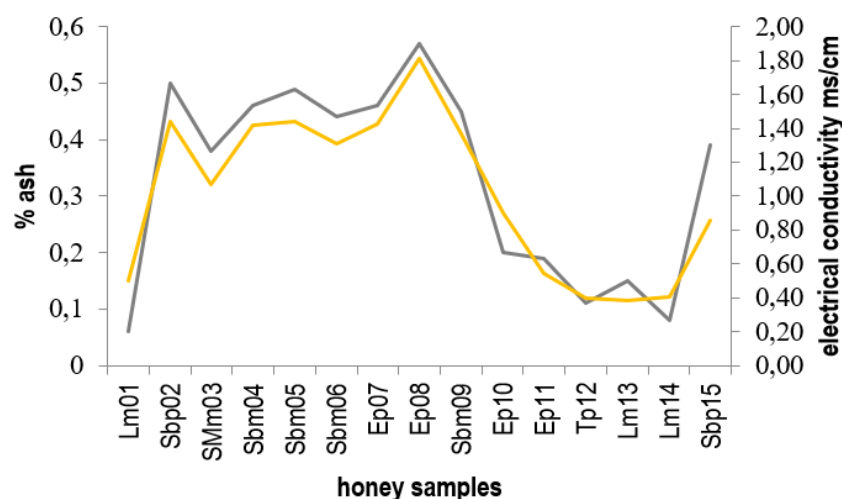

Figure 2. Ash content (gray) and electrical conductivity (yellow) relationship for the different honeys

3.2.4. Minerals

Table 4 shows the mineral content of the honeys studied. The most abundant mineral in all the honey samples was potassium (K), ranging in values between 582.3 and 3585.5 mg/kg honey; followed by sodium (Na) or calcium (Ca), depending on the honey considered, with values between 36.4-608.7 and 63.5-209.4 mg/kg

honey, respectively. For all honeys the least abundant mineral was magnesium (Mg), with values between 16.1-57.7 mg/kg honey.

In brief, the relationship between mineral content, ash and conductivity in honey is because the minerals present in honey contribute to both the mineral content and the ash, and these minerals are also responsible for its ability to conduct electricity, which affects its electrical conductivity.

Table 4. Mineral profile

Sample Code	Na	K	Ca	Mg
Lm01	114.5	783.3	63.5	16.1
Sbp02	67.8	3265.6	68.6	43.9
SMm03	46.3	2647.8	69.9	51.9
Sbm04	61.1	3137.2	195.9	57.7
Sbm05	40.0	3585.5	79.1	35.8
Sbm06	36.4	3081.0	132.9	33.5
Ep07	278.4	2931.1	169.0	53.5
Ep08	497.5	3542.6	209.4	49.6
Sbm09	57.8	3042.0	122.8	36.7
Ep10	608.7	1014.7	124.8	53.3
Ep11	127.6	892.6	148.6	31.5
Tp12	117.2	582.3	114.6	20.0
Lm13	148.3	618.8	105.1	18.6
Lm14	106.4	642.9	129.1	23.6
Sbp15	117.8	1948.2	130.8	31.1

3.2.5 Color

As for color, honeys presented a wide range of values on the Pfund scale ranging from 48 to 145 mm (extra light amber to dark amber, respectively).

Samples Sbm04, Sbm05, Sbm06 and Sbm09 defined as *Scutia buxifolia* monofloral and SMm03 (*Shinus molle* monofloral) presented conductivity values higher than those established by Codex Alimentarius of 0.8 ms/cm [20] and were amber in color. Instead, *Lotus* monofloral honeys (Lm01, Lm13, Lm14) showed conductivity values below the maximum limit established by Codex Alimentarius (0.8 ms/cm)[20], while their color ranged between light amber and extra light.

Figure 3 shows the relationship between electrical conductivity and color for the honeys studied. Similar results were previously reported correlating high conductivity values to the dark color of the honeys[21][34][35][36][37].
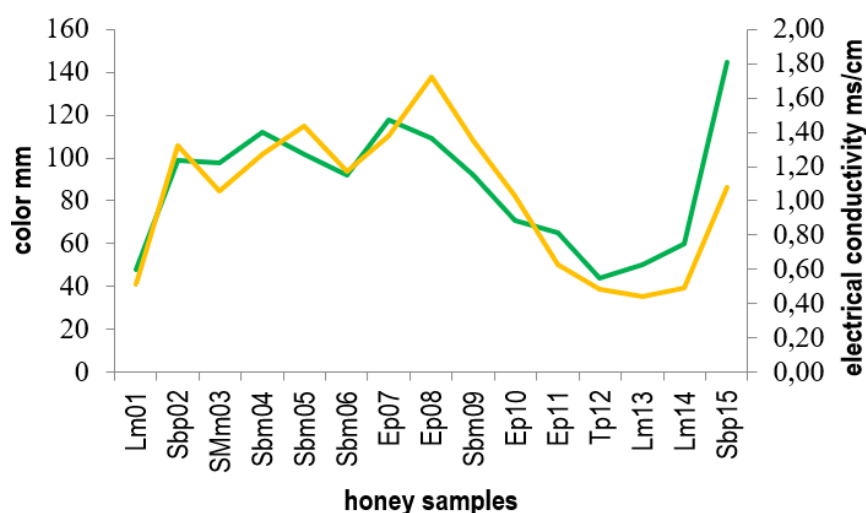
Figure 3. Color (green) and electrical conductivity (yellow) relationship for the different honeys

To confirm whether the relationship between mineral composition and parameters such as color, electrical conductivity and ash content are factors capable of differentiating the honey samples studied, multivariate statistical tools were used to perform a principal component analysis (PCA) (**Figure 4**).
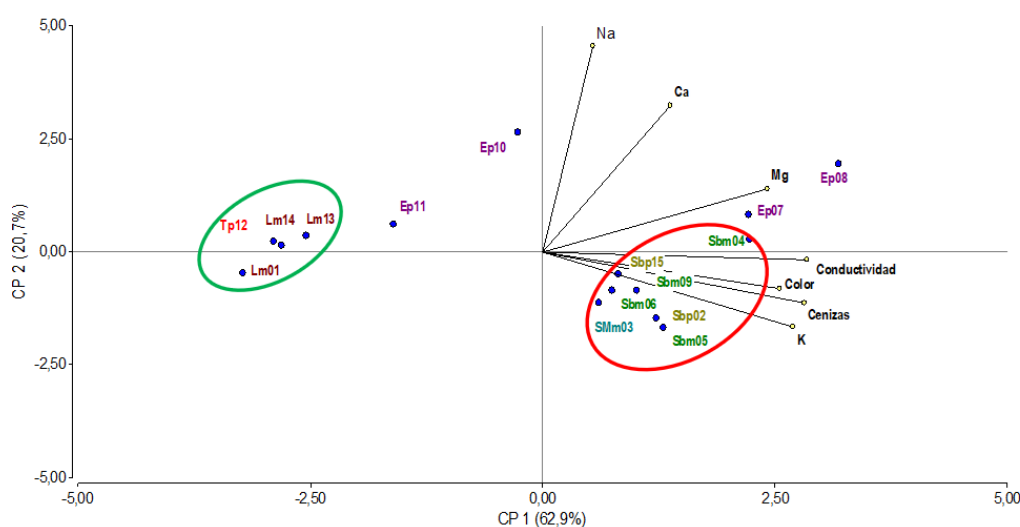

Figure 4. Discriminant analysis (PCA) of the honeys studied based on physicochemical parameters and mineral profile

3.2.6 Sugar profile

In **Table 5** the content of sugars found in each honey sample is shown. Fructose and glucose were the main sugars analyzed followed by the disaccharide maltose, sucrose and turanose. The primary sugars found in honey are fructose and glucose. These sugars, along with the sucrose content, collectively serve as key quality indicators for honeys within the European Union[38].

Table 5. Analysis of sugar content (%) present in honey samples

Sample Code	Fructose	Glucose	Sucrose	Turanose	Maltose	F+G*	F/G	G/W
Lm01	39.5	33.4	2.0	0.9	2.7	72.9	1.2	1.9
Sbp02	36.4	29.2	3.1	3.0	3.8	65.6	1.2	1.6
SMm03	36.9	29.9	2.9	1.9	3.4	66.8	1.2	1.6
Sbm04	37.0	29.3	2.9	2.2	3.0	66.3	1.3	1.6
Sbm05	36.2	28.9	2.8	2.7	3.8	65.1	1.3	1.5
Sbm06	36.1	28.9	3.2	2.8	3.9	65.0	1.2	1.5
Ep07	36.5	31.4	3.1	2.9	3.7	67.9	1.2	1.7
Ep08	38.1	29.2	3.4	2.9	4.2	67.3	1.3	1.7
Sbm09	36.2	28.9	3.1	3.0	4.5	65.1	1.3	1.6
Ep10	40.4	36.6	2.5	1.1	1.7	77.0	1.1	2.4
Ep11	40.8	30.8	2.1	0.9	2.4	71.6	1.3	1.8
Tp12	42.5	27.9	0.4	1.8	4.5	70.4	1.5	1.6
Lm13	40.0	31.7	0.9	2.4	1.8	71.7	1.3	2.1
Lm14	42.1	29.1	2.3	1.2	2.0	71.2	1.4	1.7
Sbp15	37.4	28.8	2.8	2.3	3.9	66.2	1.3	1.7
min	36.1	27.9	0.4	0.9	1.7	65.0	1.10	1.5
max	42.5	36.6	3.4	3.0	4.5	77.0	1.53	2.4

*F, fructose; G, glucose; W, water

4. Discussion

Melissopalynological analysis plays a fundamental role for monofloral honeys marketing, where at least 45% of the total number of pollen grains belong to the same plant species. There are exceptions, such as the case of *Eucalyptus* honey, in which its pollen grains are over-represented, so that to be considered monofloral it must contain at least 75% of pollen from this genus[37]. The opposite situation is represented by citrus honey, where pollen grains from citrus are underrepresented, so that a presence of this type of pollen between 10-20% is sufficient to consider citrus honeys as monofloral[15][39].

The results obtained on the pollen identification for the honeys evaluated agreed with previous works reported in the literature on the taxa of the most common plant communities in the Uruguayan regions considered[12][13][34].

When evaluating the results of the physicochemical and mineral content analyses, it was found that mineral values agree with those reported in the literature, where K is the most abundant mineral (about 80% of the total metals determined), followed in smaller proportion by Na or Ca, and lastly Mg[31][40]. Moreover, and as previously reported[31][32][33][41] honeys with higher mineral content show darker colors, higher ash content and higher conductivity values.

A statistical treatment using Principal Component Analysis (PCA) applied to the results obtained from physicochemical and mineral analyses showed that the first two components explain more than 83% of the variability of the system. The samples distribution through the projected variables (minerals, electrical conductivity, ash, and color) demonstrates that those are factors capable of differentiating the honey samples studied.

Non-native monofloral *Lotus* spp. honeys (Lm01, Lm13, Lm14) and polyfloral honeys with predominance of *Trifolium repens* and *Trifolium pratense* (Tp12) were grouped (green circle) towards negative values of PC1, and presented light colors, low ash content, low conductivity values and low mineral content.

PC1 also separated, towards positive values (red circle), the *Scutia buxifolia* native monofloral honeys (Sbm04, Sbm05, Sbm06, Sbm09), *Schinus molle* (SMm03) and polyfloral honeys with predominance of *Scutia buxifolia*

(Sbp02, Sbp15), which presented dark colors, high ash content, higher conductivity values and high metal content.

Meanwhile, samples Ep07, Ep08, Ep10 and Ep11 identified as polyfloral honeys with a higher predominance of *Eucalyptus* were distributed in this analysis according to their less abundant pollen content. Ep07 and Ep08, presenting less *Eucalyptus* pollen content with predominance of *Schinus molle* and *Scutia buxifolia*, respectively, were in the PC1 towards positive values close to the native honeys, also presenting dark color, high ash content, higher conductivity value and high metal content. Samples Ep10 and Ep11, also presenting *Eucalyptus* pollen content, less pollen content but with *Acacia longifolia* and *Lotus* sp predominance, respectively, located towards negative values of PC1, next to the non-native honeys that presented light colors, low ash content and low metal content.

The results obtained from the study of sugar profiles confirm that all the honeys analyzed pertain to flower honeys type, as the combined levels of fructose and glucose (F+G) were at least 65%, and sucrose content did not exceed 5% for flower honey[38]. The crystallization time of honey primarily depends on the relative proportions of fructose to glucose and the ratio of glucose to water[42].

Lower solubility of glucose in water than of fructose explains the F/G ratio has been used to predict the honey tendency to crystallize[43]. F/G ratio below 1 indicates fast crystallization, while the ratio exceeding 1.3 indicates slow crystallization[44]. The data obtained showed honeys Tp12 and Lm14 presented F/G ratio <1.3, so they can be considered slow crystallization honeys, while the others presented F/G values between the limits of fast and slow crystallization.

Moisture content can affect the physical properties of honey (viscosity, crystallization), so the Glucose/Moisture (G/W) ratio could be also a useful indicator to predict honey crystallization. Honey crystallization is slow when ratio glucose/G/W is less than 1.7, but it is rapid when the ratio is greater than 2[39][40]. According with our results, only the honeys samples Ep10 and Lm13 showed a ratio over 2 and so they can present faster crystallization.

5. Conclusions

The results found in this work contribute to the identification of honeys and to initiate a characterization work for Uruguayan honey valorization. Honey, being a highly complex mixture, exhibits a unique composition in every batch, making each one distinct from the other. Moreover, variations in botanical sources contribute to differences in both appearance and composition among honeys derived from native flora. Even within the same floral category, honey composition can vary significantly due to geographical location, seasonal influences, bee species, harvesting methods, storage conditions, and processing techniques. As melissopalynology remains the sole validated method for determining the botanical origin of honey, it is not without its limitations.

Studies such as the one presented here contribute with valuable information for comparative purposes, thereby aiding in the quest for improved methodologies in this field. Focus has been put on the characterization of honeys that can be classified as monofloral from native plants, where the results suggest that these honeys are in accordance with the quality standards established by national and international standards.

Transparency of data

Available data: The entire data set that supports the results of this study was published in the article itself.

Author contribution statement

AG: Conceptualization; Methodology; Investigation; Formal analysis, Writing–original draft

AB: Conceptualization; Formal analysis; Methodology; Investigation

LF: Conceptualization; Supervision

AS: Conceptualization; Formal analysis; Writing–original draft

GD: Conceptualization; Formal analysis; Writing–original draft

ED: Conceptualization; Supervision; Resources; Writing–review & editing

References

[1] Ministerio de Ganadería, Agricultura y Pesca, DIEA (UY). Anuario estadístico 2022 [Internet]. Montevideo: MGAP; 2022 [cited 2024 Jun 28]. 273p. Available from: https://bit.ly/49USu0d

[2] Alissandrakis E, Tarantilis PA, Harizanis PC, Polissiou M. Aroma investigation of unifloral Greek citrus honey using solid-phase microextraction coupled to gas chromatographic-mass spectrometric analysis. Food Chem. 2007;100(1):396-404. Doi: 10.1016/j.foodchem.2005.09.015.

[3] Fernández-Torres R, Pérez-Bernal JL, Bello-López MA, Callejón-Mochón M, Jiménez-Sánchez JC, Guiraúm-Pérez A. Mineral content and botanical origin of Spanish honeys. Talanta. 2005;65(3):686-91. Doi: 10.1016/j.talanta.2004.07.030.

[4] Sawyer R, Pickard RS. Honey identification. Cardiff: Cardiff Academic Press; 1988. 115p.

[5] Santos-Buelga C, González-Paramás AM. Chemical composition of honey. In: Alvarez-Suarez JM, editor. Bee products: chemical and biological properties. Cham: Springer; 2017. p. 43-82.

[6] Cabrera A, Willink A. Biogeografía de América Latina. 2a ed. Washington: Organización de los Estados Americanos; 1980. 122p.

[7] Ayala W, Altesor A, Paruelo J. Bases ecológicas y tecnológicas para el manejo de pastizales. Montevideo: INIA; 2011. 234p.

[8] Lapetina J. Guía de buenas prácticas ganaderas para el manejo y conservación de pastizales naturales en áreas protegidas: Quebradas del Norte. Montevideo: MVOTMA; 2012. 77p.

[9] Brazeiro A. Bosques de Uruguay: necesidades de investigación para la gestión sustentable y conservación. In: Brazeiro A, editor. Recientes avances en la investigación para la gestión y conservacion del bosque nativo de Uruguay. Montevideo: Universidad de la República; 2017. p. 12-6.

[10] Cracco P. Caracterización de mieles por su origen: valorización de la miel en Uruguay. In: VI Congreso AUPA. Montevideo: AUPA; 2018. p. 15-7.

[11] Daners G, Campá P. Evidencia palinológica de miel monofloral de coronilla (Scutia buxifolia) en Uruguay. In: Actas del V Congreso Ibero Latinoamericano de Apicultura. Mercedes: [publisher unknown]; 1996. p. 171-3.

[12] Daners G, Tellería MC. Native vs. introduced bee flora: A palynological survey of honeys from Uruguay. J Apic Res. 1998;37(4):221-9. Doi: 10.1080/00218839.1998.11100976.

[13] Tejera L, Invernizzi C, Daners G. Población y recursos alimenticios en colonias de Apis mellifera L. en Uruguay. Arch Zootec. 2013;62(240):607-10. Doi: 10.4321/S0004-05922013000400015.

[14] Carrere R. Monte indígena: mucho más que un conjunto de árboles. Montevideo: Grupo Guayubira; 2010. 50p.

[15] Louveaux J, Maurizio A, Vorwohl G. Methods of melissopalynology. Bee World. 1978;59:139-54. Doi: 10.1080/0005772X.1978.11097714.

(16) Bogdanov S. Harmonised methods of the International Honey Commission. Liebefeld: Swiss Bee Research Centre; 2002. 63p.

(17) Association of Official Analytical Chemists. Official methods of the analysis of official analytical chemists. 15th ed. Arlington: AOAC; 1990. 2v.

(18) USDA. United States standards for grades of extracted honey. Washington: USDA; 1985. 12p.

(19) Silvano MF, Varela MS, Palacio MA, Ruffinengo S, Yamul DK. Physicochemical parameters and sensory properties of honeys from Buenos Aires region. Food Chem. 2014;152:500-7. Doi: 10.1016/j.foodchem.2013.12.011.

(20) Standard for honey: CXS 12-19811. In: Joint FAO/WHO Codex Alimentarius Commission. Codex Alimentarius: International Food Standards [Internet]. Rome: FAO; 2022 [cited 2024 Jun 28]. 8p. Available from: https://bit.ly/3zAVJba

(21) Doner LW. Honey. In: Caballero P, editor. Encyclopedia of food sciences and nutrition. 2nd ed. London: Academic Press; 2003. p. 3125-30.

(22) Reglamento Bromatológico Nacional: decreto nro. 315/994 de fecha 05/07/1994: anotado y concordado con apéndice normativo. 8a ed. Montevideo: IMPO; 2022. 784p.

(23) Schade JE, Marsh GL, Eckert JE. Diastase activity and hydroxy-methyl-furfural in honey and their usefulness in detecting heat alteration. J Food Sci. 1958;23(5):446-63. Doi: 10.1111/j.1365-621.1958.tb17592.x.

(24) Kędzierska-Matysek M, Florek M, Wolanciuk A, Skałecki P, Litwińczuk A. Characterisation of viscosity, colour, 5-hydroxymethylfurfural content and diastase activity in raw rape honey (Brassica napus) at different temperatures. J Food Sci Technol. 2016;53(4):2092-8. Doi: 10.1007/s13197-016-2194-z.

(25) Bogdanov S, Baumann E. Determination of sugar composition of honeys by HPLC. Mitt Geb Lebensmittelunters Hyg. 1988;79:198-206.

(26) Chudzinska M, Baralkiewicz D. Estimation of honey authenticity by multielements characteristics using inductively coupled plasma-mass spectrometry (ICP-MS) combined with chemometrics. Food Chem Toxicol. 2010;48(1):284-90. Doi: 10.1016/j.fct.2009.10.011.

(27) Di Rienzo JA, Casanoves F, Balzarini MG, Gonzalez L, Tablada M, Robledo CW. InfoStat [Internet]. Versión 2014. Córdoba: Universidad Nacional de Córdoba, Facultad de Ciencias Agropecuarias; 2014 [cited 2024 Jun 28]. Available from: https://bit.ly/3dDvIyu

(28) Terrab A, Escudero ML, González-Miret ML, Heredia FJ. Colour characteristics of honeys as influenced by pollen grain content: A multivariate study. J Sci Food Agric. 2004;84(4):380-6. Doi: 10.1002/jsfa.1668.

(29) Cavia Camarero MM. Estudio del envejecimiento de mieles de Burgos y Galicia: influencia de la granulación inducida [doctoral's thesis]. Burgos (ES): Universidad de Burgos; 2002. 362p.

(30) Gomes S, Dias LG, Moreira LL, Rodrigues P, Estevinho L. Physicochemical, microbiological and antimicrobial properties of commercial honeys from Portugal. Food Chem Toxicol. 2010;48(2):544-8. Doi: 10.1016/j.fct.2009.11.029.

(31) Terrab A, González AG, Díez MJ, Heredia FJ. Mineral content and electrical conductivity of the honeys produced in Northwest Morocco and their contribution to the characterisation of unifloral honeys. J Sci Food Agric. 2003;83(7):637-43. Doi: 10.1002/jsfa.1341.

(32) Pires J, Estevinho ML, Feás X, Cantalapiedra J, Iglesias A. Pollen spectrum and physico-chemical attributes of heather (*Erica* sp.) honeys of north Portugal. J Sci Food Agric. 2009;89(11):1862-70. Doi: 10.1002/jsfa.3663.

(33) Santos F, Dantas Filho A, Leite R, Aroucha E, Santos A, Oliveira T. Rheological and some physicochemical characteristics of selected floral honeys from plants of caatinga. An Acad Bras Cienc. 2014;86(2):981-94.

(34) Corbella E, Tejera L, Cernuschi F. Calidad y origen botánico de mieles del Noreste de Uruguay. Rev INIA. 2005;(3):6-7.

(35) Corbella E, Cozzolino D. Classification of the floral origin of Uruguayan honeys by chemical and physical characteristics combined with chemometrics. LWT-Food Sci Technol. 2006;39(5):534-9.
Doi: 10.1016/j.lwt.2005.03.011.

(36) Yadata D. Detection of the electrical conductivity and acidity of honey from different areas of Tepi. Food Sci Technol. 2014;2(5):59-63. Doi: 10.13189/fst.2014.020501.

(37) Santos E, Meerhoff E, García Da Rosa E, Ferreira J, Raucher M, Quintana W, Martínez A, González C, Mancebo Y. Color and electrical conductivity of honeys produced by Apis mellifera in Uruguay. INNOTEC. 2018;16:51-5. Doi: 10.26461/16.08.

(38) Council Directive 2001/110/EC of 20 December 2001 relating to honey. Official Journal of the European Communities [Internet]. 2002 [cited 2024 Jun 25]; L 10:47-52. Available from: https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2002:010:0047:0052:EN:PDF

(39) Juan-Borrás M, Periche A, Domenech E, Escriche I. Correlation between methyl anthranilate level and percentage of pollen in Spanish citrus honey. Int J Food Sci Technol. 2015;50(7):1690-6.
Doi: 10.1111/ijfs.12827.

(40) Ahmad NG, Suhag Y, Majid I, Nanda V. Discrimination of high altitude indian honey by chemometric approach according to their antioxidant properties and macro minerals. J Saudi Soc Agric Sci. 2016;17(2):200-7. Doi: 10.1016/j.jssas.2016.04.004.

(41) Pohl P. Determination of metal content in honey by atomic absorption and emission spectrometries. TrAC-Trends Anal Chem. 2009;28(1):117-28. Doi: 10.1016/j.trac.2008.09.015.

(42) 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.
Doi: 10.1016/j.foodchem.2013.10.097.

(43) Laos K, Kirs E, Pall R, Martverk K. The crystallization behaviour of Estonian honeys. Agron Res. 2011;9:427-32.

(44) Dobre I, Georgescu LA, Alexe P, Escuredo O, Seijo MC. Rheological behavior of different honey types from Romania. Food Res Int. 2012;49(1):126-32. Doi: 10.1016/j.foodres.2012.08.009.