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Proceeding Paper

Geographical Differentiation of Honeys from Entre Ríos (Argentina) through Physicochemical Analysis: A Scientific Approach for the Characterization and Authentication of Regional Honeys †

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Abstract: Argentina's prominent global position in honey production is due to its extensive agroecological diversity. This study assessed the influence of geographical origin on physicochemical properties of honeys from southeastern Entre Ríos. 104 honeys from Gualeguaychú (GU), Islas del Ibicuy (II), and Concepción del Uruguay (CU) (2020–2022) were analyzed. Statistically significant differences ($p < 0.05$) were observed among districts: II displayed the lowest values (colour, conductivity, acidity, pH and ash) GU showed moderate values, and CU exhibited the highest values, except for humidity. Chemometric analyses explained 75.5% data variability (PCA) and successfully classified 85.3% of samples by origin (LDA). These findings bear implications for product differentiation and market value enhancement in Entre Ríos' honey industry, serving as a foundation for future quality control and origin identification endeavors.

Keywords: physicochemical honey analysis; geographical origin of honey; principal component analysis; linear discriminant analysis

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1. Introduction

Argentina stands out as one of the leading countries worldwide in the production and export of honey, ranking third and second, respectively. Exports have shown a continuous growth trend during the period 2015–2022 (FOB), representing approximately 95% of the total production [1]. In particular, the province of Entre Ríos, in the noreast of Argentina, positions itself as the second-largest producer in the country, with an estimated annual production of 14,000 tonnes and beehives distributed throughout the provincial territory [2]. The quality and chemical composition of honey are influenced by various factors, including its geographical origin, botanical source, climatic conditions, and seasonality. Argentina's vast agroecological diversity offers exceptional potential for obtaining honeys with distinctive organoleptic profiles and nutritional properties [3]. Particularly, Entre Ríos presents favorable conditions for honey production, as its climate varies from subtropical with no dry season in the north to humid temperate in the plains of the south [2]. However, a high percentage of Argentine honey is sold in bulk without any geographical indication or designation of origin that could add value to the product and enhance its positioning in both international and domestic markets. In the other hand in Argentina the annual consumption per capita is lower than 200 g/hab/year [4]. In this context, efforts have been made to recognize and valorize the natural variability of honeys through the creation of the "Regional Honey Identity Map". This project is based on participatory construction and aims to describe the specific characteristics of the different honeys produced in the country. Nevertheless, the available information is currently limited, focusing mainly on data related to sensory characteristics and floral origin of honeys in each region [5]. In order to explore this potential, a study was conducted to assess the impact of geographical origin on the physicochemical characteristics of honeys from the southeastern region of Entre Ríos, using chemometric methods to discriminate and classify honey samples according to their origin.

2. Materials and Methods

2.1. Sample Collection

100 honey samples were collected during the years 2020–2022 in 3 districts, Gualeguaychú (GU), Islas del Ibicuy (II), and Concepción del Uruguay (CU) of Entre Ríos Province, Argentina. The samples were stored in a dark, cool, and dry place and analyzed within the first 3 months.

2.2. Instrumental Analysis

2.2.1. Colour Index

The colour was determined following the instructions provided by Del Moro [6], using the HANNA® Instrument HI96785 Colorimeter. Prior to conducting the measurements, the samples were tempered (<45 °C) and centrifuged (5 min; 3000 rpm). This ensured that at the time of reading, they were clear and free of bubbles, crystals, or impurities that could potentially affect the outcomes. Each sample was placed in a cuvette for colour measurement, and the results were expressed in mm Pfund.

2.2.2. Determination of Electrical Conductivity (EC)

Electrical conductivity was measured at 20 °C in solutions of honey in deionised water (with electrical conductivity <1 μs/cm), with a concentration of 20% solids on a dry basis, in accordance with the specifications outlined in IRAM 15945 of 1996 and the legislation of the European Union [7]. To carry out these measurements, the Milwaukee® MW801 Conductivity Meter was utilized.

2.2.3. Determination of pH

For pH determination, honey samples were diluted to 10% (*w*/*v*) and potentiometrically measured at 20 °C using the Milwaukee® MW801 pH meter. This procedure followed the guidelines set out in the IRAM 15938 of 1996.

2.2.4. Determination of Free Acidity

To quantify the acidity of the samples, the approach adhered to the official AOAC 962.19 method from 1995. Briefly, honey solutions were prepared (in a 1:10 ratio) and subjected to neutralisation titration with 0.1N NaOH. The equivalence point (pH 8.2) was measured as indicated at 2.2.3. Each sample was evaluated in triplicate.

2.2.5. Moisture Content

The determination of moisture content in the honey samples was conducted through the measurement of their refractive index. For this purpose, a HANNA® Instrument HI96801 Digital Refractometer for Brix Analysis in Foods was employed, having been previously calibrated using deionised water. This procedure was executed in accordance with the protocols specified in AOAC (AOAC 969.38B of 1996).

2.2.6. Ash Content

The ash content was measured using the official AOAC 920.181 method. This procedure involved the gravimetric determination of ash within a honey sample, after incineration in a Laboratory Muffle Furnace model 272. The process was conducted at a temperature of 550 °C until a constant weight and a whitish appearance were achieved.

2.3. Statistical Data Analysis

Triplicate determinations were conducted for each of the physicochemical parameters present in the samples. The obtained results were processed using the statistical software STATGRAPHICS Centurion XV.II. A one-way variance analysis (ANOVA) was carried out to ascertain which physicochemical parameters displayed significant differences in accordance with the geographical origin of the honeys ($p < 0.05$). Following this, the Bonferroni multiple comparisons test was employed as a post hoc analysis to identify the mean disparities in those parameters that displayed noteworthy variations. Furthermore, the collected data underwent chemometric analyses. These assessments encompassed the application of techniques such as principal component analysis (PCA), which facilitated the determination of the physicochemical parameters that made a more significant contribution to the observed variations. Similarly, linear discriminant analysis (LDA) was used to characterise the distinctions among the different districts that were subjects of analysis.

3. Results and Discussion

3.1. Colour Index (CI)

The color of honey from the southeast region of Entre Ríos was classified from "White" to "Dark Amber." The dominant categories were "Light Amber" (52% of the analyzed samples), "Amber" (28% of the analyzed samples), and "Extra Light Amber" (13% of the analyzed samples). II honey (21.0 to 87.0 mm Pfund) were significantly lighter than those from the other two districts (GU with 44.0–108.0 and CU 49.0–117.0 mm Pfund). The colour of honey is a complex attribute influenced by various factors as mineral content and botanical origin of honey. Buth also colour is related to the presence of hydroxymethylfurfural (HMF) and other compounds (some of them are coloured) that develop as a result of prolonged or improper honey storage or an excessive heating of honey. These results fall below the values reported for the province of Formosa in 2022 [8], but surpass the data presented for the province of Córdoba in 2007 [9], where honey varieties in the categories of white water, extra white, and white were predominant. In the case of Corrientes, a province that shares the mesopotamic region, similar values to those found in II [10] have been reported.

Table 1. Physicochemical parameters of honey samples (mean ± SD).

Parameters	**Islas del Ibicuy n = 54**	**Gualeguaychú n = 20**	**Concepción del Uruguay n = 30**
Color Index (mm Pfund)	60.1 ± 17.4 ^{b}	87.3 ± 18.5 ^{a}	85.3 ± 15.3 ^{a}
Electrical Conductivity (μS/cm)	380.0 ± 1.2 ^{c}	678.6 ± 1.4 ^{b}	1064.2 ± 1.4 ^{a}
Free Acidity (meq/kg)	15.2 ± 2.3 ^{c}	17.3 ± 2.0 ^{b}	21.3 ± 2.6 ^{a}
pH	3.8 ± 0.3 ^{c}	4.1 ± 0.3 ^{b}	4.3 ± 0.3 ^{a}
Moisture (%)	19.3 ± 0.8 ^{a}	18.4 ± 0.7 ^{b}	19.3 ± 0.6 ^{a}
Ash (%)	0.18 ± 0.1 ^{b}	0.38 ± 0.1 ^{a}	0.42 ± 0.1 ^{a}

* Means in the same row with different letters are significantly different at $p < 0.05$.

3.2. Determination of Electrical Conductivity (EC)

The electrical conductivity values for the region ranged from 240 to 1810 μS/cm. Significant differences ($p < 0.05$) were observed among districts. II had the lowest electrical conductivity (240.0–630.0 μS/cm). In contrast, CU exhibited the highest values in the region (389.0 to 1813.0 μS/cm). Finally, GU had values that fell in between the previously mentioned districts (334.0 to 1218.0 μS/cm). II and GU were below the limits set by the Codex Alimentarius for unclassified honeys (<0.8 mS/cm), but some samples from II had higher EC. EC can be related to various factors, such as the sugar concentration, the presence and quantity of minerals, acidity, and other chemical components. EC is sometimes used as an indicator of honey quality and authenticity, as different honeys can have unique mineral profiles due to their geographical and floral origin. The results obtained for the II index are in line with the data presented by Acquarone y cols. (2007) for the province of Entre Ríos [10]. In the case of GU, it could be compared to the values reported for the Argentine provinces of Formosa, Chaco, and Corrientes, while CU exceeds even these values [10,12–14].

3.3. Determination of Free Acidity (FA)

The analysis of free acidity resulted in a range of 10.6 to 24.8 meq/kg, which complies with the regulations established by the Codex Alimentarius for fresh honeys (50 meq/kg). II had the lowest acidity, ranging from 10.6 to 20.0 meq/kg. The highest values in the region were reported in CU (16.8–24.8 meq/kg). The acidity of honey is associated with its chemical profile including organic acids. The acidity not only contribute to the distinct flavor but acts as a natural preservative, inhibiting the growth of microorganisms and helping to prevent spoilage, ensuring that honey remains a stable and delicious product over time. The FA results obtained for CU resembled the data presented by Ciapini y cols. (2022) for the Entre Ríos delta [8]. However, the regional average fell below the values reported by Acquarone y cols. for the province of Entre Ríos [10] and by other authors for other Argentine provinces [10,12–14].

3.4. Determination of pH

Regarding pH values, they ranged from 3.3 to 4.8. Samples from II had the lowest pH values, ranging from 3.3 to 4.5. Gualeguychú samples displayed intermediate values in this parameter, with a range of 3.5 to 4.6. Finally, CU had the highest pH values, ranging from 3.5 to 4.8. The pH results exhibited similarities with those reported for Entre Ríos in Acquarone y cols. (2007). In the case of GU, values are comparable to those recorded in the provinces of Chaco in 2014 [12] and Formosa in 2022 [15].

3.5. Moisture Content (MC)

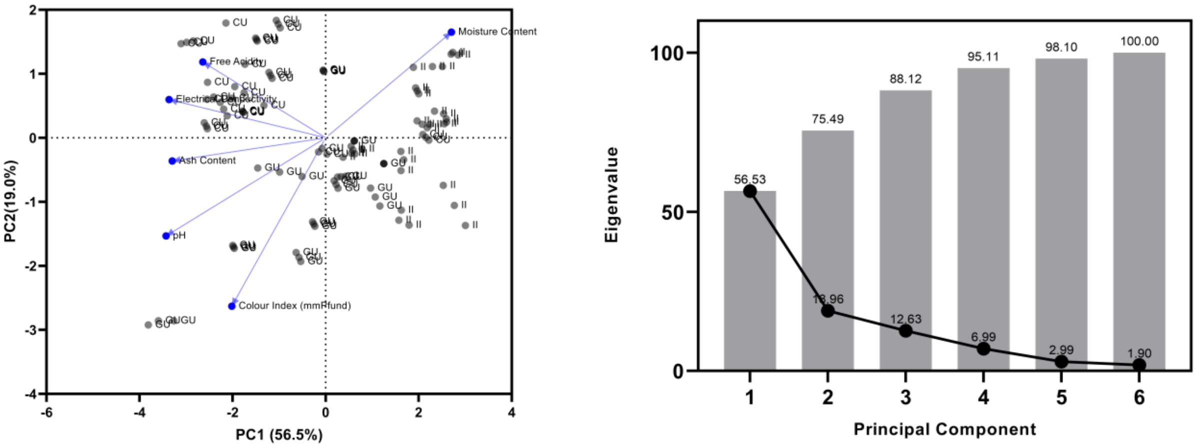
The southeastern Entre Rios region varied in moisture content from 17.0% to 21.1%. II showed a range of 17.4% to 21.1%, in accordance with the reported by Ciapinni 2022 [8]. GU ranged from 17.0% to 19.3%, while CU ranged from 18.2% to 20.20%. Notably, GU samples were significantly drier. It was observed that some samples slightly exceeded the maximum allowed for unclassified honeys according to the Codex (20%). The moisture content of honey is intricately linked to factors such as climate, floral source, geographical location, and the maturation process. These variables collectively influence the level of water present in honey, impacting its quality and stability. The moisture results for the southeastern Entre Ríos region aligned with the data presented by Acquarone y cols. [10]. Furthermore, the MC obtained values were slightly greater than the reported for other Argentine provinces, such as Jujuy, Corrientes, and Chaco [10,12,13].

3.6. Ash Content (AC)

Regarding the ash content percentage, II had significantly lower values compared to CO and GU, with ranges of 0.04–0.4, 0.29–0.48, and 0.26–0.67, respectively. Almost all the samples remained below the maximum allowable (0.6% for flower honeys). The ash content in honey is influenced by various factors, including the floral source, geographical origin, and environmental conditions, as well as whether the honey is derived from nectar or honeydew. These variables collectively contribute to the mineral content in honey, affecting its overall composition and potential uses. The ash content results obtained for Entre Ríos region was found to be above the results reported by other authors [11].

3.7. Principal Component Analysis (PCA)

A Principal Component Analysis (PCA) was conducted on the physicochemical data of the 104 samples to identify the primary vectors capturing the majority of the variability and simultaneously to scrutinize the trends exhibited by samples from different districts, as well as to assess the discriminating characteristics of the specified parameters. Figure 1a displayed a biplot graph obtained for the first two principal components and the arrangement of samples identified by geographic origin. As depicted in Figure 1b, the aforementioned two components enabled the explanation of 75.5% of the data variability.



(**a**) (**b**)

Figure 1. Principal components analysis of the studied variables: (**a**) PCA Bi-plot; (**b**) Principal Component Analysis Sedimentation Plot.

In the first principal component, the dominant variables were pH, electrical conductivity, and ash content. Conversely, in the second principal component, the variables carrying more weight were humidity and free acidity.

The majority of the samples from II positioned themselves on the right-hand side of the biplot graph, which was associated with inverse values in the pH, electrical conductivity, ash content, color, and free acidity variables. Conversely, the honeys from CU situated themselves on the left-hand side of the graph, aligning with the vectors of pH, electrical conductivity, ash content, color, and free acidity, indicating higher values in these parameters. As for the samples from GU, they were situated in the lower middle part with a tendency to the left, in contrast to the humidity vector.

3.8. Linear Discriminant Analysis (LDA)

In the LDA, the studied physicochemical parameters successfully discriminated 90.9% of the samples from II (n = 54), 80.0% from GU (n = 20), and 84.6% from CU (n = 30). This resulted in an average discrimination power of 85.3% for the model. Figure 2 illustrates the distribution of samples differentiated by their geographical origin based on the two obtained functions. II samples were primarily located in the upper left quadrant, with the majority falling between scores of −2.5 to −0.5 on function 1 and −0.5 to 2.5 on function 2. CU exhibited greater dispersion along the axis of function 1, ranging from −0.5 to 4.5, whereas function 2 displayed values ranging from −1.5 to 1.5. Lastly, GU was situated in the lower right quadrant, ranging from −2.5 to −0.5 on function 1 and −4.5 to −0.5 on function 2.

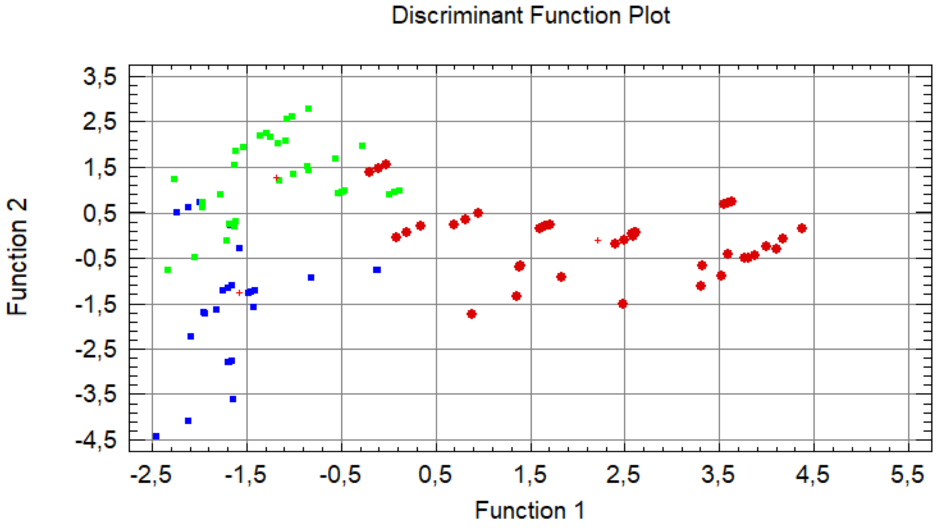


Figure 2. Discriminant Function Plot. Green squares correspond to **Islas del Ibibuy** samples, blue squares to **Gualeguaychu**, and red circles to **Concepción del Uruguay**. Red crosses correspond to the centroids of each group of observations.

4. Conclusions

Based on the results obtained from two honey harvests in the southeastern region of Entre Ríos, the significant impact of geographical origin on the studied parameters has been evident, both individually and collectively, through the use of multivariate analyses. PCA has proven its utility in simplifying the complexity of the data matrix and revealing inherent patterns in the variability of samples from the different districts analyzed. Additionally, LDA has demonstrated its ability to accurately classify a high percentage of the samples. These findings represent a significant contribution to the understanding of honey from both the Entre Ríos province and Argentina as a whole. Furthermore, these discoveries could serve as a foundation for the implementation of geographical indications and designations of origin to enhance the value or improved the classification process of honeys produced in this region.

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