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## Chapter

# Palynological and Physicochemical Characterization of Honey from *Butia yatay* Palm Savannas in Argentina

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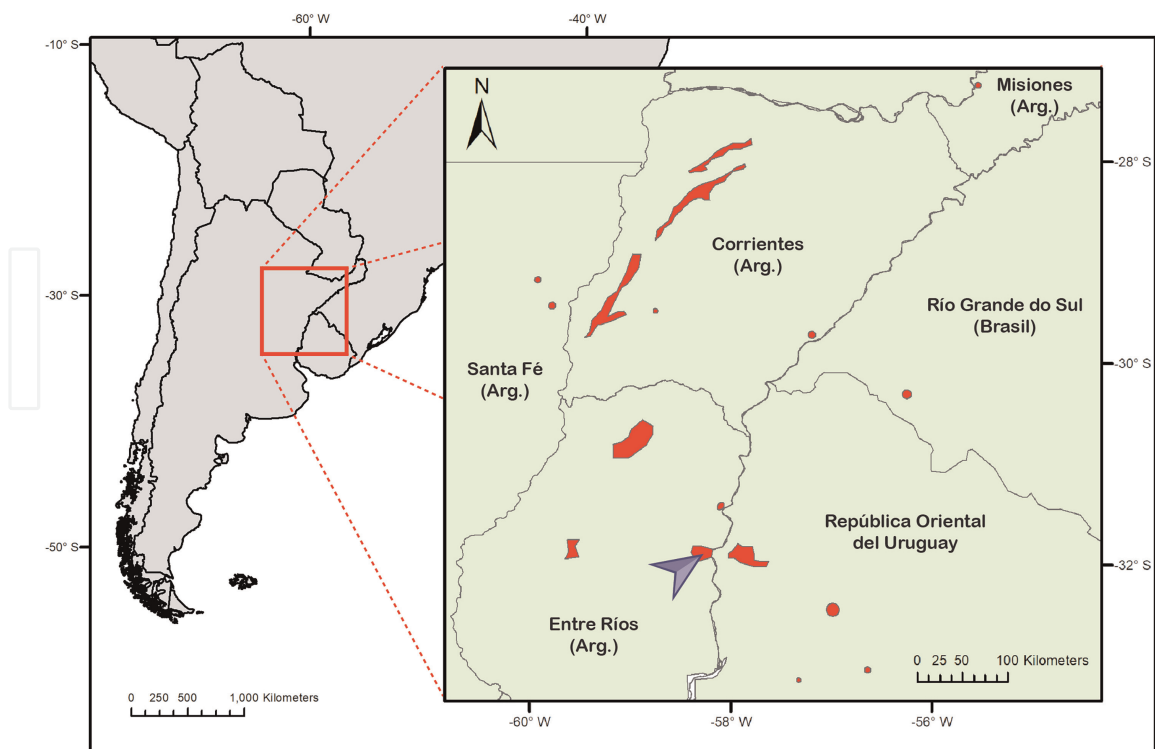
## Abstract

*Butia yatay* palm savannas (*palmares de yatay*) of Eastern Argentina constitute a unique natural and cultural landscape threatened by land conversion. Honey production, as a non-timber forest product, can become a conservation-through-use strategy for this landscape if shown to be a valuable product. Therefore, here we describe palynological and physicochemical parameters of honey obtained from hives situated in one of the remaining largest *Butia yatay* palm savannas in Entre Ríos, Argentina, during the palms' blooming peak. Melissopalynological analysis showed that three pollen types (Myrtaceae type, *Butia yatay*, and *Eryngium horridum*) accounted for 88–96% of the total pollen counted. Palm pollen was consistently present in all the analyzed samples as secondary pollen regarding its frequency with an average of 33.5% of the total pollen counted. This honey presented high proline content, high conductivity, a color range from light amber to amber, significant polyphenol bioactivity, and rheologically behaved as a Newtonian fluid. This is the first instance of producing and characterizing honey from this peculiar botanical and geographical origin, thus contributing to Argentinian efforts to hierarchize regional and local honey types. It is also the first report of *Butia yatay* palms as a significant nectar source for honey production.

**Keywords:** Argentinian honey, Entre Ríos, palm grove, honey analysis, melissopalynology, botanical origin, rheology

## 1. Introduction

*Butia* Becc. (Arecaceae: Cocoeae) is a South American endemic palm genre comprising 24 species distributed in Argentina, Brazil, Paraguay, and Uruguay [1]. *Butia yatay* (Mart.) Becc. is one of the most widely distributed species occurring in three of the aforementioned countries (**Figure 1**). Adult palms of *B. yatay* can reach up to 12 meters high and bloom between the months of November and February during the austral summer. Individuals usually occur in dense and extensive spatial aggregations such as palm savannas where they are the dominant element on the arborescent



**Figure 1.** (Left) Distribution map of remnant *Butia yatay* palm savannas (adapted from Brazeiro et al. [2]); the arrowhead shows the apiary's location. (Right) Study site vegetation physiognomy where palms are the dominant arborescent element within an extensive scrubland.

stratum (**Figure 1**). These savannas, called ‘palmares’ in Spanish (‘palmar’ in singular), have been suffering serious threats due to land conversion for agriculture and overgrazing by cattle raising. Consequently, the global conservation status of *B. yatay* has been proposed as vulnerable [2].

Products derived from native palm populations, without causing their destruction, are considered Non-Timber Forest Products (NTFPs) and possess enormous potential for both conserving the ecosystem and enhancing local economies through their utilization and commercialization [3]. There are numerous examples of conservation-through-use strategies specifically focusing on palms [3–6]. *Butia yatay* palm savannas constitute not only a valuable natural and cultural landscape but also a local economic resource due to its late valorization as a tourism attraction and as a food source for local gastronomic products [7]. Many studies show the contribution of several species of palms as an important part of the vegetation that nourishes bees and their hives [8–17]. Nonetheless, there are no records of *Butia yatay* as a resource for bees in the literature reviewed to date.

Argentina exports annually around 70 tons of honey for an amount of 240 million USD. This takes it among the top 3 honey exporters in the world together with New Zealand and China [18]. However, almost all these exports are made in bulk without specifying their regional or botanical origin. The physicochemical and sensory properties of honey largely depend on the nectar collected by bees. Therefore, a specific region with distinct flora and climate will produce a unique and unfamiliar variety of honey. In order to increase the value of these exports the Argentinian government has been promoting diverse strategies such as Protected Geographical Indication (PGI) and Protected Designation of Origin (PDO) starting with a regional map of honeys’ identities [19].

As an effort to contribute to this national characterization of kinds of honey and to add value to *B. yatay*'s NTFPs, we hereby describe palynological and physicochemical parameters of honey obtained from hives situated in one of the remaining largest *Butia yatay* palm savannas.

## 2. Study site and studied honey samples

The apiary where samples were taken from is placed in a private natural park (*La Aurora del Palmar*; Entre Ríos province, Argentina, 31.82° S, 58.33° W) which is within one of the remaining most extensive *Butia yatay* populations (**Figure 1**). The site belongs to the Mesopotamian district of the Pampean phytogeographical province [20] and has a humid subtropical climate (Cfa) according to Köppen's classification. The local landscape is a mosaic of natural fields subjected to light cattle raising or preserved as a strict reserve, *Pinus* plantations, cultivated lots, and riparian vegetation associated with streams. Natural fields are grasslands or scrublands with a variable density of *B. yatay* individuals. A detailed description of the vegetation of these fields can be found in Batista et al. [21].

Six langstroth beehives each 100 m apart were marked, fed, and checked for their sanitary status during the winter of 2021. Honey supers with empty frames and freshly stamped wax were added at the beginning of October before the first palms started to bloom. At the end of December when the frames were already full (>90% cells capped) and the peak of palms' bloom had passed the supers were taken to an extraction room. Honey from each hive was extracted by centrifugation at room temperature, homogenized, and labeled. Samples had, in general, a fruity aroma and an exceptionally sweet taste and were preserved at 4° until analysis.

## 3. Melissopalynology

Honey samples were subjected to standard melissopalynological qualitative studies [22]. From each sample 10 g of honey was dissolved in 20 ml of warm distilled water, centrifuged at 600 g for 10 m, discarded the supernatant, resuspended in the same amount of water, and centrifuged again at the same speed. The remaining pollen sediment was then mounted in glycerine jelly with basic fuchsin and inspected under an optical microscope [23]. To estimate frequency classes, at least 1000 pollen grains were identified and counted per sample [22]. These classes are according to the percentage of grains counted: predominant pollen (D:  $\geq 45\%$ ), secondary pollen (S: 45–16%), important minor pollen (M: 15–3%), minor pollen (m: 3–1%) and present (+: <1%). The samples in which one pollen type represented  $\geq 45\%$  were classified as monofloral, and those in which no pollen type reached this percentage were classified as multifloral [22].

Pollen types were identified by comparing them with a reference collection that was made from the plants in the study site. When possible, they were identified to species level, otherwise to the minimum taxonomic level. The surrounding vegetation was inspected regularly every 2 weeks to estimate the period in which species were in bloom and if they were actively visited by bees.

A total of 24 pollen types were recognized in the samples. Most of them appeared as very few grains in only one or two samples and were pooled as 'other trace types' (**Table 1**). The results show a predominance of three types in the pollen spectrum

Family	Pollen type	Sample [pollen frequency/frequency class]												
		1	2	3	4	5	6	Av %						
Myrtaceae	t. Myrtaceae	4.1	M	21.9	S	53.2	D	38.8	S	29.1	S	48.5	D	32.6
Arecaceae	<i>Butia yatay</i>	38.0	S	34.4	S	22.7	S	39.6	S	33.4	S	33.0	S	33.5
Apiaceae	<i>Eryngium horridum</i>	54.0	D	37.1	S	12.4	M	15.5	M	30.1	S	11.9	M	26.8
Apiaceae	t. <i>Ammi</i>	3.6	M	2.0	m	1.1	m	1.1	m	3.5	M	2.1	m	2.3
Boraginaceae	<i>Echium plantagineum</i>	0.2	+	1.6	m	3.6	m	1.9	m	0.9	+	0.3	+	1.4
Salicaceae	<i>Salix</i> sp.	0.2	+	0.4	+	0.3	+	0.4	+	0.5	+	0.5	+	0.4
Alismataceae	<i>Hydrocleis nymphoides</i>			0.6	+	2.3	+	0.4	+			1.5	+	1.2
	other trace types			2.0		4.3		2.2		2.5		2.0		2.6

**Table 1.**

Main pollen types and their frequency and frequency classes in samples of honey from *Butia yatay* palm savannas. Abbreviations: D, predominant pollen: (>45%); S, secondary pollen (45–15%); M, important minor pollen (15–3%); m, minor pollen (3–1%); +, present sporadic pollen (<1%).



**Figure 2.**

Pollen types present in all the samples. A. *Butia yatay*. B. *Eryngium horridum*. C. Type Myrtaceae. D. Type *Ammi*. E. *Echium plantagineum*. F. *Salix* sp. e: equatorial view. p: polar view.

along the samples: Myrtaceae type (Myrtle family), *Butia yatay* palm pollen, and *Eryngium horridum* (Apiaceae) pollen (Figure 2). Together, the three predominant types accounted for 88–96% of the total pollen counted. The fraction of *B. yatay* pollen ranged from 22.7% to 39.6%, with an average of 33.5%. Instead, the fraction of Myrtaceae and *E. horridum* ranged from 4.1% to 53.2% in the former and 11.9% to 54.0% in the latter. These two types had an almost perfect negative correlation (–0.98), where samples enriched with Myrtaceae were poor in *E. horridum* and vice versa. Along with these predominant types always appeared a small proportion of pollen of *Ammi* type (bishop’s weed, Apiaceae), *Echium plantagineum* (purple viper’s-bugloss, Boraginaceae), and *Salix* species (willows, Salicaceae) (Table 1 and Figure 2). In some samples, a very low proportion of pollen of *Hydrocleis nymphoides*

(waterpoppy, Alismataceae) also appeared. Three samples have predominant pollen frequencies ( $\geq 45\%$ ) and can be regarded as monofloral according to literature [22] and to Argentinian legislation: sample 1 with 54.0% of *E. horridum* and samples 3 and 6 with 53.2% and 48.5% of Myrtaceae type. In all the samples *Butia yatay* consistently achieved a secondary pollen frequency (45–15%).

The predominant pollen types found are in accordance with the surrounding vegetation and the specific months when suppers were filled with honey. Myrtaceae species such as *Blepharocalyx salicifolius*, *Eugenia uniflora*, and some species of *Myrcianthes* and *Myrceugenia* are common components of local riparian vegetation that bloom in the late spring and early summer [24]. These, like many other Myrtaceae, are very nectariferous species and are expected to have a high pollen load per volume of nectar which usually overrepresents them in honey samples [25]. *Butia yatay* and *E. horridum* started blooming at the end of October and peaked during the second half of November and throughout December. Field inspections confirmed that bees actively visited inflorescences of these species which were the most conspicuous and abundant floral resource during this period in the grazed palm savanna where the hives were located. *Eryngium* species have previously been informed as an important palynological element of local regional honey types [14, 26–28], sometimes even surpassing the 45% threshold to be categorized as the dominant type and to define them as monofloral [14]. On the other hand, there are very few records of palm pollen in Argentinian honey samples [14, 15, 27, 29], but none of them are of *B. yatay*. To our knowledge, this is the first report of the presence of pollen of *Butia yatay* palm in honey in all its occurring area. Nonetheless, there are two reports [10, 11] regarding *Butia odorata*, a species restricted to Eastern Uruguay and Brazil, in which honey samples from hives placed in extensive palm savannas had up to 90% of *B. odorata* pollen.

#### 4. Physicochemical analysis

Several physicochemical analyses could help to a comprehensive understanding of honey's quality and composition shedding light on how these factors influence its properties.

Among these factors, moisture content significantly impacts the properties of honey as it can affect its texture, stability, and susceptibility to spoilage. High humidity conditions during honey harvesting can be associated with the collection of immature or prematurely harvested honey, as well as poor beekeeping practices. Naturally, honey contains a certain amount of moisture, and this level plays a crucial role in determining its quality. Excessive moisture in honey can lead to undesirable consequences such as fermentation or the growth of harmful microorganisms. These issues can compromise the overall quality and shelf life of the honey. On the other hand, insufficient moisture content can cause honey to crystallize and alter its texture. To maintain the desirable properties of honey, it is essential to carefully manage its moisture content. This involves ensuring that the honey is harvested at the appropriate time, when it has reached the optimal moisture level. Additionally, proper manufacturing practices, including adequate storage and handling techniques, are crucial for preserving honey's quality and preventing moisture-related issues. By managing moisture levels effectively, beekeepers can ensure that honey retains its desired characteristics, such as its texture, stability, and extended shelf life [30, 31].

Another important factor is the hydroxymethylfurfural (HMF) that is generated as a result of the thermal degradation of sugars during its processing and storage. The formation of HMF is influenced by various factors, including temperature, time, pH, and sugar concentration. The presence of elevated levels of HMF in honey can serve as an indicative parameter of inadequate storage or processing practices, which may negatively impact the quality of the honey. High HMF content can lead to changes in the color, flavor, and aroma of honey, thereby diminishing its sensory appeal to consumers. Furthermore, the consumption of honey with excessive HMF content in significant quantities may potentially pose adverse health effects. Some studies have suggested that HMF can react with certain amino acids and proteins, forming compounds that could be detrimental to human health. It is important to highlight that honey produced in regions characterized by high ambient temperatures tends to exhibit higher levels of HMF. The elevated temperatures accelerate the rate of sugar degradation and consequently contribute to the formation of HMF during honey processing and storage. To ensure the maintenance of honey quality, it is crucial to adopt appropriate storage and processing techniques that minimize HMF formation. This entails proper temperature control, limiting exposure to heat, and utilizing efficient packaging materials that offer protection against external factors. Moreover, adherence to established quality standards and regulations is essential to ensure that honey maintains an acceptable level of HMF. By implementing these measures, honey producers and beekeepers can safeguard the desirable properties and nutritional integrity of honey, providing consumers with a high-quality and safe product. Continuous research and monitoring in the field of honey production are imperative to further enhance our understanding of HMF formation and its impact on honey quality and human health [32].

The acidity of honey contributes to its sensory properties, stability against microorganisms, improvement of chemical reactions, and antibacterial and antioxidant activities. It can be evaluated by determining pH and acidity parameters. The former provides information about ionized organic acids and inorganic ions such as phosphate and chloride, which are relevant to enzymatic activity, product texture, and inhibition of microbial growth. On the other hand, the acidity parameter is characterized by the presence of organic acids, mainly gluconic acid, resulting from the enzymatic action of glucose oxidase on glucose, and is in equilibrium with its corresponding lactones and aforementioned inorganic ions, which also contribute to acidity. The values of these parameters are influenced by the botanical source, harvesting time and conditions, freshness, and storage state of the honey. It is important to highlight that while acidity is a desirable characteristic in honey, excessively high acidity levels can indicate the presence of fungal and yeast growth. Measuring the pH and acidity parameters of honey provides valuable insights into its quality. The pH level indicates the presence of ionized organic acids and inorganic ions, which are essential for various chemical reactions and contribute to the stability of honey against microorganisms. However, it is crucial to maintain a balance, as very high levels of acidity indicate fungal and yeast growth, compromising the quality and safety of the honey. By monitoring and controlling acidity levels, honey producers can ensure the integrity and safety of their product [33, 34].

Among the amino acids present in honey, proline stands out as the most relevant as it is one of the parameters that can indicate the genuineness of honey. The presence and quantity of proline in honey thus can provide insights into whether it has been adulterated or mixed with other sugars or syrups. Pure and unadulterated honey typically exhibits higher levels of proline compared to honey that has been altered or diluted.

The proline content in honey depends on the time bees process the nectar and their maturity. Indirectly, the levels of this amino acid reflect the botanical origin of honey, fluctuating according to floral sources and being related to geographical origin [35–37].

Sugars constitute the predominant fraction in the composition of honey, with more than 22 types identified, wherein fructose and glucose are the most relevant reducing sugars. Their ratio and proportion serve as key indicators of the quality and crystallization capacity of honey, which vary according to the botanical diversity of the collected nectar, climatic conditions, and geographical location of the beehive. A higher fructose-to-glucose ratio is associated with a reduction in the glycemic index, which is of interest for both direct consumption and the development of functional products. Moreover, the detailed analysis of the specific sugar content enables the detection of adulterants and provides valuable information regarding the maturity level of honey [38, 39].

The mineral substances present in honey contribute to its nutritional value. The presence of approximately 37 macro and microelements has been discovered, with potassium (K) being the most abundant, followed by sodium (Na), magnesium (Mg), and calcium (Ca). The concentrations of these elements, both macro, and micro, vary in the mineral fraction of honey depending on the soil where the nectar-bearing plants grow. The mineral richness in honey can be estimated through the determination of ash, a parameter currently used to classify its origin and as a quality criterion [40]. It is important to note that the ash content of honey can serve as an indicator of environmental pollution, as high levels of toxic minerals pose a risk to human health. Furthermore, the mineral content of honey is related to other physicochemical parameters, such as color. In general, it is observed that light floral honey has lower ash content (0.2–0.3%) compared to darker honey (0.5–0.6%) [41–43].

It has been demonstrated that honey contains a wide variety of components with antioxidant activity, with phenolic acids and flavonoids cited as the main contributors to this activity due to their mechanisms of action and reactions based on the transfer of hydrogen atoms or simple electrons. This capacity, along with other parameters mentioned earlier, depends on the botanical origin, environmental and seasonal conditions of the harvest, as well as the processing of the honey. Furthermore, a positive correlation has been observed between antioxidant capacity and the color of honey, with darker varieties exhibiting higher antioxidant capacity compared to lighter ones. The interest in investigating the antioxidant potential of honey and analyzing its phenolic compounds and flavonoids has increased due to its potential as a food and functional ingredient, thanks to its bioactive properties [44, 45].

In the present work, physicochemical analyses were conducted on fresh honey samples from *Butia yatay* palm savannas. Moisture content was determined using refractometry, following the AOAC 969.38 B [46]. The acidity content was quantified through neutralization titration, and pH was measured using a pH meter, following the AOAC 962.19 [47] and Bogdanov and Marcazzan [48] methodologies, respectively. Additionally, total reducing sugar content was evaluated using spectrophotometric techniques, as described in Ávila Núñez et al. [49]. Glucose was determined following the method proposed by Goñi et al. [50], and total polyphenols were quantified according to Singleton et al. [51]. HMF was quantified using HPLC, following Rivero et al. [43]. Furthermore, the content of insoluble solids in water was determined using the validated V22 MAFF method from the J. Assoc. Public Analysts. The ash content of the honey samples was determined by combustion of the sample at 550° C until constant weight, following AOA 923.03. Additionally, proline analysis was conducted following the guidelines outlined in the IRAM N 15940-2.



Honey samples	1	2	3	4	5	6
Moisture (%)	17.0 ± 0.1 <sup>b</sup>	16.3 ± 0.1 <sup>c</sup>	16.5 ± 0.1 <sup>c</sup>	17.6 ± 0.1 <sup>a</sup>	17.0 ± 0.1 <sup>b</sup>	16.5 ± 0.1 <sup>c</sup>
HMF (mg/kg)	nd	nd	nd	nd	nd	nd
Acidity (meq/kg)	40.0 ± 0.1 <sup>a</sup>	36.5 ± 4.7 <sup>ab</sup>	36.3 ± 2.1 <sup>b</sup>	35.5 ± 1.3 <sup>ab</sup>	43.3 ± 2.0 <sup>c</sup>	35.0 ± 0.7 <sup>b</sup>
pH	4.3 ± 0.2 <sup>ab</sup>	4.3 ± 0.1 <sup>b</sup>	4.5 ± 0.2 <sup>a</sup>	4.5 ± 0.2 <sup>a</sup>	4.4 ± 0.4 <sup>a</sup>	4.7 ± 0.2 <sup>a</sup>
Water-insoluble solid (%)	0.03 ± 0.02 <sup>a</sup>	0.05 ± 0.01 <sup>a</sup>	0.02 ± 0.01 <sup>a</sup>	0.05 ± 0.17 <sup>a</sup>	0.02 ± 0.02 <sup>a</sup>	0.04 ± 0.01 <sup>a</sup>
Proline (mg/kg)	677 ± 21 <sup>a</sup>	690 ± 101 <sup>a</sup>	673 ± 77 <sup>a</sup>	651 ± 61 <sup>a</sup>	424 ± 43 <sup>a</sup>	582 ± 45 <sup>a</sup>
Reducing sugars (%)	71.5 ± 1.1 <sup>a</sup>	79.8 ± 1.7 <sup>a</sup>	71.9 ± 1.4 <sup>a</sup>	68.1 ± 4.3 <sup>a</sup>	75.7 ± 0.7 <sup>a</sup>	74.7 ± 7.5 <sup>a</sup>
Glucose (%)	24.2 ± 0.2 <sup>cd</sup>	30.4 ± 0.4 <sup>a</sup>	22.5 ± 0.2 <sup>ef</sup>	21.2 ± 0.2 <sup>f</sup>	28.0 ± 0.5 <sup>b</sup>	24.9 ± 0.5 <sup>c</sup>
Ash (%)	0.51 ± 0.01 <sup>ab</sup>	0.50 ± 0.02 <sup>a</sup>	0.57 ± 0.01 <sup>c</sup>	0.59 ± 0.002 <sup>c</sup>	0.53 ± 0.04 <sup>b</sup>	0.53 ± 0.01 <sup>ab</sup>
Total phenolic content (mg GA/100 g)	623 ± 3 <sup>a</sup>	654 ± 36 <sup>a</sup>	583 ± 1 <sup>a</sup>	668 ± 36 <sup>a</sup>	578 ± 33 <sup>a</sup>	618 ± 20 <sup>a</sup>

**Table 2.**

Physicochemical parameters of honey samples. The means ± standard deviation values are reported. Different letters in the same optimal powder indicates significant difference ( $P < 0.05$ ,  $n = 3$ ).

**Table 2** shows the values obtained of the physicochemical parameters of the 6 honey samples analyzed in this study. The moisture content ranged from 16.3% to 17.6%, values that are below the maximum limit of 20.0% suggested by the Codex Alimentarius for unclassified honey. These results are consistent with those reported by Acquarone et al. [52] and Salgado [16] for Argentine honey samples, who obtained ranges of 15.3–19.0% and 15.4–20.0%, respectively. These values indicate that honey is mature, with no risk of fermentation and good stability. Additionally, the determination of hydroxymethylfurfural (HMF) by HPLC resulted in non-detectable amounts in the analyzed samples, which could be attributed to their freshness and the absence of prior thermal treatments before analysis.

Free acidity and pH are good indicators of quality, and according to the Codex Alimentarius, these parameters should not exceed 50.0 milliequivalents/kg and should be between 3.4 and 6.1, respectively. The palm savanna honey samples comply with these international regulations, presenting values of 35.0–43.3 milliequivalents/kg and 4.3–4.7, respectively. These results are expected in fresh honey samples with optimal moisture content and they are similar to those results reported by Salgado [16] of 8.0–68.0 milliequivalents/kg of honey and a pH of 3.2–6.4 for other Argentinian honey samples. Other similar results were obtained in a study conducted by Cabrera and Santander [53] on honey from the province of Formosa (Argentina), where a content of 28.9 milliequivalents/kg of honey and an average pH of 4.01 was found.

Regarding the total reducing sugars content, a range of 68.1% to 79.8% was observed, with glucose contents ranging from 21.2% to 30.4%. These values are similar to those reported by Ciappini et al. [54] of 63.2–70.6% for reducing sugars and 23.6–38% for glucose (*Trifolium* spp., *Medicago sativa*, *Eucalyptus* spp. and *Melilotus* spp. floral honey from Santa Fe province, Argentina). Maldonado et al. [55] also reported glucose values in the range of 23.7–38.2% (*Citrus limon* honey, Tucuman province, Argentina). These results comply with international regulations for genuine honey.

The ash content values obtained through gravimetry were below the upper limit established by the Codex ( $<0.6\%$  for unidentified honey samples). However, the observed range for this parameter was from  $0.50\%$  to  $0.59\%$ , significantly higher than those reported by Baroni et al. [56] and Acquarone et al. [52] for Argentine honey samples. This is consistent with the values obtained for conductivity and color, suggesting that the samples analyzed here possess a substantial mineral content.

The amino acid proline is a quality criterion specified in the Codex Alimentarius standards, which recommends a minimum of  $180\text{ mg/kg}$  for genuine honey. In these honey samples, a higher content of proline was found in the range of  $424\text{--}690\text{ mg/kg}$  of honey. These values are also higher than the range of  $50\text{--}499\text{ mg/kg}$  reported by Montenegro for honey samples from the province of Chaco (Argentina).

Regarding water-insoluble solids, they were found within the limits established by the Codex, with values of  $0.02\text{--}0.05\%$ . According to the regulations, honey obtained by centrifugation should not contain more than  $0.1\text{ g/100 g}$ .

Finally, the total polyphenol content in these honey samples was  $578\text{--}668\text{ mg/100 g}$ , indicating a higher antioxidant capacity compared to the values reported by Maldonado et al. [57] of  $130\text{--}290\text{ mg/100 g}$ . These findings suggest that *Butia yatay* palm savanna honey may have potential health benefits for humans.

The overall physicochemical characteristics obtained for *Butia yatay* palm savanna honey indicate high quality, freshness, absence of thermal treatment, and production under good apicultural practices. Furthermore, the high polyphenols content and proline indicate high bioactivity and confirm its genuineness. These positive attributes contribute to the overall excellence of the honey and its potential health benefits for consumers.

## 5. Electrical conductivity

The conductivity of honey is an indicator of the amount of ions present in the liquid. Conductivity refers to the ability of a material to conduct electrical current. The conductivity of honey can vary depending on its composition and origin. Generally, a higher conductivity indicates a higher content of mineral salts and other dissolved compounds. This may be associated with factors such as the quality of the nectar used by bees and the processing of the honey. Besides, the conductivity of honey can indicate its authenticity and quality. For example, some adulterated or diluted honey may have lower conductivity due to the dilution of dissolved components.

Furthermore, it has been shown that the trace element content in honey is related to its botanical origin and color. Floral honey generally has a lower mineral content compared to dark honey types such as honeydew, chestnut, and heather honey [41]. However, this determination is not only useful for assessing the botanical origin of honey but also its geographical origin. In Acquarone et al. [52], statistically significant differences were observed in the maximum electrical conductivity values depending on the geographical origin of the analyzed samples. This is interesting because by studying the variation of conductivity concerning soluble solids, it is possible to obtain curves that differentiate honey from a particular region.

In the present work conductivity of honey was measured at different solid content by using a Milwaukee MW801 conductometer. The dependence of specific electrical conductivity (K) on honey concentration was characterized by a maximum at a  $C_{\text{max}}$

value corresponding to a dry solids of honey concentration of 30–35% (w/w) [52]. The obtained results were fitted using a nonlinear regression with the following equation (Eq. (1)):

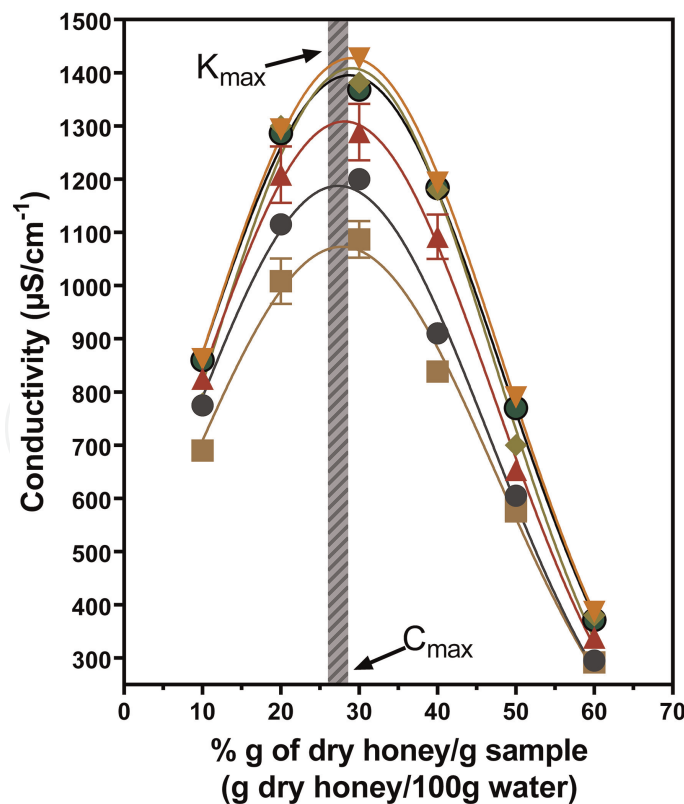
$$K = K_{\max} * e^{\left(-0.5 * \frac{(C-C_{\max})}{SD^2}\right)} \quad (1)$$

where  $K$  and  $K_{\max}$  are the electrical conductivities at concentration  $C$  and at maximum ( $C_{\max}$ ), respectively, and  $SD$  is the amplitude of Gaussian curve.

**Figure 3** presents the results of the dependence between electrical conductivity and honey concentration. It can be observed that when the solids concentration in honey was high (60%), the electrical conductivity values decreased due to the increased viscosity (high honey content). Towards the origin of the x-axis, as the solids concentration decreases, a peak corresponding to the maximum ion concentration is observed, followed by a decrease as these ions are diluted. The electrical conductivity values depend on the concentration and mobility of ions present in the honey solution.

Regarding the obtained values from the nonlinear regression, it was observed that all samples exhibited the same behavior. The used model predicts very well the experimental data as R-squared value greater than 0.9844. The peaks corresponding to the maximum conductivity ( $K_{\max}$ ) values for *Butia yatay* savanna honey samples ranged from 26.96 to 29.14% solids.

Other honey samples from Argentina showed lower curves for conductivity but similar  $C_{\max}$ . These could be related to a lower mineral content and ash content [52].



**Figure 3.** Conductivity of *Butia yatay* savanna honey samples at different solid concentrations (%). The gray zone indicates the solid concentration range ( $C_{\max}$ ) for the maxima conductivity ( $K_{\max}$ ). Sample 1 ● (black circles), sample 2 ■ (brown squares), sample 3 ▲ (red up triangles), sample 4 ▼ (orange down triangles), sample 5 ◆ (green diamonds), sample 6 ● (green circles).

Similarly to the present *Butia yatay* palm savanna honey, other honey from Spain with different botanical origins showed similar high conductivity values. These samples showed 976  $\mu\text{S}/\text{cm}$  for heather honey and 986  $\mu\text{S}/\text{cm}$  for forest honey at 20% solids [58]. Samples from Argentina of *Condalia microphylla* also showed high conductivity values (841  $\mu\text{S}/\text{cm}$ , 20% w/v solids). Considering these authors evaluated only a concentration, it is possible to say that those honey samples would have a higher conductivity at  $C_{\text{max}}$  (close to 30%) similar to those reported in the present work.

## 6. Rheology

Understanding and controlling the rheological properties of materials is vital for optimizing industrial processes across various sectors, including food and drink, cosmetics, pharmaceuticals, and oil and gas.

In industrial processes, changes in rheology can significantly impact pump performance as they must overcome the resistance and viscosity of the transported fluid. Higher viscosity fluids require more pumping force, leading to increased energy consumption or reduced throughput. The flow behavior of material also affects the efficiency of transport systems like pipelines, where pressure drops and flow rates depend on the rheological characteristics of the medium being transported.

Rheology is also crucial in the mixing process. In applications where different components or additives need to be uniformly dispersed or blended, the rheological properties of the materials involved influence mixing efficiency and final product quality. Understanding flow behavior and viscosity allows the selection of appropriate mixing equipment, optimization of mixing parameters, and prevention of issues such as uneven distribution or clumping.

In summary, rheology studies and understanding how materials respond to external forces are critical for optimizing industrial processes. By characterizing and controlling the rheological properties of materials, companies can enhance process efficiency, reduce energy consumption, improve product quality, and ensure the reliability of transportation systems.

The power law viscosity, also known as Ostwald's law of viscosity or the flow behavior index, is a rheological property that describes the relationship between shear rate and shear stress in fluids. According to the power law model, the viscosity ( $\eta$ ) of a fluid can be represented by the equation (Eq. (2)) and if taking logarithm (Eq. (3)):

$$\eta = K * (\dot{\gamma})^n \quad (2)$$

$$\log (\eta) = n * \log (\dot{\gamma}) \quad (3)$$

where:  $\eta$  represents the viscosity of the fluid,  $K$  is the consistency index or coefficient of consistency,  $\dot{\gamma}$  is the shear rate (velocity gradient), and  $n$  is the flow index or consistency exponent.

The flow index ( $n$ ) characterizes the flow properties of the fluid. For Newtonian fluids, which have a constant viscosity regardless of the shear rate,  $n$  is equal to 1. Non-Newtonian fluids, however, can exhibit different flow behavior indexes: If  $n < 1$ , the fluid is pseudoplastic or shear-thinning, meaning its viscosity decreases as the shear rate increases. If  $n > 1$ , the fluid is dilatant or shear-thickening, indicating its viscosity increases with increasing shear rate.

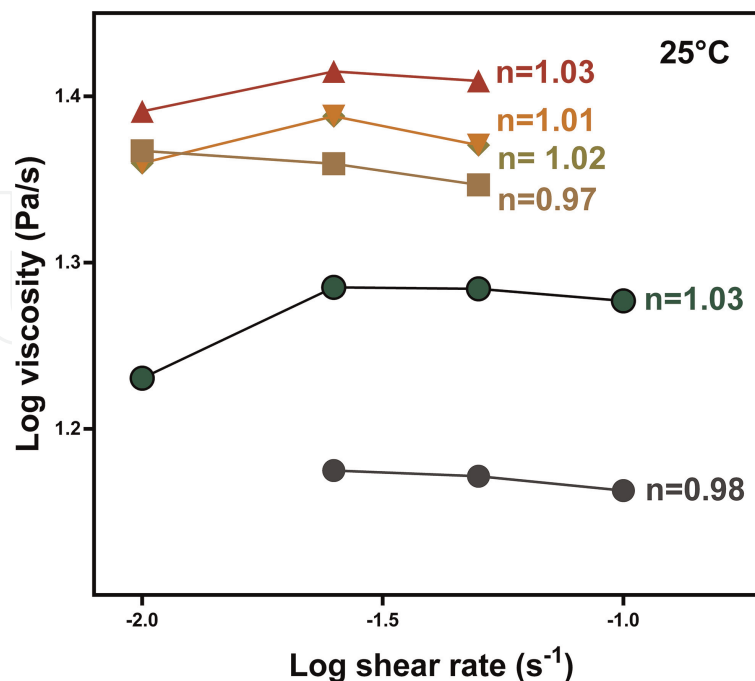
The rheological properties of honey can vary significantly based on botanical origin, moisture content, sugar content and composition, and processing. Temperature also modifies the viscosity, higher temperatures are related to lower viscosities. Another factor that may affect this is the glucose monohydrate crystallization process occurring during honey storage.

Once honey undergoes crystallization, it transforms into what is known as set honey. Set honey is a semi-solid, two-phase structure that differs significantly in its properties compared to its liquid state, which is referred to as strained honey. The crystallization process brings about significant alterations in the sensory properties of honey, specifically affecting its texture and water activity. Moreover, the rheological properties of crystallized honey undergo continuous modifications during storage as a consequence of the crystallization process [59].

In the current study, honey samples were subjected to a heating process at 40°C to dissolve all existing crystals, followed by incubation at 25°C for 30 minutes. The viscosity of the honey samples was then measured at various shear rates (0.3, 0.6, 1.5, 3.0, 6.0 rpm) while maintaining a temperature of 25°C ± 0.1°C, using a NDJ8s Digital Portable intelligent viscometer.

**Figure 4** illustrates the logarithmic relationship between viscosity and shear rate (using Eq. (3)). The linear regression analysis of these curves determined the flow index of the honey samples, whose values were close to 1 for all samples at 25°C (n obtained values at **Figure 4**). Therefore, *Butia yatay* palm savanna honey exhibited a Newtonian behavior at 25°C while the differences among samples could be attributed to natural variations in composition (individual sugars and water content) [60].

Other authors showed that steady shear and dynamic rheological tests revealed Newtonian behavior with a viscosity Arrhenius model dependence on temperature: for Greek honey samples in the range of 20–60°C [60]; for Ethiopian honey samples



**Figure 4.**

Log viscosity vs. log shear rate of honey samples at 25°C. The law power flow index is indicated for each honey sample. Sample 1 ● (black circles), sample 2 ■ (brown squares), sample 3 ▲ (red up triangles), sample 4 ▼ (orange down triangles), sample 5 ◆ (green diamonds), sample 6 ● (green circles).

in the 25–45°C range [61]; and for Portuguese honey samples in the 30–70°C range [62]. Da Silva et al. [62] proposed a model combining both temperature and solid content to predict honey viscosity behavior with good results for 40 Brazilian honey samples in the 10–60°C range. Furthermore, honey samples from the northeast of Argentina also proved to be Newtonian with Arrhenius temperature dependence in the range of 10–50°C [57]. These authors have found that some differences in rheological properties between Argentina regions may be attributed to natural variations in honey composition related to climate and flora variation. Higher temperatures and environmental moisture might also influence the maturation of honey in the beehive, affecting its physicochemical properties and leading to differences in honey rheology [57].

## 7. Color

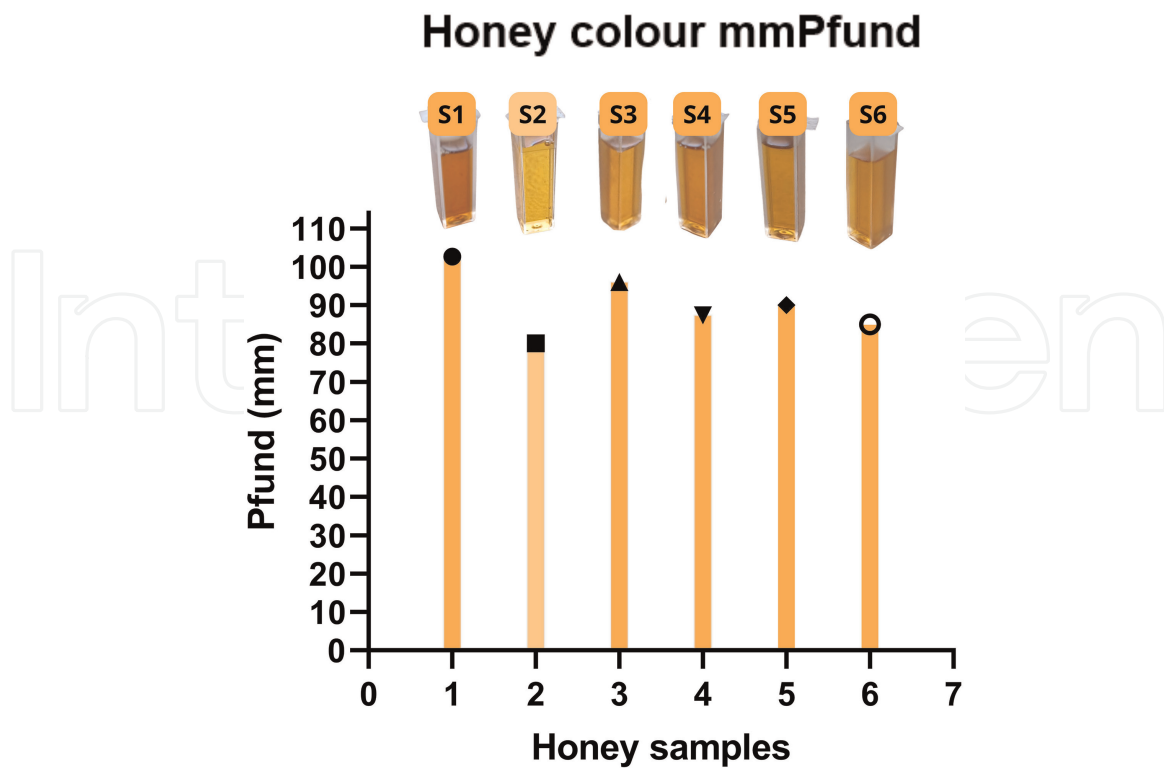
The color of honey exhibits a proportional variation based on the presence of pigments (carotenoids and xanthophylls), polyphenols (flavonoids), minerals, as well as the source of nectar (flowers or honeydew). Monofloral honey types possess distinctive and defined hues. Honey manifests a broad spectrum of colors, ranging from pale white to deep amber, including tinges of red, yellow, and green. However, lighter brown or amber tones dominate [63]. These discrepancies hold significance in studies concerning geographical and origin denominations, as well as commercial relevance, facilitating honey exports and allowing exporters to target favorable markets.

The color of honey is internationally standardized using the Pfund color scale, which provides a recognized and standardized technique for rapid, effortless, and cost-effective color measurement. This scale ranges from 0 to 140 Pfund millimeters, classifying honey into distinct categories such as water white, extra white, white, extra light amber, light amber, amber, and dark amber [64, 65].

For color analyses a portable Hanna Honey Colorimeter was used. This equipment (calibrated with glycerol) enables the assessment of light transmission through a clear honey sample into the cell, ensuring it is devoid of any residues or air bubbles that may impact the measurement. **Figure 5** shows the color of the analyzed sample expressed in Pfund millimeters. Meanwhile, **Table 3** shows the color categories. It can be observed that the analyzed samples corresponded to amber honey, with only sample 2 samples falling within the upper limit for light amber honey. These findings align with those obtained for total polyphenols and conductivity, signifying that *Butia yatay* palm savanna honey possesses higher mineral and total polyphenol contents compared to other lighter honey variants from Argentina. For example, other authors found that honey from central Argentina showed color ranging from water white to light amber categories, corresponding to a very low mineral and ash content [66]. On the other hand, Uruguayan honey samples with high pollen content (>50%) of the palm *Butia odorata* showed similar values of color (75 mm Pfund, light amber) to those obtained in the present study [11].

## 8. Conclusions

In this work, we characterize honey produced in the *Butia yatay* palm savanna in the province of Entre Ríos, Argentina, during the palms' blooming peak. Palm pollen was consistently present in all the analyzed samples as secondary pollen regarding its



**Figure 5.** Color of honey samples (mm Pfund). A photo of each sample is shown.

Honey color	Pfund range (mm)
Water white	$x \leq 8$
Extra white	$8 < x \leq 17$
White	$17 < x \leq 34$
Extra light amber	$34 < x \leq 50$
Light amber	$50 < x \leq 85$
Amber	$85 < x \leq 114$
Dark amber	$x > 114$

**Table 3.** Color categories of honeys. The mm Pfund range is shown for each color category.

frequency with an average of 33.5% of the total pollen grains. Thus, the contribution of this species as a nectar source for honey production is significant and it is here informed for the first time. This honey also showed interesting characteristics such as high proline content, elevated conductivity, a color range from light amber to amber, and significant polyphenol bioactivity. These properties together with a fruity aroma and an exceptionally sweet taste distinguish this honey as an attractive non-timber forest product (better instead, palm savanna product) with potentially higher market values. This could contribute to local beekeepers' economy by improving this honey trading while contributing to the conservation of palm populations through their use as well.

This is the first instance of producing and characterizing honey from this peculiar botanical and geographical origin, thus contributing to Argentinian efforts to hierarchize regional and local honey types to add value to them in the market, as well as contributing to general mellitological knowledge. Consequently, we plan to carry on organoleptic and further physicochemical studies in the near future to enhance our knowledge of this palm honey in its entirety.

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