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Impact of botanical origin on the chemical composition of Amazonian monofloral honey produced by *Apis mellifera*

[Impacto da origem botânica na composição química de méis monoflorais amazônicos produzidos por *Apis melífera*]

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Abstract: Beekeeping plays a significant role in the economy of northern Brazil, with honey being the primary product. Due to its biodiversity, the Amazon region offers a variety of floral sources for honey production. Monofloral honey is predominantly obtained from the nectar of a single floral species, imparting distinct characteristics, such as color, aroma, and flavor. Therefore, this study aimed to analyze and compare three *Apis mellifera* honey samples collected in the state of Pará: monofloral honey from açaí (*Euterpe oleracea*), orange (*Citrus* × *sinensis*), and mangrove (*Rhizophora mangle* L.). Pollen analysis identified the botanical origin and confirmed the monofloral classification. The samples were evaluated for their physicochemical parameters, which complied with current regulations. The mineral and proline contents were determined, yielding values consistent with the literature. The bioactive composition was assessed by analyzing the total phenolic compound (50.5–168.08 mg GAE/g), flavonoid (8.08–38.01 mg QE/100 g), and anthocyanin contents (0.26–2.07 mg/g) and antioxidant capacity via ABTS (1.1–3.1 µM TE/g), FRAP (2.38–5.72 µM Fe(II)/g), and DPPH (0.26–0.55 µM TE/g) assays. Among the samples, *Euterpe oleracea* honey presented the highest bioactive and antioxidant values, surpassing the other honey samples, as described through data analysis. These results highlight the apicultural potential of the Amazon region.

Keywords: açai; Amazon; beekeeping; bioactive compounds.

Resumo: A apicultura desempenha um papel significativo na economia do norte do Brasil, sendo o mel o principal produto. Devido à sua vasta biodiversidade, a região amazônica oferece uma ampla variedade de fontes florais para a produção de mel. O mel monofloral é obtido predominantemente do néctar de uma única espécie floral, conferindo características distintas como cor, aroma e sabor. Portanto, este estudo teve como objetivo analisar e comparar três amostras de mel de *Apis mellifera* coletadas no estado do Pará: méis monoflorais de açaí (*Euterpe oleracea*), laranjeira (*Citrus* × *sinensis*) e mangue (*Rhizophora mangle* L.). A análise polínica foi realizada para identificar a origem botânica e confirmar a classificação monofloral. As amostras foram avaliadas quanto aos parâmetros físico-químicos, todos em conformidade com a legislação vigente. O teor de minerais e os níveis de prolina também foram determinados, apresentando valores consistentes com a literatura. A composição bioativa foi avaliada por meio de análises de compostos fenólicos totais (50,5 a 168.08 mg EAG/ g), flavonoides (8,08 A 38,01 mg EQ/100 g), antocianinas (0,26 a 2,07 mg/ g), capacidade

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antioxidante ABTS (1,1 a 3,1 µM ET/g), FRAP (2,38 a 5,72 µM ESF/ g) e DPPH (0,26 a 0,55 µM ET/ g). Dentre as amostras, o mel de *Euterpe oleracea* apresentou os maiores valores bioativos e antioxidantes, superando os demais méis sendo descrito através da análise de dados, o que destaca o notável potencial apícola da região amazônica.

Palavras-chave: açaí; Amazônia; apicultura; compostos bioativos.

1. Introduction

Beekeeping is an important ancient activity, as bees play a crucial role in pollination and produce key products, such as honey, royal jelly, propolis, beeswax, and pollen. Brazil contains a diversity of climates and vegetation, which enables the production of honey with distinct characteristics that vary from region to region, showcasing unique properties, such as color, aroma, and flavor [1,2].

Honey is a natural organic product produced by bees through the collection of nectar from plants, and its characteristics vary considering flowering, the climate, soil, storage, and species of honey-producing bees. This results in honey with different characteristics, which can be classified as monofloral and multifloral. Monofloral honey is honey in which the pollen content originates from flowers of the same family, genus, or species, with an incidence of at least 45 % from a single pollen source; its classification must meet specific criteria for each floral source [3]. Honey is primarily composed of glucose and fructose, with smaller quantities of minerals, vitamins, enzymes, organic acids, flavonoids, phenolic acids, and other phytochemicals [4,5]. Flavonoids and phenolic acids, in particular, are responsible for the antioxidant capacity of honey [6].

The botanical and productive diversity of the Amazon, especially in the state of Pará, form a significant basis for resource utilization. In this context, landscapes associated with açaí (*Euterpe oleracea*) cultivation stand out, significantly driving the production chain and having great economic and social importance in Pará [7]. Citrus cultivation in the northern region, specifically orange (*Citrus* × *sinensis*), is also of socioeconomic importance for northeastern Pará; the municipality of Capitão Poço is one of the region's major producers [8]. Amazonian mangroves form extensive areas of coastal vegetation and constitute important environments for maintaining biodiversity and the floral resources available to pollinators [9].

The equatorial climate of the northern region is characterized by high temperatures, high humidity, and rainfall seasonality, which directly influence flowering and nectar and pollen availability, consequently affecting foraging behavior and honey production. Research conducted in western Pará has shown that higher honey production is associated with the equatorial climate during the dry season, when increased flowering increases food resource availability for bees [10]. Thus, the interactions among plant diversity, climate seasonality, and bee activity facilitate the production of monofloral honey derived from açaí, orange, and mangrove sources, imparting distinct characteristics linked to their botanical and environmental origins.

Honey from the Amazon region has specific chemical properties due to the equatorial climate and botanical diversity. Research has shown that the humid climate and high temperatures influence the physicochemical parameters, leading to changes in the moisture, acidity, and hydroxymethylfurfural (HMF) content. Therefore, quality control standards for honey are enforced more rigorously to slow these reactions [11,12]. Vegetation is one of the main factors influencing the bioactive composition of honey, and the presence of these compounds originates from the pollen and nectar collected by bees [6].

This study is significant given the remarkable plant and productive diversity in the state of Pará, which offers important floral sources for producing honey with a distinct regional identity. The state's prominence in açaí production, extensive citrus farming in northeastern Pará, and

vast Amazonian mangrove forests create environments with a high potential for producing monofloral honey. In addition contributing to the appreciation of natural resources and Amazonian biodiversity, these products add value to regional beekeeping by exhibiting chemical and functional characteristics linked to their botanical origin. Therefore, this study aimed to evaluate the influence of açaí, orange, and mangrove floral sources on the chemical composition of monofloral honey produced by *Apis mellifera* in the Amazon, contributing to their characterization and recognition as products with a distinct Amazonian identity.

2. Material and methods

2.1 Honey samples

This research was conducted using three honey samples from bee species *A*. *mellifera*. Monofloral orange blossom honey (*C*. × *sinensis*) was obtained from the municipality of Capitão Poço, PA (01°45'54.1"S 47°13'29.4"W) (Figure 1 - B). Monofloral açai honey (*E*. *oleracea*) was obtained from the municipality of Santa Maria, PA (1°20'06.6"S 47°30'01.6"W) (Figure 1 - A), and monofloral mangrove honey (*Rhizophora mangle* L.) was obtained from the municipality of Santa Maria, PA (01°20'13.9"S 47°30'20.1"W) (Figure 1 - C).

Honey production in the communities studied relies on a traditional management system for *A*. *mellifera*, where beekeeping serves as a complementary activity linked to pollination. Harvesting took place once per year, typically between June and August, which is the period most favorable for honey production. Collection was preferably carried out in the morning at ambient temperatures of around 29°C, targeting only strong, populous colonies that had stored sufficient honey for harvest.

The bees were kept in standardized hives consisting of a brood chamber and attached honey supers, where they store surplus honey. During harvest, the honey supers and other storage compartments were collected using traditional methods, involving pooling the contents from different hives to form a single batch of honey. After collection, the product was bottled in polyethylene terephthalate containers—without undergoing industrial processing—and sold through traditional channels within the communities themselves and at local markets. This harvesting method is traditional to the region, and the researchers did not influence the collection process. The honey samples were transported in insulated containers for analysis at the Chemistry Laboratory of the State University of Pará (CCNT campus in Belém, Pará).

The study areas are privately owned and feature a landscape typical of Amazonian environments, with available water resources and surrounding vegetation, creating favorable conditions for colony maintenance and foraging activity.

Honey associated with the açai palm flowering period was collected from an *E*. *oleracea* cultivation area of approximately 18 ha, where 16 colonies were distributed, corresponding to a density of approximately 0.89 colonies/ha. Of the colonies present in the area, 15 were selected for honey collection because they showed adequate development, were considered healthy and strong, and had a sufficient quantity of stored honey. Honey collection was carried out during the açai palm flowering period. The honey obtained from the colonies in each group was pooled and homogenized, resulting in a composite sample. Approximately 18 kg of honey were obtained, corresponding to an average yield of 1.20 kg of honey per colony.

Orange blossom honey production was carried out in an area of approximately 13 ha planted with orange trees (*C*. × *sinensis*), where 17 hives were distributed, corresponding to an average density of 1.31 hives/ha. Among the colonies, 14 (82.35 %) showed adequate development

and were considered healthy. The hives were distributed in an area predominantly composed of orange trees, ensuring high availability of floral resources during the flowering period and favoring bee foraging.

Mangrove honey production was conducted in an area of approximately 8 ha of mangrove vegetation—an ecosystem subject to periodic tidal flooding—where 10 hives were distributed, corresponding to an average density of 1.25 hives/ha. Among the established colonies, nine hives showed adequate development and were considered healthy; therefore, they were selected for honey collection. The location of the hives in the mangrove environment provided the bees with access to the floral resources characteristic of this ecosystem—including nectar and pollen sources—which influence the botanical origin and chemical composition of honey.

The honey samples were labeled with the following acronyms: *C*. × *sinensis* (CS); *E*. *oleracea* (EO); and *R*. *mangle* L. (RM).

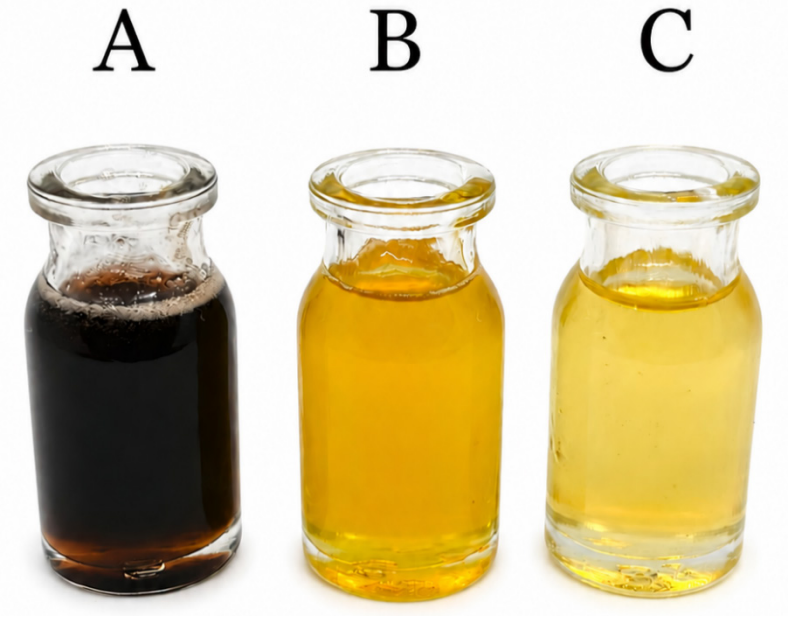


Figure 1. A – Açai palm honey with pollen predominantly from *Euterpe oleracea*; B –Orange tree honey with pollen predominantly from *Citrus* × *sinensis*; C – Mangrove honey with pollen predominantly from *Rhizophora mangle L*.

2.2 Methods

2.2.1 Melissopalynological analysis of honey

Pollen identification was adapted from Louveaux et al. [3]. Briefly, the sample was diluted 50 % in water and centrifuged for 20 min at 3400 rpm. Three slides were prepared for each sample with one or two drops of the centrifuged sediment, and a drop of iodized glycerin was added between the slide and cover slip and visualized at 1000× magnification under an optical microscope. The following were counted and classified: dominant pollen (PD > 45 %), accessory pollen (PA 16–45 %), and isolated pollen (PI 3–15 %). The EO pollen was identified following Muto et al. [13], who evaluated the impacts of the introduction of stingless bees on açaí production. The CS pollen was identified based on the pollen catalog “Pollen in Brazilian Honey” [14], and RM pollen was identified using a pollen catalog describing spores and pollen grains of aquatic plants from the coastal plain of southeastern Brazil [15].

2.2.2 Physicochemical analyses

Free, lactonic, and total acidity and pH were determined following AOAC 962.19 [16]. The moisture content was determined using the method described in AOAC 969.38 [16]. The HMF content was determined following the method described by White [17]. The diastatic activity was determined

as described by Bogdanov, Martin, and Lüllmann [18]. Honey color was assessed as described by Bianchi [19]. Reducing and non-reducing sugars were quantified using the 3,5-dinitrosalicylic acid method according to Miller [20]. The ash content was determined following the AOAC 923.03 method [16]. Electrical conductivity was measured using a conductivity meter in a 50 % honey solution [21]. The mineral content was determined through sample digestion in HNO_3 and quantified with microwave plasma atomic emission spectrometry using Model G8003A. The results were expressed in mg/kg [22].

The proline content was carried out following the methodology described by Ough [23] and adapted by Bogdanov et al. [24]. The method is based on the reaction of a honey solution with formic acid and a ninhydrin solution in isopropanol. For the analysis, 0.5 mL of a honey solution (0.05 g/mL) was reacted with 1 mL of 80 % formic acid and 1 mL of 3 % ninhydrin solution in ethylene glycol and stirred. It was then placed in a boiling water bath for 10 min, and after cooling, 5 mL of 50 % isopropanol was added. The solution was analyzed on a spectrophotometer at 510 nm. A proline solution was prepared and used as a standard.

2.2.3 Bioactive compounds and antioxidant capacity

The extraction of bioactive compounds from the honey samples was performed using a hydro-organic extraction procedure with acetone and methanol as solvents. Briefly, 2.0 g of honey was weighed and transferred to an appropriate container, and 5 mL of 70 % (v/v) acetone was added. The mixture was subjected to orbital shaking at 180 rpm for 1 h at room temperature. Subsequently, 5 mL of methanol was added, and the mixture was shaken at 180 rpm for an additional 1 h. After extraction, the resulting suspension was filtered through filter paper, and the filtrate was transferred to amber glass vials and stored under refrigeration at approximately 4°C until analysis of the bioactive compounds. The total phenolic compound content was determined as described by Singleton, Orthofer, and Lamuela-Raventós [25]. The total flavonoid content was determined according to the methodology described by Chang et al. [26]. The total anthocyanin content was determined following the method described by Francis [27].

The antioxidant activity was determined using the ABTS radical as described by Re et al. [28], FRAP as described by Benzie et al. [29], and the DPPH radical-scavenging assay as described by Mensor et al. [30], with modifications. The main modification consisted of using Trolox as the antioxidant standard to construct the calibration curve. The antioxidant activity of the honey extracts was calculated from the Trolox calibration curve and expressed as µmol Trolox equivalents (TE) per gram of honey (µmol TE/g).

2.3. Data processing analysis

All analyses were performed in triplicate, and the results were expressed as the mean ± standard deviation (SD). The coefficient of variation (CV%) was calculated to determine the analytical variability among replicates. As honey samples from multiple hives of each botanical origin were homogenized to obtain a single composite sample per honey type, the analytical replicates were considered technical replicates and not treated as independent biological replicates. Therefore, the data were evaluated descriptively, considering the mean, standard deviation, coefficients of variation, and relative differences among honey types.

3. Results and discussion

3.1 Melissopalynological analysis of honey

In Figure 2, images A and B represent the *E. oleracea* pollen found in açai palm honey, images C and D show the *C.* × *sinensis* pollen observed in orange blossom honey, and images E and F show the *R. mangle* pollen found in mangrove honey.

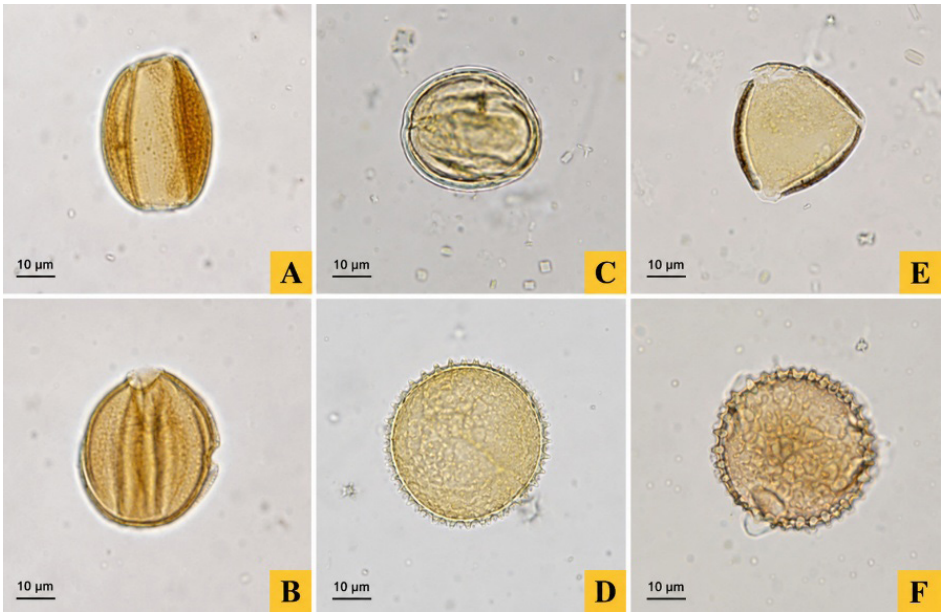


Figure 2. Equatorial and polar views of pollen grains of *Euterpe oleracea* (A and B), *Citrus* × *sinensis* (C and D), and *Rhizophora mangle* L. (E and F).

Equatorial views of pollen: Figure A, The grain presents a regular elliptical outline and an elongated shape, with a longitudinally oriented colpus-type aperture along the polar axis. The exine exhibits striate ornamentation, with parallel striae running along the entire body of the grain, imparting a characteristic ribbed appearance. Figure B, A pattern similar to the previous one was observed, with a sub-elliptical outline and a slight constriction near the apertural region. The ornamentation is also striate, but the striae are thicker and slightly undulate; the colpus region is more distinct, suggesting thickened margins (margo). Figure C, The grain displays an elliptical outline with an irregularly folded surface, indicating possible partial exine collapse due to dehydration during preparation. The ornamentation appears to be rugulate to foveolate, featuring irregular grooves that make it difficult to distinguish the primary pattern due to the overlapping folds.

Pollen grains viewed from the pole (polar view): Figure D, There is a circular outline with a slightly serrated margin due to small, regular exine projections. The ornamentation is micro-echinate (short, numerous, evenly distributed spines) superimposed on a finely granular (scabrate) background surface. Figure E, The grain has a subtriangular amb and three convex angles corresponding to the probable position of the apertures (a configuration consistent with a tricolporate grain). The exine exhibits ornamentation ranging from psilate to micro-reticulate, with evident thickening at the angles. Figure F, The grain has a circular outline that is darker in color than the other grains, suggesting greater exine thickness (prominent sexine). The ornamentation is echinate, featuring longer, rigid, and regularly spaced spines along the entire margin, associated with a verrucate-to-rugulate background surface visible in the central region of the grain.

Table 1 shows the pollen count performed on the monofloral honey samples. In the identification of the pollen from the açaí honey sample, 88.02 % was *E. oleracea* pollen. In orange blossom honey, 84.4 % was *C.* × *sinensis* pollen. In mangrove honey, 68.42 % of pollen was from *R. mangle* flowers.

Table 1. Quantification of pollen in *Euterpe oleracea*, *Citrus* × *sinensis*, and *Rhizophora mangle* L. honey samples.

Species	Total Pollen	Predominant Pollen	Classification
Euterpe oleracea	192	168 (88.02 %)	PD
Citrus* × *sinensis	188	158 (84.40 %)	PD
***Rhizophora mangle* L.**	228	156 (68.42 %)	PD

Predominant pollen (PD > 45 %), accessory pollen (PA 16–45 %), and isolated pollen (PI 3–15 %).

The three honey samples were classified as monofloral (or unifloral) according to the criteria established by Louveaux et al. [3], and they showed a predominance of a single pollen type, accounting for more than 45 % of the total identified pollen grains. This indicates that the samples were predominantly derived from the nectar of a particular plant species or botanical group, characterizing a predominant floral origin.

In addition to the predominant pollen type, pollen grains from other botanical species were detected at lower frequencies. However, these pollen types occurred below the threshold established for predominant pollen and, therefore, were not considered in the characterization of the floral origin of the samples.

3.2 Physicochemical analyses

Table 2 presents the mean, standard deviation, and respective coefficients of variation for the physicochemical, mineral, and proline content analyses of monofloral honey.

Table 2. Physicochemical parameters, minerals, and proline content determination in honey.

Parameters	EO	CV%	CS	CV%	RM	CV%
Free acidity (meQ/kg)	41.38 ± 1.61	3.89	32.62 ± 1.32	4.04	17.61 ± 0.77	4.37
Lactonic acidity (meQ/kg)	6.41 ± 0.49	7.64	6.07 ± 0.74	12.9	4.43 ± 0.5	11.2
Total acidity (meQ/kg)	47.71 ± 1.18	2.47	38.8 ± 0.86	2.21	22.5 ± 0.49	2.17
Moisture (%)	20 ± 0.01	0.05	19.8 ± 0.01	0.05	18.8 ± 0.01	0.05
pH	3.61 ± 0.01	0.27	3.87 ± 0.01	0.25	4.28 ± 0.01	0.23
HMF (mg/kg)	36.45 ± 0.96	2.63	5.91 ± 0.23	3.89	58.59 ± 1.14	1.94
DA (GOTHE g-1)	8.57 ± 0.02	0.23	60.0 ± 0.02	0.03	12.0 ± 0.02	0.16
Color	Amber	-	Light Amber	-	Extra Light Amber	-
Reducing sugars (%)	71.73 ± 0.05	0.06	76.50 ± 0.7	0.91	72.07 ± 0.49	0.67
Sucrose (%)	4.60 ± 0.6	13.0	1.70 ± 0.12	7.05	4.96 ± 0.15	3.02
Ash content (%)	0.12 ± 0.02	16.6	0.52 ± 0.08	15.3	0.17 ± 0.01	5.88
EC (mS/cm)	0.35 ± 0.01	2.85	0.87 ± 0.01	1.14	0.46 ± 0.01	2.17
Fe (mg/Kg)	7.63 ± 0.95	12.4	3.71 ± 0.11	2.96	3.23 ± 0.32	9.90
Zn (mg/Kg)	5.43 ± 0.54	9.94	5.01 ± 0.45	8.98	4.43 ± 0.58	13.0
Cu (mg/Kg)	1.01 ± 0.08	7.92	0.83 ± 0.02	2.40	0.85 ± 0.03	3.52
Mn (mg/Kg)	3.03 ± 0.16	5.28	3.40 ± 0.09	2.82	1.12 ± 0.06	5.35
P (mg/Kg)	105.89 ± 2.14	2.02	133.19 ± 1.6	0.87	196.35 ± 2.3	1.17
Ca (mg/Kg)	80.24 ± 1.11	1.38	180.94 ± 2.01	1.11	77.44 ± 0.99	1.27
Mg (mg/Kg)	19.17 ± 0.87	4.53	51.17 ± 0.98	1.91	25.07 ± 0.91	3.62
Proline (mg/ Kg)	294.4 ± 7.13	2.42	321.4 ± 4.99	1.55	204.1 ± 3.10	1.51

HMF – Hydroxymethylfurfural; DA – Diastase activity. EC – Electrical conductivity; CV – Coefficient of variation. The results are expressed as the mean ± standard deviation.

Free acidity results from the organic acids present in the floral nectar used by bees to produce honey [31]. The findings of this study are consistent with those reported by Feás et al. [32], who observed values ranging from 17.2 to 45.2 meq/kg in 45 honey samples from northwestern Portugal. Nascimento et al. [33] also obtained averages within the same ranges for monofloral honey produced in Rio Grande do Sul. All samples complied with the regulatory limit of 50 meq/kg established by Codex Alimentarius [34].

The lactonic acidity results of this study were higher than those reported by Brunelli, Imaizumi, Venturini Filho [35], who found a mean of 1.32 meq/kg. Lactonic acidity originates from lactones, which are volatile compounds responsible for the characteristic aromas of floral nectars [36].

The moisture levels obtained in this study are similar to those recorded by Lira et al. [37], who reported a range of 15.2–20 % in *A. mellifera* honey. This data convergence reinforces the relationship between moisture, acidity, and honey maturation. Moisture is a critical parameter in honey quality assessment, as it directly affects maturation, viscosity, crystallization behavior, and the sensory profile. Post-harvest changes in the moisture content may occur under inadequate storage conditions. An elevated moisture content (>20 %) creates a favorable environment for osmophilic microorganisms, triggering fermentation and compromising product stability [38]. All samples complied with the 20 % maximum limit established by the Codex Alimentarius [34], confirming adherence to international quality standards.

These pH values fall within the range reported by Rodríguez-Flores [39] (4.0–4.1) and Ghorab et al. [22] (3.5–4.4 in 30 honey samples). Honey typically exhibits a low pH (3.5–5.5), and it is influenced by bee species, nectar composition, mandibular secretions added during transport to the hive, and acid and mineral concentrations [40]. Although the pH is not legally regulated, values outside this range indicate fermentation or adulteration.

The Amazonian honey showed results higher than those reported by Da Silva et al. [4] (2.8–30.43 mg/kg in *A. mellifera* honey from multiple continents) but similar to those of Lira et al. [37] (7.49–56.80 mg/kg).

Normally, 5-HMF is considered an important indicator of honey quality, freshness, and excessive heat exposure, and it is generally present at low concentrations in fresh honey. Elevated HMF contents may result from the degradation of hexoses, particularly fructose and glucose, through acid-catalyzed dehydration and Maillard-related reactions. Although thermal processing contributes to HMF formation, its concentration is also influenced by intrinsic and extrinsic factors, including honey pH and acidity, sugar composition, water activity, mineral content, botanical origin, storage time, and storage temperature [41, 42]. Therefore, HMF contents should be interpreted as a result of the interaction between the chemical characteristics of the honey and its processing and storage history rather than as a consequence of temperature alone.

In the present study, EO and RM honey showed higher HMF contents than CS honey. Although the tropical climatic conditions of the Amazon region, particularly the relatively high ambient temperatures, favor HMF formation during storage, this factor alone does not explain the differences observed among the samples since all honey was produced in the same geographic region. Differences in the botanical origin and intrinsic chemical composition of the honey, including pH, acidity, sugar profile, water activity, and mineral composition, may also contribute to the observed variation in the HMF content. In addition, differences in post-harvest handling, processing conditions, storage time, and temperature may influence the final HMF content. Recent studies have emphasized that HMF formation in honey is a multifactorial process, in which temperature and storage duration interact with the physicochemical characteristics of the honey matrix [11,43]. Thus, the higher HMF content observed in EO and RM honey should be interpreted as resulting from the combined effects of honey composition, botanical origin, and post-harvest conditions, rather than being attributed exclusively to the tropical climate.

The HMF content obtained in this study was determined according to the limits established by the applicable honey quality standards. The Codex Alimentarius establishes a maximum HMF level of 40 mg/kg for honey, with specific provisions and exceptions depending on the honey type

and origin [34]. Therefore, the observed HMF content provides useful information regarding the quality and freshness of the evaluated samples, although they should be interpreted together with other quality parameters.

The CS honey exhibited the highest activity, which was associated with its low HMF content. These results are consistent with those reported by Da Silva et al. [4] for Spanish (11.50–45.80) and Moroccan honey (7.40–29.29) [44]. The diastase activity reflects the presence of α-amylase and is sensitive to heat treatment; low values may indicate overheating, improper storage, or adulteration [44, 45]. This activity also shows an inverse relationship with the HMF content [41]. All samples met the minimum limit of 8 (Gothe scale) established by the Codex Alimentarius [34].

The color of the Amazonian honey evaluated in this study ranged from light to dark amber, in agreement with color ranges previously reported for honey from Pará and other geographical regions [11, 45]. Honey color is a complex characteristic influenced by the botanical origin, phenolic composition, mineral content, enzymatic and non-enzymatic reactions, and processing and storage conditions. Darker honey frequently exhibits higher phenolic compound concentrations and greater antioxidant potential, but the relationship between color and individual chemical constituents is not necessarily linear. Prolonged heating and storage may promote Maillard-type reactions and melanoidin formation, which contribute to honey darkening and may occur concomitantly with increased HMF formation [43]. Therefore, the color differences observed among EO, CS, and RM honey should be interpreted as the result of multiple interacting factors rather than being attributed to climate alone.

The data obtained in this study fall within the range reported by Adalina and Heryati [46] for Indonesian mangrove honey (55.84–77.94 %). Low values indicate a premature harvest [47]. All samples exceeded the 60 % minimum requirement [34]. The sucrose levels in Amazonian honey were consistent with those reported by Da Costa and Toro [48] for Pará honey (0.16–8.26 %). Elevated levels may indicate adulteration or early harvest [42]. All values were below the 5 % maximum [34].

The CS sample had a significantly higher ash content compared to the others. These results align with those reported by Grosso, Tangarife, and Méndez [49] for Colombian (0.19–0.20 %) and Peruvian honey (0.14–0.53 %) [50]. The ash content reflects the mineral composition and processing quality [21]. All samples were below the 0.6 % maximum limit [24].

The CS sample's conductivity was consistent with its high ash content [4]. These values exceeded those reported by Gürbüz [45] for Turkish honey (0.15–0.31 mS/cm) but matched the findings for Iberian honey (0.5-0.8 mS/cm) [39]. Only the CS sample slightly surpassed the 0.8 mS/cm limit [34].

The mineral profile revealed calcium and phosphorus as the most abundant elements, and they are associated with carbohydrate transport and energy storage in plants [51]. Minerals originate from the soil and are transferred through nectar. Toxic metals indicate environmental pollution or improper handling [51]. All values fell within the ranges reported in the literature [52].

The proline content in the studied honey exceeded that reported by Da Costa and Toro [48] for Pará honey (6.0–130.0 mg/kg) but were lower than those found in Pakistani honey (287.6–511.1 mg/kg) [53]. This amino acid serves as an indicator of honey maturation and adulteration [54]. All samples surpassed the minimum quality threshold of 180 [24].

3.3 Bioactive compounds and antioxidant capacity

The results of bioactive compounds in the honey samples of *E. oleracea*, *C.* × *sinensis*, and *R. mangle* L. are presented in Table 3.

Table 3. Characterization of bioactive compounds and antioxidant capacity in honey.

Bioactive honey	*EO*	CV%	*CS*	CV%	*RM*	CV%
TPC (mg GAE/100 g)	168.08 ± 3.83	2.27	74.9 ± 1.06	1.41	50.5 ± 0.95	1.88
FT (mg EQ/100 g)	38.01 ± 0.68	1.78	9.35 ± 0.34	3.63	8.08 ± 0.32	3,96
Anthocyanins (mg/100 g)	2.07 ± 0.22	10,9	0.48 ± 0.28	58.3	0.26 ± 0.12	46,1
ABTS (µM TE/g)	3.10 ± 0.01	0.32	1.70 ± 0.01	0,58	1.10 ± 0.01	0.90
FRAP (µM FSE/g)	5.72 ± 0.08	1.39	3.12 ± 0.01	0.32	2.38 ± 0.04	1.68
DPPH (µM TE/g)	0.55 ± 0.01	1.81	0.35 ± 0.01	2.85	0.26 ± 0.01	3.84

TPC - Total Phenolic Compounds; GAE - Gallic Acid Equivalent; TF - Total Flavonoids; EQ – Quercetin Equivalent; TE – Trolox Equivalent; FSE - Ferrous Sulfate Equivalent. The results are expressed as mean ± standard deviation.

The analysis of bioactive compounds revealed a consistent pattern among the Amazonian monofloral honey evaluated in this study. Total phenolic compounds ranged from 50.5 mg GAE/100 g in RM to 168.08 mg GAE/100 g in EO, while total flavonoid contents ranged from 8.08 mg QE/100 g in RM to 38.01 mg QE/100 g in EO.

A similar pattern was observed for the total anthocyanins, with concentrations of 0.26, 0.48, and 2.07 mg/100 g in RM, CS, and EO honey, respectively. Thus, EO honey consistently exhibited the highest concentrations of the three groups of bioactive compounds evaluated, whereas RM presented the lowest values. This consistent chemical pattern suggests that the botanical origin of the honey may have played an important role in determining their bioactive composition. Previous studies with Brazilian honey have demonstrated that monofloral honey exhibits distinct phenolic profiles and antioxidant capacities according to the botanical origin, even when produced in the same country [33].

The predominance of bioactive compounds in EO honey is relevant considering the botanical origin of this honey—*E*. *oleracea*. The chemical composition of honey is influenced by the plant-derived substances transferred from nectar and, to a lesser extent, pollen during foraging and honey processing by bees. Consequently, differences in the floral resources available to the colonies contribute to differences in the secondary metabolite concentrations and profiles in the resulting honey. In this context, the higher phenolic and flavonoid contents observed in EO honey reflect the contribution of metabolites associated with the *E*. *oleracea* floral resource, but the present study did not determine the individual phenolic compounds or establish specific chemical markers of this botanical origin. Honey composition is also influenced by the geographical origin and environmental conditions, which may act together with botanical origin to determine the final chemical profile [33, 55].

The EO sample presented a total phenolic content of 168.08 mg GAE/100 g, considerably exceeding the range reported by Galhardo et al. [56] for *A*. *mellifera* honey from Paraná (11.39–61.27 mg GAE/100 g) and Bueno-Costa et al. [57] for honey from southern Brazil (61.16–111.37 mg GAE/100 g). Rather than indicating that EO honey is inherently richer in phenolics than all Brazilian honey, these differences should be interpreted in the context of the botanical and geographical origin since these factors affect the phenolic composition of honey. The presence and concentration of phenolic acids and flavonoids in honey are closely associated with the plant resources exploited by bees, but environmental and post-harvest conditions may also influence the final composition. Therefore, the higher phenolic content observed in EO honey may be related to the floral resource represented by *E*. *oleracea* and the environmental conditions of the Amazonian production system.

A similar trend was observed for total flavonoids. EO honey exhibited a concentration of 38.01 mg QE/100 g, which was approximately 4 times higher than those detected in RM and CS honey. This value was higher than that reported by Nayaka et al. [58] for Indonesian honey (0.074–2.053 mg QE/100 g) and from Piauí (5.43–13.02 mg QE/100 g) [6]. Flavonoids are an important group of phenolic secondary metabolites found in honey and may contribute to its antioxidant properties. However, their concentration varies based on floral and geographical origin. Brazilian studies have shown differences in the flavonoid composition among monofloral honey, reinforcing the role of botanical origin in determining the chemical characteristics of honey [33,55]. Thus, the higher flavonoid concentration in EO honey, together with its higher total phenolic content, confirms the existence of a distinct bioactive profile associated with this honey.

The differences among the three honey types were reflected in their antioxidant capacity. ABTS values were 1.1, 1.7, and 3.1 μM TE/g for RM, CS, and EO honey, respectively, and FRAP values were 2.38, 3.12, and 5.72 μM SF/g, respectively. DPPH values followed the same trend, increasing from 0.26 μM TE/g in RM honey and 0.35 μM TE/g in CS honey to 0.55 μM TE/g in EO honey. Therefore, the three antioxidant assays showed the same overall pattern: EO honey had the greatest antioxidant capacity, followed by CS and RM honey. This agreement among analytical approaches strengthens the interpretation that the distinct chemical composition of EO honey is reflected in its antioxidant potential. Brazilian studies involving monofloral and polyfloral honey have similarly demonstrated that the botanical and geographical origin influence the phenolic composition and antioxidant activity [33, 55].

The ABTS results for the present samples exceeded those reported by Srećković et al. [59] for honey from Central Serbia (0.026–0.59 μM TE/g) and Galhardo et al. [56] for 67 multifloral *A. mellifera* honey (0.43–1.54 μM TE/g). Similarly, the FRAP value observed for EO was higher than the range reported by Galhardo et al. [56] (1.59–3.54 μM SF/g) and within the range previously reported for honey from Pará (0.98–5.99 μM SF/g) (43). DPPH values were also higher than those reported by Galhardo et al. [56] for honey from Paraná (0.04–0.16 μM TE/g) and Srećković et al. [59] for honey from Central Serbia (0.008–0.26 μM TE/g). Although these comparisons demonstrate the high antioxidant capacity of the evaluated samples, differences in extraction procedures, analytical conditions, botanical origin, geographical origin, and expression units should be considered when comparing antioxidant results among studies.

The relationship between bioactive composition and antioxidant capacity was evident in EO honey, which exhibited the highest concentrations of total phenolics, flavonoids, and anthocyanins and highest values in ABTS, FRAP, and DPPH assays. This pattern is consistent with that in previous studies showing associations between phenolic/flavonoid composition and antioxidant activity in Brazilian honey [60, 33]. In particular, Sant'Ana et al. [60] reported relationships among honey color, total phenolics, flavonoids, and antioxidant capacity, suggesting that these characteristics are interconnected. However, the antioxidant activity of honey should not be attributed exclusively to phenolic compounds since other constituents, including Maillard reaction products, peptides, organic acids, minerals, enzymes, and other reducing substances, may also contribute to the overall antioxidant response [60, 33].

The higher anthocyanin content observed in EO honey (2.07 mg/100 g) compared to RM (0.26 mg/100 g) and CS honey (0.48 mg/100 g) also contributes to the distinctive bioactive profile of this sample. The EO value was comparable to the concentration reported by Habryka, Socha, and Juszczak [61] in Polish honey (2.01 mg/100 g). Anthocyanins are flavonoid pigments with antioxidant properties that may contribute to the coloration of food matrices. The relationship

between the anthocyanin concentration and honey color should be interpreted cautiously because honey coloration is a complex characteristic influenced by multiple compounds and processes. The higher anthocyanin and flavonoid contents in EO honey may have contributed to its darker appearance, but the present results do not enable the darker color to be exclusively attributed to anthocyanins [62].

The color–composition relationship is particularly relevant when interpreting the results for the EO sample. Its darker pigmentation occurred together with higher phenolic compound and flavonoid concentrations and greater antioxidant capacity. This pattern is consistent with studies of Brazilian honey reporting associations among darker color, phenolic/flavonoid content, and antioxidant activity [60]. More recent research with Brazilian monofloral and polyfloral honey have also shown correlations between flavonoid content and honey color and demonstrated that botanical and geographical origins strongly influence the chemical composition and antioxidant activity [55]. Thus, the darker color of EO honey may be considered an external characteristic associated with its distinctive chemical composition, but color alone cannot be used as a direct indicator of the concentration of a specific bioactive compound.

The visual similarity between EO and açai-derived products should be interpreted with caution. Although *E. oleracea* is widely recognized for its anthocyanin-rich fruit, the fruit composition does not necessarily reflect the composition of its nectar or of honey produced from floral resources. Therefore, the darker coloration observed in EO honey should not be interpreted as evidence that the honey has a chemical composition equivalent to that of açai fruit. Instead, the similarity should be understood as a visual characteristic, and the chemical differences observed among EO, CS, and RM honey are more appropriately discussed in relation to their botanical origin and the compounds transferred or modified during honey production.

The distinctive profile of EO honey may also be interpreted in the context of the botanical characteristics of *E. oleracea*. The chemical composition of a monofloral honey does not represent a direct chemical replica of the plant from which bees collect nectar. Instead, honey results from a complex process involving the nectar composition, bee metabolism, enzymatic transformations, dehydration, maturation, and incorporation of compounds derived from the floral environment. Consequently, the higher bioactive compound concentrations observed in EO honey may reflect the combined effect of the floral resource associated with *E. oleracea* and the biochemical transformations occurring during honey formation. Further studies using chromatographic profiling of individual phenolic compounds are necessary to identify specific chemical markers associated with *E. oleracea* honey [33, 63].

In contrast, CS showed intermediate phenolic compound, flavonoid, and anthocyanin concentrations and an intermediate antioxidant capacity. This profile is consistent with the known characteristics of citrus honey, which generally contain a diverse range of phenolics and other secondary metabolites and exhibit antioxidant properties, but their composition varies according to citrus species, geographical origin, and production conditions [63]. Therefore, the intermediate position of CS honey between EO and RM honey suggests that its bioactive composition reflects the characteristics of the *C. × sinensis* floral resource and its production environment.

RM honey presented the lowest concentrations of total phenolics, flavonoids, and anthocyanins and consequently the lowest values for all three antioxidant assays. This indicates that the three groups of bioactive compounds evaluated did not vary independently but rather showed a coordinated pattern that was also reflected in the antioxidant capacity. Nevertheless, the lower antioxidant activity of RM honey should not be interpreted as evidence of inferior honey quality since the antioxidant capacity represents only one aspect of honey composition and

functionality. Differences in botanical origin, mineral composition, environmental conditions, and other compounds not quantified in the present study could contribute to its chemical and biological characteristics.

Overall, the results indicate a coherent association among the botanical origin, bioactive composition, and antioxidant capacity in monofloral honey. EO honey, associated with *E. oleracea*, exhibited the highest concentrations of total phenolics, flavonoids, and anthocyanins and consistently showed the greatest antioxidant capacity in ABTS, FRAP, and DPPH assays. CS honey displayed intermediate values, and RM honey showed the lowest values. Rather than attributing this pattern exclusively to a single class of compounds, the results suggest that differences in the floral resource and resulting chemical composition of each honey may have contributed to the observed antioxidant response. This interpretation is consistent with studies of Brazilian honey demonstrating that botanical origin is a major determinant of phenolic composition and antioxidant properties [33, 55].

The present findings reinforce the importance of evaluating monofloral honey based on its specific botanical origin rather than assuming that all honey produced within the same geographical region shares similar bioactive profiles. Although the three samples originated from the Amazonian region, their distinct floral sources were associated with different bioactive compound concentrations and antioxidant responses. Thus, the results support the hypothesis that botanical origin, together with environmental and geographical factors, contributes to the chemical differentiation of Amazonian honey.

4. Conclusion

The honey analyzed in this study was classified as monofloral honey and showed results within the physicochemical parameters allowed by Brazilian and international regulations. Regarding their bioactive composition, all honey showed the presence of compounds associated with the antioxidant potential, with Açai palm honey with pollen predominantly from Euterpe oleracea exhibiting the most expressive overall profile in terms of bioactive compounds and antioxidant capacity compared to the other honey samples. These findings highlight the potential influence of botanical origin on the chemical and functional characteristics of honey. Overall, the results confirm the importance of promoting research and beekeeping activities in the Amazon region, where the rich plant biodiversity provides a valuable source of distinctive honey with promising bioactive properties and potential for greater scientific and commercial valorization.

Conflict of interest statement

The authors declare no conflict of interest.

Data availability statement

The original contributions presented in the study are included in the article. Further inquiries can be directed to the corresponding author.

Author contributions

Conceptualization: Cunha J.S., Silva W.O., Toro M.J.U. Data curation: Cunha J.S., Silva W.O., Silva M.E., Toro M.J.U. Formal analysis: Cunha J.S., Silva W.O., Silva M.E. Funding acquisition: Toro M.J.U. Investigation: Cunha J.S., Silva W.O., Silva M.E. Methodology: Cunha J.S., Silva W.O., Silva M.E., Toro M.J.U. Project administration: Toro M.J.U. Resources: Toro M.J.U. Supervision: Toro M.J.U. Validation: Cunha J.S., Silva W.O., Silva M.E. Writing — original draft: Cunha J.S, Silva W.O., Silva M.E. Writing — review and editing: Cunha J.S., Toro M.J.U.

Generative AI use statement

The authors declare that no generative artificial intelligence (AI) tools or AI-assisted technologies were used in the writing, preparation, revision, interpretation, or organization of the scientific content of this manuscript. The entire manuscript was written exclusively by the authors, who assume full responsibility for the originality, accuracy, and integrity of the information presented, in accordance with the editorial policies of Ciência Animal Brasileira/ Brazilian Animal Science (CAB).

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