

Discriminating honeys from Brazilian Pantanal using neutron activation analysis

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Abstract

The potential of elemental profiling for discriminating the geographical origin of honey produced by Africanized honey bees (*Apis mellifera*) in the Brazilian Pantanal was investigated using neutron activation analysis (NAA). The elements Br, Co, Cs, K, Na, Rb, and Zn were evaluated as potential indicators of origin, and principal component analysis (PCA) was applied to assess patterns of differentiation among honey samples from five Pantanal subregions. The study aimed to contribute to the protection, valorization, and consolidation of the geographical indication (GI) for Pantanal honey.

Introduction

Brazil encompasses a vast geographical area and is characterized by a remarkable diversity of honey plant species [1], offering considerable potential to produce high-quality bee products, including honey, propolis, beeswax, and pollen. These products meet the rigorous quality standards of the most demanding international markets, placing Brazil among the world's leading producers [2]. Brazilian beekeeping has experienced significant growth over the past two decades. Honey production, predominantly from Africanized honey bees, increased from approximately 34,000 tons in 2012 to 67,000 tons in 2024 [3]. During the same period, the country improved its position among the world's leading honey producers, rising from 12th place in 2012 to 9th place in 2024 [4].

The Pantanal is a biome located in central South America, covering parts of Brazil, Bolivia, and Paraguay. This region is not homogeneous; its 138,183 km² are divided into eleven distinct sub-regions, each characterized by specific soil types, vegetation, and climatic conditions [5]. The Pantanal's vegetation is highly resilient and adaptable, having evolved to withstand seasonal and decadal cycles of flooding and drought [6]. Both grassland and aquatic plant species are strongly influenced by the alternating flood and drought cycles associated with the region's flood pulse [7]. Fire is another important factor shaping the landscape and plant communities of the biome [8], with fire-prone weather conditions persisting for increasingly longer periods each year and showing a steady increase over the past four decades [9]. Despite these environmental pressures, the Pantanal remains the best-conserved biome in Brazil [6].

The year-round flowering patterns of melliferous plants, as well as the types of floral resources they provide, were monitored over three consecutive years along the western edge of the Brazilian Pantanal. A total of 160 flowering species visited by Africanized honey bees and/or native Brazilian bees were identified. Of these, 73 species were classified as melliferous plants, comprising 34 herbs, 17 trees, 15 shrubs, and 7 lianas, distributed among 59 genera and 28 botanical families. Fabaceae, Malvaceae, and Euphorbiaceae were the most represented families, with 20, 9, and 4 species, respectively. Melliferous plants were found flowering throughout the year, with the highest species richness observed in summer and the lowest in winter. Most of these species (89%) are native to the region, whereas only 11% are cultivated or naturalized [10]. Consequently, Brazilian Pantanal honey is mostly derived from the nectar of wild plant species [11]. This scenario is becoming increasingly uncommon worldwide, as global honey

production relies primarily on agricultural and forestry monocultures. In contrast, the Brazilian Pantanal is characterized by extensive beef cattle ranching as its main economic activity [2, 12].

Honey produced in the Pantanal biome exhibits a wide range of organoleptic properties, largely reflecting its predominantly wild floral origin. Notable monofloral honeys include *Aroeira* (*Myracrodruon urundeuva*), characterized by an extremely dark color (> 114 on the Pfund scale, Ps [13]), a caramelized flavor, and slight bitterness, and *Cambará* (*Vochysia divergens*), which exhibits a very light amber color (9–17 Ps) and a mild flavor. In addition to these monofloral honeys, the region produces a variety of multifloral honeys with a broad spectrum of colors and shades between these extremes. For example, honeys containing varying proportions of nectar from *Hyptis crenata*, locally known as *Hortelã-do-Campo*, are characterized by a light amber color (51–85 Ps) and a mild flavor. These unique attributes, together with collaborative efforts involving the Brazilian Agricultural Research Corporation (Embrapa Pantanal), research institutions, and beekeepers from the states of Mato Grosso do Sul and Mato Grosso, resulted in the geographical indication (GI) for Pantanal honey. This was the first GI granted to a honey-producing region in Brazil [14].

The combination of intrinsic characteristics and machine learning algorithms has proven to be an effective approach for determining the geographical origin of food and agricultural products [15], including those bearing GIs [16]. In this context, several studies have investigated elemental profiles as a means of discriminating the geographical origin of honey [17–19] and assessing the presence of potentially toxic metals [20–22]. Various analytical techniques have been employed for this purpose, including inductively coupled plasma mass spectrometry (ICP-MS) [23, 24, 27], inductively coupled plasma optical emission spectrometry (ICP-OES) [17, 19], multi-collector inductively coupled plasma mass spectrometry (MC-ICP-MS) [25], and neutron activation analysis (NAA) [26, 27]. NAA stands out for its high analytical precision and metrological rigor [28]. This technique has previously been applied to characterize elemental composition and discriminate the geographical origin of different types of Brazilian honey produced in the Cerrado and Atlantic Forest biomes [29, 30].

Therefore, the objective of this study was to evaluate whether the elemental profile of honey produced by Africanized honey bees in the Brazilian Pantanal is associated with its geographical origin and can serve as a fingerprint for honey traceability. By identifying elemental signatures associated with geographical origin through NAA, this approach may provide complementary evidence to support the authenticity of Pantanal honey and contribute to the consolidation of its GI.

Material and methods

A total of 29 honey samples produced by Africanized honey bees (*Apis mellifera*), with complete traceability of extraction dates and georeferenced sourcing locations, were collected from five subregions of the Brazilian Pantanal: Aquidauana (n = 1), Miranda (n = 1), Nhecolândia (n = 8), Paraguai (n = 12), and Poconé (n = 7) (Fig. 1).

After collection, the honey samples were transferred to hermetically sealed high-density polyethylene bottles and stored under controlled temperature and humidity conditions. Immediately before the analytical portions were transferred to vials, the honey samples were subjected to ultrasonic agitation to ensure homogenization. For NAA, analytical portions of approximately 200 mg were directly weighed into high-density polyethylene vials (Posthumus Products, Beverwijk, the Netherlands) suitable for neutron irradiation.

For analytical quality control, two certified reference materials (CRMs) were analyzed: SRM 1549 Non-Fat Milk Powder, produced by the National Institute of Standards and Technology (NIST), and CRM-Agro C1004a Tomato Pulp, produced by CRM-Agro Reference Materials for Agriculture, Livestock and Toxicology (LRI/CENA/USP). In addition, an in-house quality control material consisting of honey produced by Africanized honey bees and collected in the state of Mato Grosso do Sul was included. Ni-Cr alloy wires of known composition [31] were placed between the vials to monitor the thermal neutron flux through the reaction $^{50}\text{Cr}(n,\gamma)^{51}\text{Cr}$.

Irradiation was performed for 4 hours at the nuclear research reactor IEA-R1, Nuclear and Energy Research Institute, Brazilian National Nuclear Energy Commission (IPEN/CNEN), using an average thermal neutron flux of $3.5 \times 10^{12} \text{ cm}^{-2} \text{ s}^{-1}$. The induced radioactivity was measured at CENA/USP after approximate decay periods of 4, 7, 14 and 28 days using HPGe detectors with 50% relative efficiency at 1332 keV emission line of ^{60}Co . Counting times ranged from 15 minutes for the first measurement to 2 hours in the fourth measurement. Elemental mass fractions were determined through the k_0 -method implemented in the in-house Quantu software package [32].

Statistical analysis was performed by grouping the honey samples according to their geographical origin across the five studied subregions of the Brazilian Pantanal. The non-parametric Kruskal-Wallis test was applied to assess statistically significant differences in the mass fractions of the elements among the subregions, using a significance level of $p < 0.05$. For elements showing statistically significant differences, Dunn's *post hoc* test with Bonferroni adjustment for multiple comparisons was thereafter used to identify significant differences between pairs of subregions [33].

Subsequently, principal component analysis (PCA) was performed as an exploratory, unsupervised approach for dimensionality reduction. The results were visualized using a biplot combining sample scores and variable vectors (loadings) for the first two principal components (PC1 and PC2). To identify the elements contributing to the differentiation of honey samples according to their subregional origin, the magnitude and direction of the variable vectors were examined in conjunction with the distribution and clustering patterns of the samples along PC1 and PC2 [34].

Results and discussion

NAA enabled the determination of Br, Co, Cs, K, Na, Rb, and Zn in all honey samples analyzed in this study. The mean mass fractions for each subregion are presented in Table 1. Considering the individual

sample results, substantial variation was observed, with values ranging from 0.15 to 4.22 mg kg⁻¹ for Br, 0.0007 to 0.063 mg kg⁻¹ for Co, 0.0012 to 0.156 mg kg⁻¹ for Cs, 492 to 2677 mg kg⁻¹ for K, 3.5 to 13.9 mg kg⁻¹ for Na, 1.1 to 13.7 mg kg⁻¹ for Rb, and 0.088 to 3.97 mg kg⁻¹ for Zn.

Table 1

Mass fractions of chemical elements (mg kg⁻¹, fresh weight basis) and standard deviations in honey samples from five subregions of Brazilian Pantanal

Br	Aquidauana (n = 1)	Miranda (n = 1)	Nhecolândia (n = 8)	Paraguai (n = 12)	Poconé (n = 7)
	1.65	0.6	1.84 ± 1.72	1.11 ± 1.56	2.13 ± 1.26
Co	0.0066	0.036	0.0173 ± 0.0165	0.0293 ± 0.0199	0.0123 ± 0.0221
Cs	0.00585	0.00461	0.037 ± 0.062	0.00535 ± 0.00231	0.0432 ± 0.0624
K	1220	852	1130 ± 788	758 ± 176	1490 ± 565
Na	7.65	4.76	7.28 ± 3.16	5.85 ± 1.05	5.02 ± 1.15
Rb	1.8	1.89	4.58 ± 5.02	1.36 ± 0.18	5.84 ± 4.78
Zn	2.08	0.102	1.08 ± 1.25	1.02 ± 0.65	0.426 ± 0.284

The Kruskal-Wallis test revealed a statistically significant difference in Rb mass fractions among the subregions ($p < 0.05$), while Dunn's *post hoc* test with Bonferroni correction identified a significant difference specifically between the Paraguai and Poconé subregions. Notably, Rb has also been reported as an important discriminating element for distinguishing the geographical origins of honey produced by two bee species, *Tetragonisca angustula* and *Apis mellifera*, in the Atlantic Forest and Cerrado biomes [30]. The recurring contribution of Rb to geographical discrimination across different Brazilian biomes suggests that this element may have potential as a marker of honey origin.

No statistically significant differences were detected among the subregions for the other elements. The absence of significant differences for most elements, together with the variability observed within individual subregions, indicates substantial overlap in the elemental profiles. This variability may be associated with differences in floral composition, environmental conditions, or other factors influencing the elemental composition of honey. Although Rb was the only element showing statistically significant differences among the subregions, the discriminatory potential of the elemental profile should be interpreted in conjunction with the PCA, which considers the elements collectively and may reveal patterns of differentiation that are not evident from univariate analyses alone.

The first two principal components of PCA explained 69.86% of the total variance, with PC1 accounting for 54.94% and PC2 for 14.92% (Fig. 2). The arrangement of the variable vectors (loadings) in the biplot indicated a positive association among Cs, K, and Rb. Their loadings align with the positive direction of PC1, indicating their contribution to sample separation along this component. In contrast, Co was

oriented in the opposite direction, in the upper-left quadrant, indicating a negative association with Cs, K, and Rb. Variation along PC2 was primarily associated with Na and Zn, whose vectors pointed toward the upper-right quadrant, whereas Br was positioned in the lower-right quadrant, corresponding to positive PC1 and negative PC2 scores.

The distribution of the samples in the multidimensional space revealed distinct patterns associated with the Pantanal subregions (Fig. 2). Samples from the Paraguai subregion were predominantly distributed on the left side of the plot, showing a weaker association with K, Cs, Rb, and Br and a comparatively stronger association with Co. In contrast, samples from Poconé were mainly projected toward the lower portion of PC2, showing a stronger association with Br. Samples from Nhecolândia exhibited greater dispersion along the PC1 axis, indicating substantial variability in their elemental profiles. The Aquidauana sample was positioned in the upper-right quadrant, in the direction of the Na and Zn vectors, suggesting a stronger association with these elements. Conversely, the Miranda sample was in the lower-left quadrant, opposite to most of the variable vectors oriented toward the positive side of PC1, indicating a comparatively weaker association with the elements contributing to the positive PC1 direction.

The Pantanal biome encompasses a heterogeneous landscape composed of different vegetation types, each potentially contributing distinct elemental signatures to the floral resources accessible to bees. In addition, variations in soil characteristics, hydrological conditions, and the availability and distribution of mineral elements may influence their uptake by plants and subsequent transfer to nectar and honeydew. The foraging behavior of Africanized honey bees, including the range of plant species visited within and among different subregions, may further contribute to variations in the elemental profile of honey. Local beekeeping practices may also influence these characteristics, although their contribution is likely to be secondary compared with environmental and botanical factors. Thus, the elemental composition of Pantanal honey may result from the combined influence of botanical, environmental, and management factors, potentially providing information that can be explored for geographical discrimination and traceability of honey produced in the biome.

Conclusion

The elemental profile of honey produced by Africanized honey bees revealed patterns associated with geographical origin within the Brazilian Pantanal. Principal component analysis highlighted distinct clustering trends among the subregions and identified Br and Co as potential elements contributing to the differentiation of honeys from the Paraguai and Poconé subregions. This finding was further supported by the statistically significant difference in Rb mass fractions observed between these two subregions. Overall, the results demonstrate the potential of elemental profiling as a complementary approach for characterizing and discriminating the geographical origin of honey produced in the Brazilian Pantanal.

Elemental profiling by NAA may provide complementary analytical evidence to support the geographical indication (GI) of Pantanal honey by strengthening the characterization of its authenticity. This approach may enhance the territorial identity and market differentiation of Pantanal honey, while adding value to its distinctive environmental and botanical characteristics. The application of such analytical approaches may contribute to the valorization of Pantanal honey and to the consolidation of beekeeping as an economically relevant activity closely linked to the unique environmental, botanical, and productive features of the Brazilian Pantanal.

Declarations

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Author Contribution

VDAdR: Conceptualization, Writing: original draft, writing-review and editing the manuscript text, Investigation, Formal analysis, Data Curation; EADNF: Conceptualization, Supervision, Investigation, Project administration, Funding acquisition; MAB: Software, Formal analysis, Investigation, Data curation; RCdL: Formal analysis, Software, Prepared figures 1-2; GRM: Methodology; All authors reviewed the manuscript.

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Data Availability

Available from the corresponding author on request.

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Figures

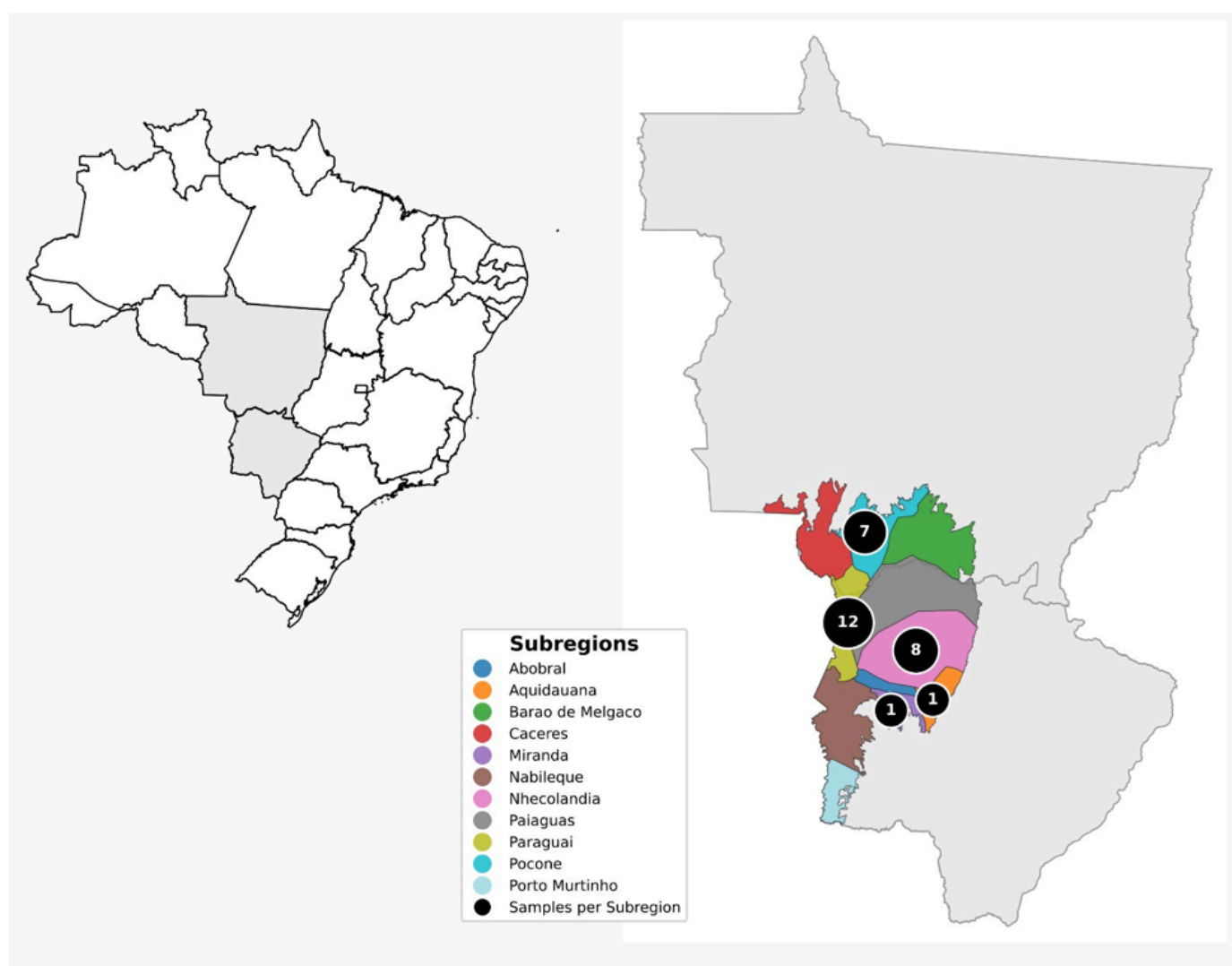


Figure 1

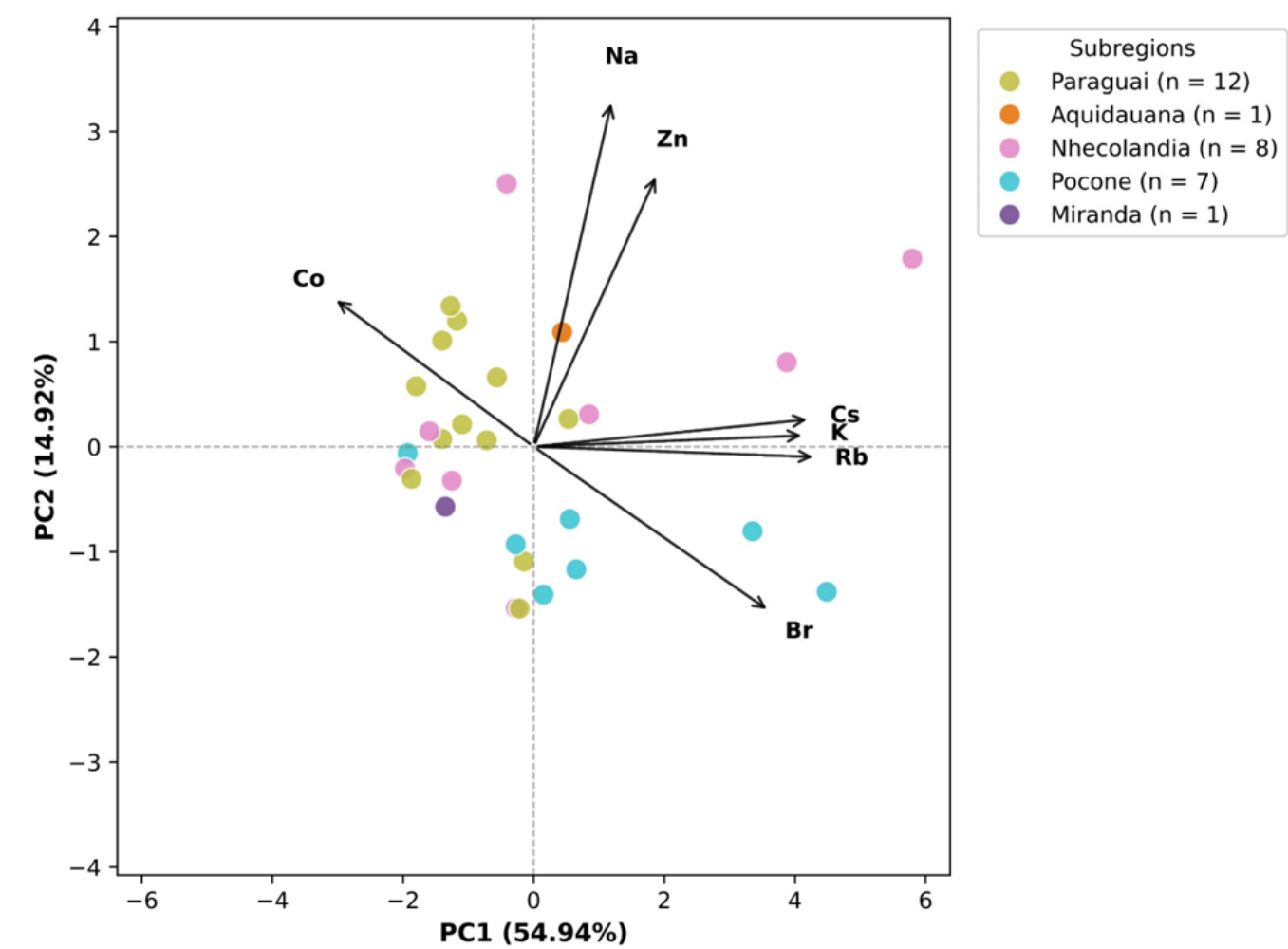


Figure 2

Principal component analysis biplot of honey samples from five subregions of the Brazilian Pantanal based on the mass fractions of Br, Co, Cs, K, Na, Rb, and Zn