



AVALIAÇÃO INTEGRADA DO PERFIL FÍSICO-QUÍMICO, CROMÁTICO E DAS PROPRIEDADES ANTIOXIDANTES DO MEL (APIS MELLIFERA) DA CAATINGA DO PERNAMBUCO

EVALUACIÓN INTEGRADA DEL PERFIL FISICOQUÍMICO, CROMÁTICO Y DE LAS PROPIEDADES ANTIOXIDANTES DE LA MIEL (APIS MELLIFERA) DE LA CAATINGA DE PERNAMBUCO

INTEGRATED EVALUATION OF THE PHYSICOCHEMICAL, CHROMATIC PROFILE AND ANTIOXIDANT PROPERTIES OF HONEY (APIS MELLIFERA) FROM CAATINGA OF PERNAMBUCO

Apresentação: Comunicação Oral

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RESUMO

O mel é um produto natural cuja composição físico-química e propriedades bioativas são diretamente influenciadas pela origem botânica, condições ambientais e características geográficas. Nesse contexto, o presente estudo teve como objetivo caracterizar o mel produzido na Caatinga Pernambuco por meio de análises físico-químicas, cromáticas e antioxidantes, contribuindo para a valorização de produtos regionais e o entendimento de seus atributos de qualidade. Três amostras de mel (M1, M2 e M3) foram avaliadas quanto a sólidos solúveis totais, pH, umidade, extrato seco, cinzas, acidez total, proteínas, lipídios, ácido ascórbico, açúcares redutores, atividade de água, parâmetros cromáticos (L^* , a^* , b^* , ângulo de matiz e croma), compostos fenólicos e atividade antioxidante. As análises físico-químicas revelaram sólidos solúveis variando de 75,3 a 75,6 °Brix, valores de pH entre 3,5 e 3,9, teor de umidade de 11,9 a 14,5% e açúcares redutores entre 42,20 e 87,90 g/100 mL. A atividade de água permaneceu constante em 0,60 em todas as amostras, enquanto nenhum teor de lipídios ou adulteração com amido foi detectado. A avaliação colorimétrica demonstrou diferenças significativas entre as amostras, com luminosidade (L^*) variando de 53,99 a 75,51 e intensidade de cor (C^*) de 27,27 a 35,62. A análise de compostos bioativos mostrou teores totais de flavonoides entre 14,52 e 15,89 mg/100 mL, taninos de 4,42 a 12,42 mg/100 mL, flavonóis de 2,14 a 3,60 mg/100 mL e polifenóis totais de 80,64 a 144,06 mg/100 mL. A atividade antioxidante apresentou considerável variação, com valores de sequestro do radical DPPH variando de 48,01 a 73,89% e inibição do ABTS acima de 90% para todas as amostras. Os resultados demonstram diferenças significativas entre méis produzidos na mesma região geográfica, destacando a influência da diversidade floral e das condições ambientais locais em suas características físico-químicas e funcionais. Esses achados reforçam a importância do terroir da Caatinga de Pernambuco como fonte de méis diferenciados com relevante potencial antioxidante e valor comercial.

Palavras-Chave: Deve conter no mínimo 3 e no máximo 5 palavras-chaves, separadas por vírgula. São

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palavras características do tema que servem para indexar o artigo.

RESUMEN

La miel es un producto natural cuya composición fisicoquímica y propiedades bioactivas están directamente influenciadas por el origen botánico, las condiciones ambientales y las características geográficas. En este contexto, el presente estudio tuvo como objetivo caracterizar la miel producida en la Caatinga de Pernambuco mediante análisis fisicoquímicos, cromáticos y antioxidantes, contribuyendo a la valorización de los productos regionales y a la comprensión de sus atributos de calidad. Se evaluaron tres muestras de miel (M1, M2 y M3) en cuanto a sólidos solubles totales, pH, humedad, extracto seco, cenizas, acidez total, proteínas, lípidos, ácido ascórbico, azúcares reductores, actividad de agua, parámetros cromáticos (L^* , a^* , b^* , ángulo de tono y croma), compuestos fenólicos y actividad antioxidante. Los análisis fisicoquímicos revelaron sólidos solubles entre 75,3 y 75,6 °Brix, valores de pH entre 3,5 y 3,9, contenido de humedad entre 11,9 y 14,5 %, y azúcares reductores entre 42,20 y 87,90 g/100 mL. La actividad del agua se mantuvo constante en 0,60 en todas las muestras, y no se detectó contenido lipídico ni adulteración de almidón. La evaluación colorimétrica demostró diferencias significativas entre las muestras, con una luminosidad (L^*) que osciló entre 53,99 y 75,51 y una intensidad de color (C^*) entre 27,27 y 35,62. El análisis de compuestos bioactivos mostró contenidos totales de flavonoides entre 14,52 y 15,89 mg/100 mL, taninos de 4,42 a 12,42 mg/100 mL, flavonoles de 2,14 a 3,60 mg/100 mL y polifenoles totales de 80,64 a 144,06 mg/100 mL. La actividad antioxidante presentó una variación considerable, con valores de eliminación de radicales DPPH que oscilaron entre el 48,01 y el 73,89 % e inhibición de ABTS superior al 90 % para todas las muestras. Los resultados demuestran diferencias significativas entre las mieles producidas dentro de la misma región geográfica, lo que resalta la influencia de la diversidad floral y las condiciones ambientales locales en sus características fisicoquímicas y funcionales. Estos hallazgos refuerzan la importancia del terruño de la Caatinga de Pernambuco como fuente de una miel distintiva con un potencial antioxidante relevante y valor comercial.

Palabras Clave: Debe contener un mínimo de 3 y un máximo de 5 palabras clave, separadas por comas. Estas son palabras características del tema que sirven para indexar el artículo.

ABSTRACT

Honey is a natural product whose physicochemical composition and bioactive properties are directly influenced by botanical origin, environmental conditions, and geographical characteristics. In this context, the present study aimed to characterize honey produced in the Pernambuco Caatinga through physicochemical, chromatic, and antioxidant analyses, contributing to the valorization of regional products and the understanding of their quality attributes. Three honey samples (M1, M2, and M3) were evaluated regarding total soluble solids, pH, moisture, dry extract, ash, total acidity, proteins, lipids, ascorbic acid, reducing sugars, water activity, chromatic parameters (L^* , a^* , b^* , Hue angle, and Chroma), phenolic compounds, and antioxidant activity. The physicochemical analyses revealed soluble solids ranging from 75.3 to 75.6 °Brix, pH values between 3.5 and 3.9, moisture contents from 11.9 to 14.5%, and reducing sugars between 42.20 and 87.90 g/100 mL. Water activity remained constant at 0.60 in all samples, while no lipid content or starch adulteration was detected. Colorimetric evaluation demonstrated significant differences among samples, with luminosity (L^*) ranging from 53.99 to 75.51 and color intensity (C^*) from 27.27 to 35.62. Bioactive compound analysis showed total flavonoid contents between 14.52 and 15.89 mg/100 mL, tannins from 4.42 to 12.42 mg/100 mL, flavonols from 2.14 to 3.60 mg/100 mL, and total polyphenols from 80.64 to 144.06 mg/100 mL. Antioxidant activity exhibited considerable variation, with DPPH radical scavenging values ranging from 48.01 to 73.89% and ABTS inhibition above 90% for all samples. The results demonstrate significant differences among honeys produced within the same geographical region, highlighting the influence of floral diversity and local environmental conditions on their physicochemical and functional characteristics. These findings reinforce the importance of Pernambuco's Caatinga terroir as a source of distinctive honey with relevant antioxidant potential and commercial value.

Keywords: Honey, Caatinga, Antioxidant activity, Phenolic compounds, Physicochemical characterization, Terroir.

INTRODUCTION

Honey is one of the most economically important products of beekeeping, valued not only as a natural sweetener but also as a source of bioactive compounds with recognized nutritional and functional properties. Its chemical composition is highly complex and depends on several factors, including botanical origin, geographical location, climatic conditions, and beekeeping practices. Consequently, honey exhibits considerable variability in its physicochemical characteristics, sensory attributes, and biological activities, making compositional characterization essential for quality control, authenticity assessment, and commercial valorization (Vrabcová et al., 2020; Gebretsadik et al., 2026).

Among the constituents responsible for honey quality, phenolic compounds have received increasing scientific attention because of their antioxidant, antimicrobial, and anti-inflammatory properties. These compounds contribute to the neutralization of reactive oxygen species and are considered important indicators of the functional potential of honey. In addition, studies have demonstrated positive relationships between phenolic content, antioxidant capacity, and color parameters, reinforcing the importance of integrating physicochemical and bioactive analyses to achieve a more comprehensive evaluation of honey quality (Sarmiento et al., 2025; Wilczyńska et al., 2024).

Brazil is one of the world's leading honey producers, and the northeastern region is recognized for its rich botanical diversity, which favors the production of honeys with distinctive chemical and functional characteristics. Within this context, the Agreste region of Pernambuco represents a transition zone between the Atlantic Forest and the Caatinga biomes, providing a diverse floral composition that may directly influence honey quality. However, despite the growing economic relevance of regional beekeeping, studies integrating physicochemical characterization, authenticity assessment, phenolic composition, and antioxidant activity of honey produced in this region remain scarce. Most available investigations focus on isolated quality parameters or on honeys from other Brazilian regions, limiting the scientific understanding of the compositional variability and functional potential of Agreste honey. This knowledge gap hinders the valorization of regional products and the generation of scientific evidence to support quality certification and market competitiveness.

Based on the environmental characteristics of the Agreste region and its botanical diversity, we hypothesized that honey produced in this region presents distinctive physicochemical characteristics, complies with quality and authenticity standards, and exhibits elevated phenolic content and antioxidant activity, reflecting its potential as a functional food with high added value. Therefore, this study aimed to comprehensively characterize honey produced in the Agreste region

of Pernambuco by evaluating its physicochemical properties, color parameters, authenticity indicators, total phenolic content, and antioxidant activity. The integration of these analyses provides a broader understanding of the quality and functional potential of regional honey, contributing to its scientific characterization, commercial valorization, and future application in the food industry.

THEORETICAL FOUNDATION

The beekeeping sector represents one of the most strategic activities within the global bioeconomy, contributing simultaneously to environmental preservation, agricultural productivity, food security, and rural development. Through pollination services, bees play an indispensable role in maintaining ecosystem stability and biodiversity, being responsible for the reproduction of approximately 75% of cultivated crops and a substantial proportion of wild plant species worldwide. Beyond their ecological significance, bee-derived products have gained increasing prominence due to their nutritional, pharmaceutical, and biotechnological applications. Honey, beeswax, propolis, royal jelly and pollen, constitute important raw materials for the development of functional foods, nutraceuticals, cosmetics, and therapeutic products. Among these products, honey remains the most economically relevant, representing a growing market segment driven by consumer demand for natural products with health-promoting properties and minimal industrial processing (Prodanovic *et al*, 2024).

Honey is recognized as one of the oldest foods consumed by humanity. Archaeological evidence indicates that honey collection predates the development of agriculture, with cave paintings dating back more than 8,000 years depicting honey harvesting activities. Ancient civilizations, including the Egyptians, Greeks, Romans, and Chinese, not only consumed honey as a natural sweetener but also utilized it for medicinal, cosmetic, and religious purposes due to its preservative and therapeutic characteristics. In modern times, scientific advances have transformed the perception of honey from a simple carbohydrate-rich food into a highly complex biological matrix containing numerous compounds of nutritional and functional importance (Dunne *et al.*, 2021).

In Brazil, beekeeping was formally introduced in 1839 through the arrival of European honeybee colonies in Rio de Janeiro, subsequently expanding throughout the country and becoming an important agricultural activity (Nunes, 2019). The introduction and adaptation of *Apis mellifera* to Brazilian environmental conditions, particularly following the establishment of Africanized bee populations, resulted in increased productivity and enhanced resistance to tropical climatic conditions. Consequently, Brazil has become one of the world's leading honey producers and exporters, with production systems distributed across diverse ecosystems, each contributing unique

botanical and geographical characteristics that influence honey composition and quality (Gomes *et al.*, 2025).

Honey is defined as a natural substance produced by bees from floral nectar, plant secretions, or excretions of plant-sucking insects found on living plant tissues, which are transformed through enzymatic action, dehydration, and maturation within honeycombs. According to Brazilian Normative Instruction No. 11/2000, honey is a product resulting from biological and biochemical processes carried out by bees, emphasizing its natural origin and complex composition (Brazil, 2000). Chemically, honey consists predominantly of carbohydrates, especially fructose and glucose, which account for approximately 80% of its composition. However, its biological value extends far beyond its sugar content, as honey contains a diverse range of minor compounds, including proteins, amino acids, vitamins, minerals, organic acids, enzymes, volatile compounds, pigments, and bioactive phytochemicals (Schiassi *et al.*, 2021; Antunes *et al.*, 2023).

The chemical complexity of honey is largely determined by its botanical and geographical origin. Environmental conditions, climate, soil characteristics, floral diversity, bee species, harvesting season, processing methods, and storage conditions collectively influence the physicochemical properties and bioactive profile of honey. Consequently, honey produced in different regions exhibits considerable variation in color, aroma, flavor, nutritional composition, and biological activity. This variability has attracted increasing scientific interest, particularly regarding the identification of biomarkers capable of differentiating honey according to its floral and geographical origin (Kiran *et al.*, 2026; Jang *et al.*, 2026).

From a biotechnological perspective, honey can be considered a natural reservoir of biologically active molecules. Among its constituents, phenolic compounds represent one of the most extensively studied groups due to their association with numerous health-promoting effects. These compounds include flavonoids, phenolic acids, tannins, and other secondary plant metabolites transferred from nectar and pollen into honey during production. The concentration and diversity of phenolic compounds are strongly influenced by floral source, climatic conditions, and environmental factors, making them valuable indicators of honey quality and authenticity (Schiassi *et al.*, 2021).

Phenolic compounds are recognized for their ability to act as potent antioxidants through multiple mechanisms, including free radical scavenging, metal ion chelation, inhibition of oxidative enzymes, and protection against lipid peroxidation. These mechanisms contribute to reducing oxidative stress, a physiological condition associated with cellular damage and the development of chronic diseases such as cardiovascular disorders, diabetes mellitus, neurodegenerative diseases, inflammatory syndromes, and certain types of cancer. Consequently, the determination of phenolic

compounds and antioxidant activity has become an essential aspect of honey characterization, particularly in the context of functional food development and nutraceutical applications (Ahmad *et al.*, 2025).

Recent advances in food biotechnology have highlighted the growing importance of natural antioxidants as alternatives to synthetic additives commonly used in the food industry. Consumer concerns regarding the safety of artificial preservatives have stimulated research into natural sources of antioxidant compounds capable of enhancing food stability while simultaneously providing health benefits. In this context, honey emerges as a promising ingredient due to its combination of nutritional value, antimicrobial activity, and antioxidant potential. The incorporation of honey into food formulations has been investigated as a strategy to improve shelf life, reduce oxidative deterioration, and increase the functional value of food products (Pedrouso *et al.*, 2026).

The antioxidant activity of honey results from a synergistic interaction among various compounds, including phenolic acids, flavonoids, enzymes such as catalase and glucose oxidase, vitamins, carotenoids, Maillard reaction products, and organic acids. Although phenolic compounds are considered the primary contributors to antioxidant capacity, the overall biological activity of honey cannot be attributed to a single group of molecules. Instead, it reflects the complex interactions among multiple constituents present within the honey matrix. This complexity has motivated extensive research aimed at understanding the relationship between chemical composition and biological functionality (Tlak Gajger *et al.*, 2025).

Several studies have demonstrated a direct relationship between honey color and antioxidant activity. Darker honeys generally exhibit higher concentrations of phenolic compounds and consequently greater antioxidant potential compared to lighter varieties. Color intensity may serve as an indirect indicator of the concentration of antioxidant molecules present in honey. Therefore, colorimetric analyses have become increasingly relevant as complementary tools for evaluating honey quality and predicting its functional properties (Shakoori *et al.*, 2024).

Beyond antioxidant activity, honey has been reported to exhibit antimicrobial, anti-inflammatory, immunomodulatory, antiviral, wound-healing, and anticancer properties. These biological effects have encouraged investigations into its potential applications in biotechnology, medicine, pharmaceuticals, and food science. The development of honey-based biomaterials, bioactive coatings, therapeutic formulations, and functional beverages represents a growing area of research that reinforces the value of honey as a multifunctional natural resource (Dunne *et al.*, 2021).

The global honey market has experienced substantial growth in recent years, driven not only by increased consumption but also by the recognition of honey as a functional food. According to the Brazilian Institute of Geography and Statistics (IBGE), honey production in Brazil generated

R\$503.6 million in 2018 and increased progressively to R\$957.8 million in 2022, corresponding to an annual production exceeding 60 million kilograms. This growth reflects both domestic consumption and the expansion of export markets, which increasingly demand products with proven quality, authenticity, and functional attributes. Within this scenario, Pernambuco has emerged as an important honey-producing state, occupying a prominent position in national production and demonstrating significant economic potential for rural communities (IBGE, 2022).

The Agreste region of Pernambuco constitutes a unique ecological environment located between the humid Atlantic Forest and the semi-arid Sertão. This transitional zone is characterized by diverse vegetation formations, predominantly hypoxerophilous Caatinga, moderate rainfall, and environmental conditions that favor the development of a rich and heterogeneous flora. Such botanical diversity provides bees with access to a wide variety of nectar and pollen sources, potentially resulting in honey with distinctive physicochemical characteristics and elevated concentrations of bioactive compounds (Oliveira *et al.*, 2020).

Despite the increasing importance of honey production in the region, scientific information regarding the physicochemical quality, antioxidant properties, phenolic composition, and authenticity of Agreste honey remains limited. Considering that geographical origin and floral diversity are key determinants of honey quality, comprehensive characterization studies are essential to establish quality standards, identify regional specificities, support geographical valorization strategies, and strengthen the competitiveness of local beekeeping systems. Furthermore, understanding the bioactive profile of regional honey may contribute to the development of value-added products and promote its application as a functional ingredient in food, pharmaceutical, and biotechnological sectors (Gomes *et al.*, 2022).

METHODOLOGY

Samples

Three multifloral honey samples were obtained from a single beekeeper located in the municipality of Taquaritinga do Norte, Pernambuco, Brazil. All samples belonged to the same production batch and were collected during the spring harvest of 2023. The honey was produced from nectar collected by bees from multiple floral sources naturally available in the region, without the identification of a predominant botanical origin, and was therefore classified as multifloral honey.

Before packaging, the honey harvested from different hives within the same apiary was homogenized by the producer, resulting in a single representative production batch. Consequently, the analyses performed in this study were conducted on this homogenized batch, representing the

overall characteristics of the apiary production rather than individual hives. The use of honey from a single producer and a single production batch minimized variations associated with beekeeping management and post-harvest handling, allowing the study to focus on the physicochemical characteristics, authenticity indicators, total phenolic content, and antioxidant activity of multifloral honey produced in the Agreste region of Pernambuco.

Image 1: honey samples used in the study.



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Physicochemical characterization

Honey samples were characterized through the determination of total soluble solids (°Brix), pH, moisture, dry matter, ash content, total titratable acidity, protein content, lipid content, vitamin C, reducing sugars, water activity (A_w), and adulteration indicators.

Total soluble solids were determined by direct measurement using a digital refractometer (MA871, Milwaukee Instruments, USA), while pH was measured using a calibrated digital pH meter (MYLABOR MY140®) according to AOAC (1990) procedures. Moisture, dry matter, ash, total acidity, proteins, lipids, vitamin C, and reducing sugars were determined following the official methodologies described by the Adolfo Lutz Institute (IAL, 2008). Protein content was quantified using the Kjeldahl method, whereas lipid content was determined according to a modified Bligh and Dyer extraction procedure adapted for honey matrices.

Water activity (A_w) was measured using an AquaLab® water activity meter based on the chilled mirror dew-point technique (Picanço, 2018). All analyses were performed in triplicate.

Adulteration analysis

The presence of starch and dextrins, indicative of possible adulteration, was evaluated using the Lugol reaction according to the methodology described by Lucena (2020). The development of a reddish-brown to blue coloration was considered indicative of adulterating agents, with color intensity proportional to the concentration of starch-derived compounds present in the sample.

Color characterization

Color parameters were determined using a Minolta CR-300 colorimeter equipped with a D65 standard illuminant and a 2° observer angle. The CIELAB coordinates L* (lightness), a* (red-green axis), and b* (yellow-blue axis) were directly measured. Subsequently, Chroma (C*) and Hue angle (h°) values were calculated to provide a comprehensive evaluation of honey color characteristics (Marcus, 1998).

Determination of bioactive compounds

The bioactive profile of honey samples was assessed through the determination of total phenolic compounds, total flavonoids, flavanols, and condensed tannins using spectrophotometric methods. Total phenolic compounds were quantified using the Folin-Ciocalteu reagent according to Giovanelli and Buratti (2009), with results expressed as gallic acid equivalents (GAE). Total flavonoids were determined using the aluminum chloride colorimetric method described by Dewanto et al. (2002). Flavanol content was quantified according to the methodology proposed by Lees and Francis (1972), while condensed tannins were determined using the vanillin-HCl assay adapted from Queiroz (2002).

Absorbance measurements were performed using UV–Vis spectrophotometers (EDUTECH® and Rayleigh UV-2601®) at their respective analytical wavelengths.

Antioxidant activity

The antioxidant potential of honey samples was evaluated by radical scavenging assays against DPPH• (2,2-diphenyl-1-picrylhydrazyl) and ABTS•+ (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radicals. The DPPH assay was performed according to Porgali and Büyüktuncel (2012), with antioxidant activity expressed as percentage inhibition of the radical. The ABTS assay was conducted following Rao and Fuller (2018), and the antioxidant capacity was likewise expressed as percentage radical inhibition. Absorbance readings were obtained at 517 and 734 nm for DPPH and ABTS assays, respectively.

Statistical analysis

All analyses were conducted in triplicate and results were expressed as mean ± standard deviation. Data were subjected to one-way analysis of variance (ANOVA), followed by Tukey's post hoc test at a significance level of 5% ($p < 0.05$). Pearson correlation analysis was performed to investigate relationships among physicochemical properties, color parameters, bioactive compounds, and

antioxidant activity. Statistical analyses were conducted using PAST® software.

Results

The results obtained allowed for the characterization of different aspects of honey quality through physicochemical, colorimetric, and bioactive analyses. Initially, parameters related to the composition and stability of the product are presented, including total soluble solids, pH, moisture, dry extract, ash, acidity, proteins, lipids, ascorbic acid, reducing sugars, and water activity. Next, the chromatic characteristics of the samples are described, evaluated by the parameters of luminosity, color coordinates, hue angle, and color intensity. Finally, the results regarding phenolic compounds and antioxidant activity are presented, encompassing total flavonoids, total tannins, flavonols, total polyphenols, and the antioxidant capacity determined by the DPPH and ABTS methods. Together, these parameters provide a comprehensive view of the physicochemical, visual, and functional properties of the honey samples analyzed.

Table 1 presents the physicochemical characterization of three honey samples (M1, M2, and M3), covering parameters related to the composition and quality of the product. Total soluble solids content was determined, ranging from 75.3 to 75.6 °Brix; pH between 3.5 and 3.9; moisture between 11.9 and 14.5%; dry extract between 85.4 and 88.0%; and ash between 0.1 and 0.3%. Total acidity was also evaluated, with values between 0.026 and 0.034 mEq/kg; protein between 0.4 and 0.5 g/100 mL; and lipids, which were not detected in the analyzed samples. Furthermore, ascorbic acid content was quantified, ranging from 5.59 to 20.52 g/100 mL, reducing sugars between 42.20 and 87.9 g/100 mL, as well as water activity, which showed a value of 0.6 in all samples. The results are presented as mean $\pm$ standard deviation, with statistical differences indicated by distinct superscript letters.

Table 1. Physicochemical characterization of honey..

Parameter	Results		
	M1	M2	M3
Total Soluble Solids (°Brix)	75,6 $\pm$ 0,2 ^A	75,3 $\pm$ 0,1 ^B	75,5 $\pm$ 0,1 ^A
pH	3,5 $\pm$ 0,0 ^C	3,7 $\pm$ 0,0 ^B	3,9 $\pm$ 0,0 ^A
Moisture (%)	11,9 $\pm$ 0,4 ^A	14,5 $\pm$ 2,1 ^A	14,1 $\pm$ 3,7 ^A
Dry Extract (%)	88 $\pm$ 0,371 ^A	85,4 $\pm$ 2,137 ^A	85,8 $\pm$ 3,693 ^A

Ash (%)	0,1±0,1 ^A	0,1±0,1 ^A	0,3±0,1 ^A
Total Acidity (mEq/KG)	0,03±0,0 ^A	0,034±0,0 ^A	0,026±0,0 ^A
Protein (g/100mL)	0,4±0,1 ^A	0,5±0,2 ^A	0,4±0,2 ^A
Lipids (g/100mL)	ND ¹	ND ¹	ND ¹
Ascorbic Acid (g/100mL)	13,05±2,9 ^B	20,52±2,9 ^A	5,59±0,0 ^C
Reducing Sugars (g/100mL)	87,9±3,8 ^A	79,6±8,9 ^B	42,20±5,0 ^C
Water Activity (a_W)	0,6±0,0 ^A	0,6±0,0 ^A	0,6±0,0 ^A

Legend: ¹ND - Not determined by the sensitivity of the assay performed. M1: Honey 1; M2: Honey 2; M3: Honey 3; Different letters indicate a statistically significant difference (p<0.05).

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Table 2 presents the chromatic parameters of the three honey samples (M1, M2, and M3), evaluated using the colorimetric coordinates L*, a*, and b*, as well as the derived parameters H° (hue angle) and C* (color intensity). Luminosity (L*) values ranged from 53.99 to 75.51, while the a* coordinate showed values between -1.00 and -0.63. For the b* coordinate, the results ranged from 27.35 to 35.61. The hue angle (H°) showed values between 46.49 and 63.95, and the color intensity (C*) ranged from 27.27 to 35.62. The results are expressed as mean ± standard deviation, with statistical differences between samples indicated by distinct superscript letters.

Table 2: Physicochemical characterization of honey.

Parameter	Results		
	M1	M2	M3
L*	75,51±1,9 ^A	53,99±1,93 ^C	63.46±1,97 ^B
a*	-1,0±0,17 ^A	-0,85±0,34 ^A	-0,63±0,08 ^A
b*	34,12±0,73 ^B	27,35±2,22 ^C	35,61±0,46 ^A
H°	63,95±2, 55 ^A	46,49±2,32 ^C	52,50±2,55 ^B
C*	34,14±0,74 ^B	27,27±2,21 ^C	35,62±0,46 ^A

Legend: Evaluated color parameters: L*: Luminosity, a*: Red/green coordinate (+a=red; -a=green), b*: Yellow/blue coordinate (+b=yellow; -b=blue), C*: Color intensity, Ho: Hue angle; M1: Honeycomb 1; M2: Honeycomb 2; M3: Honeycomb 3; Different letters indicate a statistically significant difference (p<0.05)

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Table 3 presents the determination of phenolic compounds and antioxidant activity of the three honey samples (M1, M2, and M3). Total flavonoid content was evaluated, ranging from 14.520 to 15.889 mg/100 mL, total tannins from 4.424 to 12.417 mg/100 mL, and flavonols from 2.144 to 3.595 mg/100 mL. Additionally, antioxidant activity values were determined using the DPPH and ABTS methods, with results ranging from 48.01 to 73.89 and from 90.71 to 92.21, respectively. Total polyphenols were also quantified, with values between 80.64 and 144.06 mg/100 mL. The results are presented as mean $\pm$ standard deviation, with statistical differences between samples indicated by distinct superscript letters.

Table 3: Phenolic and antioxidant determination of honey

Parameter	Results		
	M1	M2	M3
Total Flavonoids¹	14,855 $\pm$ 0,152 ^B	15,889 $\pm$ 0,009 ^A	14,520 $\pm$ 0,066 ^B
Total Tannins¹	5,209 $\pm$ 0,048 ^B	12,417 $\pm$ 0,127 ^A	4,424 $\pm$ 0,206 ^C
Flavonols¹	3,595 $\pm$ 0,190 ^A	2,144 $\pm$ 0,060 ^B	3,068 $\pm$ 0,407 ^A
DPPH¹	48,01 $\pm$ 0,95 ^A	65,95 $\pm$ 1,78 ^B	73,89 $\pm$ 2,02 ^C
Total Polyphenols	144,06 $\pm$ 5,83 ^A	93,93 $\pm$ 0,46 ^B	80,64 $\pm$ 2,13 ^C
ABTS¹	91,07 ^A	92,21 ^A	90,71 ^A

¹Result expressed in mg/100mL. M1: Honey 1; M2: Honey 2; M3: Honey 3; Different letters indicate a statistically significant difference (p<0.05)

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The combined analysis of the evaluated parameters reveals differences between the honey samples regarding their physicochemical composition, color characteristics, and bioactive potential. The variations observed in the levels of soluble solids, moisture, sugars, phenolic compounds, and antioxidant activity, as well as in the color parameters, reflect the complexity of the honey matrix and the influence of factors related to its botanical origin, environmental conditions, and processing. Therefore, the discussion of the results below seeks to understand the behavior of each variable analyzed, relating the values obtained with those described in the literature and with aspects that may influence the quality, stability, and functional properties of the product.

Discussion

The physicochemical characterization demonstrated that the multifloral honey samples exhibited significant compositional differences despite originating from the same geographical region, highlighting the influence of botanical diversity on honey quality. Variations in nectar composition among plant species directly affect the concentrations of carbohydrates, organic acids, minerals, and

phytochemicals transferred to honey.

Total soluble solids (75.3–75.6 °Brix) were characteristic of mature honey with high sugar concentration, indicating adequate ripening and low moisture. Likewise, moisture content (11.9–14.5%) remained well below the legal limit (20%), ensuring low water availability and reduced susceptibility to fermentation by osmophilic yeasts. The acidic pH (3.5–3.9) also contributes to microbiological stability and reflects differences in organic acid composition associated with floral origin (Gomes et al., 2022; Pop et al., 2023).

No significant differences were observed for ash, total acidity, proteins, lipids, or water activity, suggesting similar mineral composition and overall physicochemical stability among samples. In contrast, vitamin C and reducing sugars differed significantly, indicating that botanical origin influenced antioxidant composition and carbohydrate profiles. The absence of starch in the Lugol assay confirmed the authenticity of all honey samples.

Color analysis revealed significant differences among samples, reflecting variations in botanical origin and phytochemical composition. Sample M2 showed lower lightness (L^*) and higher flavonoid and tannin contents, whereas M3 exhibited greater yellow intensity (b^*) and chroma. These findings agree with previous studies showing that darker honeys generally contain higher concentrations of phenolic compounds and other natural pigments, although honey color results from the combined influence of phenolics, minerals, pigments, proteins, and Maillard reaction products rather than a single chemical class (Topa et al., 2026; Rivera-Pérez et al., 2024).

The phytochemical profile confirmed botanical origin as the main determinant of honey composition. M1 presented the highest total polyphenol content, whereas M2 contained significantly greater flavonoid and tannin concentrations, demonstrating that total phenolic content alone does not fully represent honey phytochemical complexity. These compounds originate from plant secondary metabolism and are influenced by both floral diversity and environmental conditions, contributing to the antioxidant, antimicrobial, and anti-inflammatory properties of honey (Vîjan et al., 2023; Limpas-Hurtado et al., 2026).

Antioxidant assays further demonstrated the complexity of honey antioxidant systems. Although M1 showed the highest total polyphenol concentration, it exhibited the lowest DPPH scavenging activity, whereas M3 presented the highest antioxidant activity despite having lower total phenolic content. These findings indicate that antioxidant capacity depends more on the qualitative composition of bioactive compounds and their synergistic interactions than on total phenolic concentration alone. The contrasting responses between DPPH and ABTS assays reinforce that different antioxidant mechanisms coexist in honey, with ABTS indicating consistently high antioxidant potential across all samples (Becerril-Sánchez et al., 2021; Aryal et al., 2019; Platzer et al., 2021).

Overall, the results demonstrate that multifloral honeys from the Agreste region of Pernambuco possess excellent physicochemical quality, high microbiological stability, and considerable functional potential. The observed variability emphasizes the importance of integrating physicochemical, colorimetric, phytochemical, and antioxidant analyses to comprehensively characterize regional honeys and support their commercial valorization as functional foods.

CONCLUSIONS

The honey samples produced in the Agreste region of Pernambuco exhibited physicochemical characteristics consistent with current quality standards, demonstrating adequate maturation, low moisture content, low water activity, and absence of adulteration, which collectively indicate high product quality, authenticity, and microbiological stability. These findings reinforce the favorable conditions of the region for honey production and highlight the importance of local beekeeping as a source of high-value natural products.

Significant differences were observed among the evaluated samples regarding physicochemical composition, color attributes, bioactive compounds, and antioxidant activity, confirming the strong influence of floral origin and environmental conditions on honey quality. The chromatic analyses demonstrated variations in luminosity, color saturation, and hue angle, which were associated with differences in the concentration of phenolic compounds, particularly flavonoids and tannins. These results support the use of color parameters as complementary indicators of honey composition and functional potential.

The bioactive characterization revealed that all samples contained considerable concentrations of phenolic compounds and exhibited expressive antioxidant activity. Notably, the results demonstrated that antioxidant capacity was not exclusively dependent on total phenolic content, suggesting that the qualitative composition of bioactive compounds and their synergistic interactions play a decisive role in determining the antioxidant performance of honey. This finding reinforces the complexity of honey as a natural biological matrix and highlights the importance of integrated evaluations involving both phytochemical composition and functional properties.

Overall, the results demonstrate that honey produced in the Agreste region of Pernambuco constitutes a valuable source of natural antioxidants and bioactive compounds with potential health-promoting properties. The observed relationship among physicochemical characteristics, color parameters, phenolic composition, and antioxidant activity contributes to a better understanding of the factors that determine honey quality and functional value. Furthermore, these findings strengthen the scientific and commercial potential of regional honey, supporting its valorization in domestic and international markets and encouraging future studies focused on the identification of specific

phenolic compounds, geographical authentication, and the development of functional and biotechnological applications based on this natural product.

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