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PHYSICOCHEMICAL, BIOACTIVE AND SAFETY PROPERTIES OF HONEY PRODUCED BY STINGLESS BEES IN THE COLOMBIAN AMAZON PIEDMONT

PROPIEDADES FISICOQUÍMICAS, BIOACTIVAS Y DE INOCUIDAD DE MIELES PRODUCIDAS POR ABEJAS SIN AGUIJÓN EN EL PIEDEMONTE AMAZÓNICO COLOMBIANO

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ABSTRACT

Background: In the Colombian Amazonian Piedmont, a highly biodiverse ecosystem, local communities practice meliponiculture using various species of stingless bees. The honeys produced are traditionally used as food and medicine, and in many cases, they can be empirically differentiated according to origin, sensory properties, and therapeutic application. However, the comprehensive characterization of these honeys remains too limited. **Objective:** The physicochemical and bioactive properties of honey produced by stingless bee species from the Colombian Amazonian Piedmont were investigated, as well as its chemical and microbiological safety and its behavior when subjected to heat treatments. **Methods:** Twenty-eight honey samples were taken from seven species of stingless bees. The following were determined: moisture, pH, total acidity, electrical conductivity, diastase activity, hydroxymethylfurfural, ash, minerals (Mg, Mn, K, Na, Zn), insoluble solids, reducing sugars, and color. In addition, antioxidant activity, total phenols, and organic acid profile were evaluated. Safety was assessed for heavy metals (Cu, Cr, Cd, Pb, As, and Hg) and microbiologically. Selected groups of samples were heat-treated to evaluate the effect on chemical and microbiological properties. **Results:** The honeys showed high variability within and between bee species. Regarding *Apis mellifera* honey, the pot honeys presented higher moisture content (26.6 ± 3.01%), higher acidity (49.59 ± 15.68 meq/kg; pH 3.68 ± 0.27), higher insoluble solids content, and a similar total sugar content (62.85% ± 4.32%), but a lower reducing sugar content (50.77% ± 4.21%). The organic acid profile revealed a notable ascorbic acid content in all honeys (47.1–233.7 mg/100g), related to the observed antioxidant activity. Lactic acid was also found, consistent with the presence of lactic acid bacteria and their potential probiotic properties. No contamination with lead, arsenic, cadmium, or mercury was detected, and the absence of pathogenic microorganisms was confirmed. Heat treatments differentially affected lactic acid bacteria and ascorbic acid, thereby affecting functional potential. **Conclusion:** This first comprehensive evaluation of the

quality of stingless bee honey from the Colombian Amazonian Piedmont highlights its unique bioactive profile and safety, thus contributing to the sustainable development of the region.

Keywords: Lactic acid bacteria, Meliponiculture, Organic acids, Pot honey composition, Sustainable development

RESUMEN

Antecedentes: En el Piedemonte amazónico colombiano, un ecosistema con gran biodiversidad, las comunidades locales practican la meliponicultura, cuidando varias especies de abejas sin aguijón. Producen mieles que son utilizadas tradicionalmente como alimento y medicina; con frecuencia ellos mismos son capaces de diferenciarlas empíricamente por origen, propiedades sensoriales y aplicación terapéutica. Sin embargo, la caracterización integral de estas mieles aún es demasiado limitada. **Objetivo:** Se investigaron las propiedades fisicoquímicas y bioactivas de miel de especies de abejas sin aguijón, así como su inocuidad química y microbiológica, y el comportamiento al someterse a tratamientos térmicos. **Métodos:** Se tomaron 28 muestras de miel de siete especies de abejas sin aguijón. Se determinaron humedad, pH, acidez total, conductividad eléctrica, actividad de la diastasa, hidroximetilfurfural, cenizas, minerales (Mg, Mn, K, Na, Zn), sólidos insolubles, azúcares reductores y color. Además, se evaluó actividad antioxidante, fenoles totales y perfil de ácidos orgánicos. La inocuidad se evaluó en cuanto a metales pesados (Cu, Cr, Cd, Pb, As y Hg) y microbiológicamente. Grupos seleccionados de muestras fueron tratados térmicamente para evaluar el efecto sobre propiedades químicas y microbiológicas. **Resultados**: Las mieles mostraron alta variabilidad dentro y entre especies de abejas. Respecto a la miel de *Apis mellifera*, presentaron mayor contenido de humedad (26,6 ± 3,01%), mayor acidez (49,59 ± 15,68 meq/kg; pH 3,68 ± 0,27), mayor contenido de sólidos insolubles y similar contenido de azúcares totales (62,85% ± 4,32%), pero menor contenido de azúcares reductores (50,77% ± 4,21%). El perfil de ácidos orgánicos reveló destacable contenido de ácido ascórbico en todas las mieles (47,1–233,7 mg/100g), relacionado con la actividad antioxidante observada. También se encontró ácido láctico, consistente con la presencia de bacterias acido lácticas y sus posibles propiedades probióticas. No se detectó contaminación con plomo, arsénico, cadmio ni mercurio, y se confirmó la ausencia de microorganismos patógenos. Los tratamientos térmicos afectaron diferencialmente las bacterias lácticas y el ácido ascórbico y por tanto el potencial funcional. **Conclusión:** Esta primera evaluación integral de la calidad de la miel de abejas sin aguijón del Piedemonte amazónico colombiano destaca su perfil bioactivo único y su inocuidad, contribuyendo al desarrollo sostenible de la región.

Palabras clave: Bacterias acido lácticas, Meliponicultura, Ácidos orgánicos, Composición de miel de pote, Desarrollo Sostenible

1. INTRODUCTION

Meliponiculture, the practice of cultivating stingless bees (Meliponini tribe) for pot honey and other products, has deep ancestral roots in the Americas, where these bees play a vital ecological role as pollinators of wild and cultivated plants [1–3]. Historically, indigenous communities valued stingless bee products for their medicinal and nutritional properties long before the introduction of *Apis mellifera* through European colonization, a tradition that persists today, especially in regions like the Amazon [2]. Despite their resilience to climate change, many stingless bee species have suffered drastic population declines, with some now endangered [4]. The resurgence of meliponiculture as a sustainable practice has led to advancements in hive design, transitioning from rustic structures made of tree trunks and bamboo to modern prototypes that improve management, hygiene, and productivity [2,5]. However, to ensure that meliponiculture contributes effectively to community well-being and local economies, a deeper understanding of the physicochemical and microbiological properties of pot honey is essential, not only to properly value them but also to establish guidelines for their safe use and commercialization.

Globally, over 600 species of stingless bees have been identified, with more than 130 species recognized in Colombia, inhabiting environments ranging from sea level to 3,400 meters in altitude, of which approximately one-third are used in meliponiculture [6,7]. In many countries, meliponiculture is promoted as a sustainable livelihood to enhance rural living conditions [3]. In the Colombian Amazon piedmont, organizations are fostering meliponiculture to promote forest

conservation while providing economic and social development opportunities for indigenous, Afro-descendant, and rural communities. The Andean-Amazon piedmont has preserved traditional meliponiculture practices, with pot honey valued for both food and medicinal purposes [8]. Current regulations permit the subsistence harvesting of native bees within the experimental phase of the meliponiculture, establishing criteria for sustainable community management, while efforts are underway to obtain an environmental license for the commercial phase to ensure the long-term sustainable use of stingless bees in Colombia [8].

The traditional medicinal use of stingless bee honey suggests the presence of bioactive compounds with potential health benefits, yet this remains an underexplored research area. The primary stingless bee species cultivated in the region belong to the genera *Melipona, Tetragonisca, Lestrimelitta, Plebeia, Scaptotrigona,* and *Tetragona* [9]. Physicochemical analyses are essential for identifying honey varieties according to bee species, botanical and geographical origin, and for providing regulatory agencies with objective tools to prevent honey adulteration in trade. While some quality parameters such as pH, acidity, hydroxymethylfurfural content, and diastase activity, are not directly related to nutritional or bioactive value, they are crucial for ensuring authenticity and safety, leading regulatory bodies like the Codex Alimentarius [10], the International Honey Commission [11], and the European Honey Commission [12] to establish standards for these properties. Studies on pot honey from *Melipona, Tetragonisca, Trigona*, and other genera have shown that its composition is highly species-specific and influenced by botanical and geographical origins [13–19]. Compared to *Apis mellifera* honey, stingless bee honey exhibits distinct physicochemical and bioactive properties, including higher concentrations of ascorbic acid, organic acids, and polyphenols, which have been linked to antioxidant, antimicrobial, and potential probiotic benefits [9]. However, no prior studies have characterized the composition of pot honey from the Andean-Amazon piedmont, particularly for species such as *Melipona nebulosa, Melipona grandis,* and *Lestrimelitta* sp., leaving a knowledge gap regarding their chemical composition and functional properties.

A key difference between pot honey and *Apis mellifera* honey is its higher moisture content, which makes it more susceptible to microbial growth and fermentation [20]. While conservation strategies such as microbial load reduction have been proposed to prevent fermentation [20,21], their impact on quality, freshness, and bioactive properties, such as antioxidant activity and organic acid stability, remains unclear. Moreover, the advisability of reducing microbial loads is debated, as some microorganisms may possess probiotic properties, which, when combined with the prebiotic potential of certain carbohydrates and polyphenols, could confer symbiotic benefits to pot honey [22,23].

Given these considerations, this study evaluated the physicochemical, bioactive and microbiological quality of pot honey produced by seven stingless bee species (*Lestrimelitta* sp., *Melipona eburnea*, *Melipona grandis, Melipona nebulosa*, *Scaptotrigona* sp.*, Tetragona* sp., and *Tetragonisca angustula*) and established relationships between them. Additionally, it was examined the effects of thermal treatments on microbial loads and bioactive compounds in honey samples from selected species. This study expands the understanding of meliponiculture beyond the Colombian Amazon piedmont by providing novel insights into the physicochemical, bioactive, and microbiological properties of pot honey from multiple stingless bee species. While meliponiculture is gaining global recognition as a sustainable beekeeping practice, much of the existing research has focused on regions such as Southeast Asia, Australia, and Brazil, leaving significant knowledge gaps regarding the unique properties of stingless bee honey from Andean-Amazonian ecosystems. Furthermore, the findings on microbial composition and heat treatment effects, which have been poorly studied to date, provide valuable information for improving honey preservation methods while maintaining its bioactive potential. These findings have implications for food safety and functional food development worldwide, supporting the sustainable development of meliponiculture and providing a scientific foundation for integrating stingless bee products into international markets, emphasizing their ecological, economic, and nutraceutical significance.

2. MATERIALS AND METHODS

2.1 Sample collection and preparation

In 2023, a total of 28 pot honey samples were collected exclusively from sealed honey pots in both rustic hives (large, undivided wooden boxes) and technified hives (modular wooden boxes with separate compartments for the brood, as well as honey and pollen storage). Table 1 provides details on the origin of the samples, while Figure 1 illustrates their geographic locations.

The sampling was carried out in areas located between 250 and 500 m above sea level, with an average altitude of 310 meters, an average temperature of 25 °C, and a relative humidity of 88 %.

Table 1. Identification of studied samples

Sample	Origin	Bee species	Type of hive	Geographic coordinates
1	Florencia, Caquetá	*Lestrimelitta* sp	Technified	1° 36′ 57″ N 75° 36′ 52″ W
2	Piamonte, Cauca	*Melipona eburnea* Friese, 1900	Technified	1° 7′ 7″ N 76° 19′ 36″ W
3	Vereda San Antonio, Mocoa, Putumayo		Technified	1° 10′ 51″ N 70° 40′ 32″ W
4	Vereda Los Corrales, Puerto Guzmán, Putumayo		Rustic	0° 43′ 50″ N 75° 54′ 22″ W
5	Vereda Los Corrales, Puerto Guzmán, Putumayo		Rustic	0° 43′ 50″ N 75° 54′ 22″ W
6	Vereda Los Corrales, Puerto Guzmán, Putumayo		Rustic	0° 43′ 50″ N 75° 54′ 22″ W
7	Barrio La Esmeralda, Mocoa, Putumayo		Rustic	1° 8′ 58″ N 76° 38′ 48″ W
8	Barrio Los Prados, Puerto Asís, Putumayo		Technified	0° 27′ 42″ N 76° 20′ 32″ W
9	Campesino, venta callejera en Mocoa, Putumayo		Information not available	1° 8′ 48″ N 76° 38′ 58″ W
10	Vereda La Siberia, Orito, Putumayo		Technified	0° 40′ 36″ N 76° 52′ 38″ W
11	Vereda La Florida, La Hormiga, Putumayo		Technified	0° 27′ 9″ N 76° 55′ 9″ W
12	Barrio Los Prados, Puerto Asís, Putumayo		Technified	0° 27′ 42″ N 76° 20′ 32″ W
13	Vereda Los Andes Mocoa, Putumayo		Technified	1°, 7′, 71″ N 76°, 39′, 31″ W
14	Piamonte, Cauca	*Melipona grandis* Guérin-Méneville, 1844	Technified	1° 7′ 7″ N 76° 19′ 36″ W
15	Vereda Los Andes, Mocoa, Putumayo		Technified	1°, 07′, 71″ N 76°, 39′, 31″ W
16	Vereda Los Corrales, Puerto Guzmán, Putumayo		Rustic	0° 43′ 50″ N 75° 54′ 22″ W
17	Barrio La Esmeralda, Mocoa, Putumayo		Rustic	1° 8′ 58″ N 76° 38′ 48″ W
18	Vereda La Siberia, Orito, Putumayo		Technified	0° 40′ 36″ N 76° 52′ 38″ W
19	Vereda La Florida, La Hormiga, Putumayo		Technified	0° 27′ 9″ N 76° 55′ 9″ W
20	Vereda Corrales, Puerto Guzmán, Putumayo		Technified	0° 43′ 50″ N 75° 54′ 22″ W

Sample	Origin	Bee species	Type of hive	Geographic coordinates
21	Vereda Los Andes, Mocoa Putumayo		Technified	1°, 07′, 71″ N 76°, 39′, 31″ W
22	Vereda La Siberia Orito, Putumayo	*Melipona nebulosa* Camargo, 1988	Technified	0° 40′ 36″ N 76° 52′ 38″ W
23	Vereda Los Andes, Mocoa , Putumayo		Technified	1°, 07′, 71″ N 76°, 39′, 31″ W
24	Vereda La Florida, La Hormiga, Putumayo	*Scaptotrigona sp*	Rustic	0° 27′ 9″ N 76° 55′ 9″ W
25	Resguardo Santa Rosa, La Hormiga, Putumayo	*Tetragona sp*	Technified	0° 27′ 9″ N 76° 55′ 9″ W
26	Vereda Campucana, Mocoa , Putumayo		Rustic	1°12′ 73″ N 76° 42′ 55″ W
27	Vereda Los Andes, Mocoa, Putumayo	*Tetragonisca angustula* Latreille, 1811	Technified	1°, 07′, 71″ N 76°, 39′, 31″ W
28	Vereda Campucana, Mocoa , Putumayo		Rustic	1°12′ 73″ N 76° 42′ 55″ W

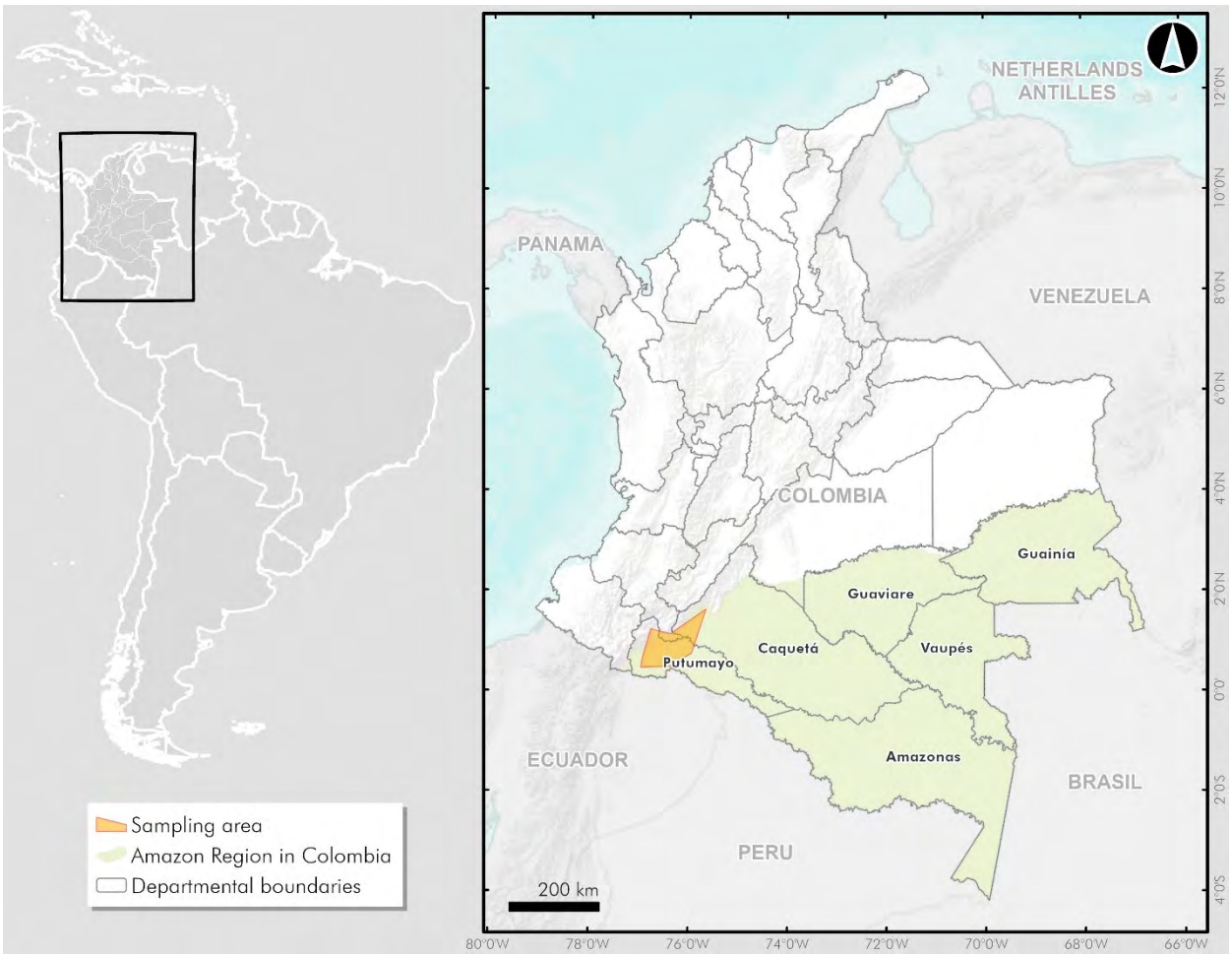

Figure 1. Sampling area (in yellow) in the Colombian Amazon piedmont, Colombia, South America

All samples were collected within the same week, with each sample corresponding to hives from the same meliponary. Honey combs with fully sealed cells were removed from the hives and taken to a facility suitable for food processing. Glass containers with lids and stainless steel utensils used in the extraction process (bowls, spatulas, forks, spoons, tongs, and strainers) were sanitized beforehand. Good food handling practices were followed during honey extraction. The comb frame was held, and each cell was hygienically pierced at the top using forks. The frame was then tilted to allow the honey to drain through a stainless steel strainer placed over a stainless steel container. This procedure was repeated with as many combs as needed, and the honey was subsequently transferred into the glass containers. The samples were stored under refrigeration at 4°C for later analysis.

2.2 Physicochemical quality indexes

Table 2 presents the list of physicochemical analyses performed on the pot honey samples [24–27]. Moisture content was measured by refractometry (AOAC 969.38B). pH was determined potentiometrically using a calibrated pH meter, and total acidity was measured by titration with 0.05 M NaOH and expressed as meq/kg (AOAC 962.19). Electrical conductivity was measured at 20 °C using a conductivity meter (IHC, 2009). Diastase activity was determined spectrophotometrically according to AOAC method 958.09 and expressed as Schade units. Hydroxymethylfurfural (HMF) content was quantified by UV–Vis spectrophotometry following AOAC method 980.23. Insoluble solids were determined gravimetrically according to IHC procedures. Total and reducing sugars were quantified using the Lane–Eynon volumetric method (AOAC 920.183 and 920.184). Reducing sugars were determined before hydrolysis, while total sugars were measured after acid inversion. Non-reducing sugars were calculated by difference. Results were expressed as g/100 g of honey.

Table 2. Applied methodologies for physicochemical and microbiological analysis

Analysis	Units	Method	Analytical technique
pH	Adimensional	IHC [11]	Potentiometry
Total acidity	meq/kg	AOAC 962.19 [24]	Volumetry
Electrical conductivity	µS/cm	IHC [11]	Conductimetry
Diastase activity	Schade Units	AOAC 958.09 [24]	Spectrophotometry UV-VIS
Hydroxymethylfurfural	mg/kg	AOAC 980.23 [24]	Spectrophotometry UV-VIS
Ash and Minerals (Mg, Mn, K, Na, Zn)	g/100 g	AOAC 928.181 [24]	Gravimetry. Atomic absorption spectrometry
Insoluble solids	g/100 g	IHC [11]	Gravimetry
Moisture	g/100g	AOAC 969.38B [24]	Refractometric
Total and reducing sugars	g/100 g	AOAC 920.183; 920.184 [24]	Gravimetric - Volumetric
Color	mm Pfund	Pfund colorimeter [25]	Pfund colorimetry
Antioxidant activity	mmol Trolox/kg	TEAC (Trolox equivalent antioxidant activity) [26]	Spectrophotometry
Total phenolics	mg caffeic acid/100 g	Folin Ciocalteu [26]	Spectrophotometry
Organic acids profile	mg/100g	AOAC 986.13 [24]	HPLC
Copper (Cu), Chrome (Cr), Cadmium (Cd), Lead (Pb)	mg/kg	AOAC 999.11[24]	Atomic absorption
Arsenic (As)	mg/kg	AOAC 986.15[24]	Atomic absorption spectrometry. Hydride generator.
Mercury (Hg)	mg/kg	AOAC 977.15 [24]	Atomic absorption spectrometry. Graphite furnace.
Aerobic mesophiles	log(CFU/g)	ISO 4833-1-2013	Plate count

Analysis	Units	Method	Analytical technique
Lactic acid bacteria	log(CFU/g)	ISO 15124:1998	Plate count
Yeast and molds	log(CFU/g)	ISO 21527-1,2:2008	Plate count
Total and fecal coliforms	MPN/g	ISO 4831:2006	Most probable number
Clostridium sulfite reducing spores	log(CFU/g)	ISO 7937:2004	Plate count
Salmonella sp.	Presence / Absence in 25g	ISO 6579-1:2017	Culture detection

Organic acids were quantified by high-performance liquid chromatography (HPLC) following AOAC method 986.13. Honey samples were filtered prior to analysis, and organic acids were identified and quantified using external standards. Results were expressed as mg/100 g of honey. Total phenolic content was determined using the Folin–Ciocalteu method and expressed as mg caffeic acid equivalents per 100 g of honey. Antioxidant activity was evaluated using the Trolox Equivalent Antioxidant Capacity (TEAC) assay, and results were expressed as mmol Trolox/kg of honey. Color was measured using a Pfund colorimeter and expressed in mm Pfund.

Ash content was determined gravimetrically. Mineral content (Mg, Mn, K, Na, Zn) and heavy metals (Cu, Cr, Cd, Pb) were analyzed by atomic absorption spectrometry following AOAC methods 928.181, 979.23, and 999.11. Arsenic and mercury were determined using hydride generation and graphite furnace atomic absorption techniques, respectively. Results were expressed as mg/kg.

2.3 Microbiological quality

Microbiological analyses of the pot honey samples were performed 2according to ISO standards (Table 2). Aerobic mesophilic microorganisms, lactic acid bacteria, molds and yeasts were quantified by plate count methods and expressed as log (CFU/g). Total and fecal coliforms were determined using the most probable number (MPN) method. The presence of Salmonella spp. was evaluated in 25 g samples using culture-based detection.

2.4 Heat treatments

To evaluate the effect of pasteurization on the microbiological load, selected pot honey samples were subjected to heat treatment at 80°C for 5 minutes, followed by rapid cooling in an ice bath until reaching 4°C. The samples were then stored at this temperature until they underwent the same microbiological analyses outlined in Table 2.

A second group of samples was analyzed for microbiological safety indicators and lactic acid bacteria content. These samples were subjected to heat treatment at 65°C for 3 minutes. The effect of this treatment on microbiological indicators, antioxidant activity, phenolic compound content, ascorbic acid content, and color was assessed using the corresponding methodologies presented in Table 2.

2.5 Statistical analyses

Mean and standard deviation were calculated when the number of samples was sufficient for statistical evaluation. Ranges (minimum – maximum) are presented on each variable to provide a clearer description of the results.

3. RESULTS

3.1 Physicochemical characterization

The physicochemical properties of the analyzed pot honey samples are summarized in Tables 3 and 4. Moisture content ranged from 20.8% to 40.5%, with an average of 26.6%, a characteristic commonly observed in stingless bee honey. Acidity levels were notably high, with an average of 49.59 meq/kg and pH values ranged from 3.22 to 4.63.

Table 3. Physicochemical analysis results of pot honey from stingless bee species of Colombian Amazon piedmont

Bee species	*Melipona eburnea*	*Melipona grandis*	*Melipona nebulosa*	*Tetragonisca angustula*	*Lestrimelitta* sp.	*Scaptotrigona* sp.	*Tetragona* sp.
Moisture content (g/100 g)	26.8 ± 2.4 [20.8 - 30.6] (12)	27.7 ± 2.5 [21.1 – 27.8] (7)	28.3± 2.0 [25.3 – 30.3] (3)	23.3 ± 0.5 [22.9 – 24.1] (3)	40.5 (1)	23.9 (1)	30.3 (1)
Acidity (meq NaOH/kg)	55.39 ± 12.86 [28.20 - 78.21] (12)	36.62 ± 15.84 [20.40 -65.11] (7)	45.10 ± 11.80 [27.40 - 60.10] (3)	64.60 ± 18.07 [47.79 - 91.70] (3)	78.21 (1)	18.80 (1)	20.30 (1)
pH	3.61 ± 0.26 [3.26 - 4.10] (12)	3.58 ± 0.50 [3.22 - 4.63] (7)	3.81 ± 0.20 [3.65 - 4.12] (3)	3.90 ± 0.14 [3.80 - 4.11] (3)	3.32 (1)	3.40 (1)	3.42 (1)
Diastase number (DN)	2.17 ± 0.91 [0.44 - 4.46] (12)	1.93 ± 1.11 [0.28 - 3.24] (7)	2.18 ± 0.62 [1.25- 2.87] (3)	19.45 ± 5.96 [12.57- 28.40] (3)	1.81 (1)	2.92 (1)	8.01 (1)
HMF (mg/kg)	18.68 ± 16.16 [3.58 – 46.00] (12)	24.80 ± 16.27 [5.00 - 52.0] (7)	27.43 ± 17.43 [1.28 - 46.00] (3)	27.79 ± 2.14 [21.74 - 27.00] (3)	102.44 (1)	4.00 (1)	24.00 (1)
Insoluble solids (g/100 g)	0.19 ± 0.15 [0.01 -0.63] (12)	0.25 ± 0.19 [0.06 – 0.49] (7)	0.19 ± 0.19 [0.01 – 0.47] (3)	0.21 ± 0.03 [0.17 – 0.24] (3)	0.64 (1)	0.63 (1)	0.34 (1)
Reducing sugars (g/100 g)	51.32 ± 2.90 [45.64 – 59.95] (8)	55.62 ± 2.43 [51.32 – 58.89] (4)	45.12 (1)	42.42 ± 1.01 [41.41 – 43.43] (2)	49.32 (1)	N.Q.	N.Q.
Total sugars (g/100 g)	63.04 ± 3.21 [58.59 - 68.52] (8)	63.53 ± 7.80 [55.89- 71.35] (4)	66.36 (1)	57.86 ± 9.12 [48.74 – 66.98] (2)	65.07 (1)	N.Q.	N.Q.
Sucrose* (g/100 g)	11.72 ± 2.61 [7.39 – 16.66] (8)	7.91 ± 5.92 [1.59 - 13.38] (4)	21.24 (1)	15.45 ± 10.13 [5.32 - 25.58] (2)	15.75 (1)	N.Q.	N.Q.
Conductivity (µS/cm)	1279 ± 828 [212-2948] (12)	856 ± 689 [200 - 2020] (7)	1230 ± 746 [450 - 2349] (3)	4029 ± 2590 [144 - 6946] (3)	8832 (1)	164 (1)	130 (1)
Ashes (g/100 g)	0.13 ± 0.09 [0.03 - 0.50] (12)	0.06 ± 0.05 [0.01 - 0.15] (7)	0.09 ± 0.04 [0.03 - 0.14] (3)	0.15 ± 0.16 [0.03 - 0.39] (3)	0.03 (1)	0.39 (1)	0.56 (1)
Mg (mg/kg)	0.86 ± 0.82 [<LOQ – 1.90] (8)	0.65 ± 0.32 [0.20 - 0.90] (4)	1.10 (1)	4.70 ± 3.10 [1.06 – 7.80] (3)	11.90 (1)	N.Q.	N.Q.
Mn (mg/kg)	2.43 ± 3.92 [<LOQ – 10.88] (8)	4.73 ± 4.00 [0.65 – 8.15] (4)	3.05 (1)	3.06 ± 0.80 [2.25 – 3.86] (2)	7.83 (1)	N.Q.	N.Q.
K (mg/kg)	65.53 ± 53.32 [6.31 - 216.28] (8)	32.44 ± 32.83 [11.53 – 68.99] (4)	68.18 (1)	154.96 ± 37.68 [117.3 - 192.6] (2)	165.21 (1)	N.Q.	N.Q.
Na (mg/kg)	5.65 ± 2.16 [1.17 - 8.28] (8)	5.30 ± 5.90 [1.52 - 12.3] (4)	5.70 (1)	17.48 ± 1.74 [15.74 - 19.22] (2)	7.16 (1)	N.Q.	N.Q.
Zn (mg/kg)	0.06 ± 0.02 [<LOQ - 0.10] (8)	0.06 ± 0.02 [<LOQ - 0.08] (4)	0.12 (1)	0.14 ± 0.05 [0.09 - 0.18] (2)	0.2 (1)	N.Q.	N.Q.
Total phenolic compounds (mg caffeic acid/100 g)	16.7 ± 6.6 [6.8 – 20.6] (4)	9.5 ± 7.8 [3.7 – 18.3] (3)	[8.4 – 9.5] (2)	29.6 *(1)*	N.Q.	7.0 *(1)*	56.6 *(1)*
TEAC (mmol Trolox/kg)	0.63 ± 0.49 [0.50 - 1.20] *(12)*	0.47 ± 0.31 [0.20 - 1.15] (7)	0.68 ± 0.25 [0.40 - 0.85] *(3)*	1.65 ± 0.45 [1.20 - 2.10] *(3)*	0.95 (*1*)	0.30 (*1*)	3.10 (*1*)

For species with $n \geq 2$. values of are presented as mean ± standard deviation [range] (*n*). where *n* is the number of samples. HMF: hydroxymethylfurfural; TEAC: Trolox equivalent antioxidant capacity; *LOQ*: limit of quantification; *LOQ* for each metal = 0.04 mg/kg; NQ: Not Quantified.

*Apparent sucrose quantified as non-reducing sugars.

Table 4. Organic acids profile of pot honeys from stingless bee species of Colombian Amazon piedmont

Bee species	Citric acid (mg/100 g)	Gluconic acid (mg/100 g)	Tartaric acid (mg/100 g)	Ascorbic acid (mg/100 g)	Succinic acid (mg/100 g)	Lactic acid (mg/100 g)	Acetic acid (mg/100 g)
Melipona eburnea	*<LOQ (12)*	260.3 ± 136.8 [146.3 - 459.2] *(4)*	37.0 ± 36.3 [*<LOQ* - 78.5] *(12)*	146.9 ± 52.5 [96.7 – 223.8] *(12)*	336.4 ± 651.0 [*<LOQ* - 2230.6] *(12)*	238.5 ± 250.6 [*<LOQ* - 702.5] *(12)*	83.4 ± 83.8 [*<LOQ* - 306.4] *(12)*
Melipona grandis	*<LOQ (7)*	221.1 ± 111.0 [94.7 – 302.3] *(3)*	3.2 ± 5.2 [*<LOQ* – 14.1] *(7)*	171.6 ± 54.9 [118.8 – 233.7] *(7)*	282.8 ± 276.3 [*<LOQ* - 777.1] *(7)*	173.6 ± 89.4 [*<LOQ* – 241.1] *(7)*	25.3 ± 35.8 [*<LOQ* - 93.6] *(7)*
Melipona nebulosa	*<LOQ (3)*	304.0 ± 199.8 [162.7 - 445.3] *(2)*	*<LOQ (3)*	183.0 ± 50.4 [124.9 – 214.5] *(3)*	18.0 ± 30.3 [*<LOQ* - 53.0] *(3)*	346.2 ± 227.1 [92.4 – 530.3] *(3)*	86.6 ± 27.7 [55.4 – 108.16] *(3)*
Tetragonisca angustula	192.5 ±203.0 [*<LOQ* – 404.9] *(3)*	1314,5 *(1)*	*<LOQ (3)*	123.2 ± 47.7 [92.0 - 178.1] *(3)*	415.0 ± 359.5 [*<LOQ* - 641.1] *(3*	265.9 ± 359.5 [*<LOQ* – 675.0] *(3)*	192.6 ± 114.2 [80.1 - 308.4] *(3)*
Scaptotrigona sp.	*<LOQ (1)*	301.0 *(1)*	*<LOQ (1)*	213.9 (1)	*<LOQ (1)*	216.5 *(1)*	71.9 *(1)*
Tetragona sp.	*<LOQ (1)*	695.1 *(1)*	*<LOQ (1)*	91.4 *(1)*	*<LOQ (1)*	2836.5 *(1)*	828.4 *(1)*
Lestrimelitta sp.	*<LOQ (1)*	NQ *(1)*	410.0 *(1)*	47.1 *(1)*	11920.0 *(1)*	3119.9 *(1)*	1754.5 *(1)*

(n): number of samples. For species with *n*>2, values are presented as mean ± standard deviation [range]. *LOQ:* Limit of quantification. *LOQ* for each acid: 0.5mg/100g. NQ: Not Quantified

Diastase enzyme activity varied significantly among samples, with values ranging from 0.28 to 28.40 Schade units, much lower than the minimum limit established for conventional honey (8 Diastase Units). As this enzyme is produced by bees to hydrolyze starch residues, its activity depends on both the flora and the bee species. For this reason, diastase activity is often used as an indicator of honey authenticity. In contrast, HMF levels were generally low, averaging 24.56 mg/kg, indicating freshness. Insoluble solids showed considerable variability, with approximately half of the honey samples exceeding 0.1%.

The total sugar content averaged 62.85 g/100g meanwhile reducing sugars ranged from 42 to 55 g/100 g. Non-reducing sugar levels varied notably among species. In half of the samples, the reducing sugar content was below the minimum value established for *Apis mellifera* honey (65%), which correlates with the higher moisture content observed.

The organic acid profile revealed the presence of citric, gluconic, tartaric, succinic, ascorbic, lactic and acetic acids. These acids are related to their floral and entomological origin, giving the honeys an identity with the Amazonian Piedmont, in addition to being related to the functional and therapeutic properties traditionally recognized in these honeys. The presence of lactic acid suggests potential probiotic benefits, while ascorbic acid may contribute antioxidant properties. The types of organic acids identified are related to the diversity of floral sources in the region and may also indicate the enzymatic activity of the bees, which convert sugars into organic acids characteristic of both the nectar and bee species.

Ash content was below 0.6% which, compared to previous studies [9,13], is under the limit established for *Apis mellifera* honey. Among the minerals, potassium was the most abundant. However, the low mineral content could be attributed to careful sampling practices that minimized soil contamination, a common factor contributing to higher electrical conductivity. Despite this, conductivity values were higher than those typically found in *Apis mellifera* honey, likely due to the presence of organic acids and possibly amino acids. Finally, the total phenolic content ranged from 3.7 to 56.6 mg/100g, with higher concentrations observed in pot honey from *Tetragonisca angustula* and *Tetragona* sp.

3.2 Heavy metals

Table 5 shows the results for heavy metals measured in the honey samples. All analyzed samples met food safety standards, with no detectable levels of cadmium, lead, arsenic, or mercury (Pb: 0.3 mg/kg; Cd: 0.05 mg/kg; As: 0.1 mg/kg; Hg: 0.01 mg/kg). However, trace amounts of copper and chromium were detected, suggesting minimal environmental exposure.

Table 5. Ranges of variation of heavy metals in pot honeys from stingless bee species of Colombian Amazon piedmont

Bee species	Cu (mg/kg)	Cr (mg/kg)	Cd (mg/kg)	Pb (mg/kg)	As (mg/kg)	Hg (mg/kg)
Melipona eburnea	<0.25 - 0.658 (*12*)	<0.5 (*12*)	<0.023 - 0.077 (8)	<0.025 *(8)*	<0.05 *(8)*	<0.25 *(8)*
Melipona grandis	<0.25 - 0.752 (7)	<0.5 (*7*)	<0.023 - 0.068 (4)	<0.025 (4)	<0.05 (*4*)	<0.25 (4)
Melipona nebulosa	<0.25 (*3*)	<0.5 (*3*)	0.033 (1)	<0.025 (*1*)	<0.05 (*1*)	<0.25 (*1*)
Tetragonisca angustula	<0.058 - 1.0 (*3*)	<0.25 (*3*)	<0.023 (*2*)	<0.025 (*2*)	<0.05 *(2)*	<0.25 (*2*)
Scaptotrigona sp.	<0.25 (*1*)	<0.5 (*1*)	N.Q.	N.Q.	N.Q.	N.Q.
Tetragona sp.	<0.25 (*1*)	<0.5 (*1*)	N.Q.	N.Q.	N.Q.	N.Q.
Lestrimelitta sp.	0.575 (*1*)	<0.25 (*1*)	<0.023 (*1*)	<0.025 (*1*)	<0.05 (*1*)	<0.25 (*1*)

Concentration of heavy metals is presented as variation range (*n*). where *n* is the number of samples. N.Q. not quantified due to insufficient sample.

3.3 Microbiological quality and effect of heat treatments on microbial loads and bioactive compounds

Table 6 presents the results of the microbiological evaluation of stingless bee honey samples after extraction, filtration, and packaging in glass jars, both before and after thermal treatment at 80°C for 5 minutes. In raw pot honey, no pathogenic microorganisms, such as heat-resistant (fecal) coliforms, *Clostridium perfringens*, or *Salmonella*, were detected. Additionally, it is shown microbiological data for samples subjected to a milder heat treatment (65°C for 3 minutes), both before and after processing. All samples complied with safety standards for fecal coliforms, sulfite-reducing *Clostridium* and *Salmonella*. Some of the samples initially contained molds or yeasts above acceptable food safety limits. However, some honeys exhibited high aerobic mesophilic bacteria before treatment, which correlated with the presence of lactic acid bacteria. Finally, Table 7 presents data on antioxidant capacity, total phenolic content, ascorbic acid, and color in honeys before and after heat treatment at 65°C for 3 minutes. These results suggest that milder heat treatment better preserved the bioactive properties of the honey while still ensuring microbiological safety.

Table 6. Microbiological content of selected pot honey samples before and after thermal treatment at 65°C, 3 min and 80°C, 5 min

Bee species	Type of hive*	Aerobic mesophiles log(CFU/g)		Molds and Yeasts log(CFU/g)		Total coli-forms MPN/g		Fecal coli-forms MPN/g		*Clostridium* sulfite reducer log(CFU/g)		*Salmonella* Presence or Absence in 25g		Lactic Acid Bacteria log(CFU/g)	
		Thermal treatment 65°C, 3 min													
		Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
Melipona grandis	T	< 1,0	< 1.0	< 1.0	< 1.0	<3	<3	<3	<3	< 1.0	< 1.0	Absence	Absence	< 1.0	< 1.0
Melipona grandis	T	3.1	3.3	< 1.0	< 1.0	<3	<3	<3	<3	< 1.0	< 1.0	Absence	Absence	6.4	3.9
Melipona grandis	T	3.5	< 1.0	< 1.0	< 1.0	<3	<3	<3	<3	< 1.0	< 1.0	Absence	Absence	5	< 1.0
Melipona eburnea	T	< 1.0	< 1.0	< 1.0	< 1.0	<3	<3	<3	<3	< 1.0	< 1.0	Absence	Absence	< 1.0	< 1.0
Melipona eburnea	T	4.7*	3.3	< 1.0	< 1.0	<3	<3	<3	<3	< 1.0	< 1.0	Absence	Absence	5.8	3.3
Melipona eburnea	T	4.1*	< 1.0	< 1.0	< 1.0	<3	<3	<3	<3	< 1.0	< 1.0	Absence	Absence	5.4	< 1.0
Melipona eburnea	T	4.2*	1.7	< 1.0	< 1.0	<3	<3	<3	<3	< 1.0	< 1.0	Absence	Absence	4.9	< 1.0
Tetragonisca angustula	R	2	2.1	< 1.0	< 1.0	<3	<3	<3	<3	< 1.0	< 1.0	Absence	Absence	< 1.0	< 1.0
Melipona nebulosa	T	2.9	2.1	< 1.0	< 1.0	<3	<3	<3	<3	< 1.0	< 1.0	Absence	Absence	4	< 1.0
Melipona nebulosa	T	2.3	2.2	< 1.0	< 1.0	<3	<3	<3	<3	< 1.0	< 1.0	Absence	Absence	< 1.0	< 1.0
Tetragona sp	T	2.3	2.8	< 1.0	< 1.0	<3	<3	<3	<3	< 1.0	< 1.0	Absence	Absence	< 1.0	< 1.0
Scaptotrigona sp	R	3.3	3.8	< 1.0	< 1.0	<3	<3	<3	<3	< 1.0	< 1.0	Absence	Absence	6.4	< 1.0
		Thermal treatment 80°C, 5 min													
		Before	After	Before	After	Before	After	Before	After	Before	After	Before	After		
Melipona grandis		2,7	< 1,0	2,6*	< 2,0	<3	<3	<3	<3	< 1,0	< 1,0	Absence	Absence		
Melipona grandis		3,0	< 1,0	< 2,0	< 2,0	<3	<3	<3	<3	< 1,0	< 1,0	Absence	Absence		
Melipona grandis		3,1	< 1,0	2,4*	< 2,0	<3	<3	<3	<3	< 1,0	< 1,0	Absence	Absence		
Melipona grandis		< 1,0	< 1,0	< 1,0	< 1,0	<3	<3	<3	<3	< 1,0	< 1,0	Absence	Absence		
Melipona eburnea		2,7	2,4	< 2,0	< 2,0	<3	<3	<3	<3	< 1,0	< 1,0	Absence	Absence		
Melipona eburnea		2,9	< 1,0	2,7*	< 2,0	<3	<3	<3	<3	< 1,0	< 1,0	Absence	Absence		
Melipona eburnea		3,8	< 1,0	< 1,0	< 1,0	<3	<3	<3	<3	< 1,0	< 1,0	Absence	Absence		
Melipona eburnea		4,0	< 1,0	< 1,0	< 1,0	<3	<3	<3	<3	< 1,0	< 1,0	Absence	Absence		
Melipona eburnea		3,9	< 1,0	< 1,0	< 1,0	<3	<3	<3	<3	< 1,0	< 1,0	Absence	Absence		
Melipona eburnea		3,6	3,8	< 1,0	< 1,0	<3	<3	<3	<3	< 1,0	< 1,0	Absence	Absence		
Melipona eburnea		1,8	1,8	< 1,0	< 1,0	<3	<3	<3	<3	< 1,0	<1,0	Absence	Absence		
Melipona eburnea		< 1,0	< 1,0	< 1,0	< 1,0	<3	<3	<3	<3	< 1,0	< 1,0	Absence	Absence		
Tetragonisca angustula		3,1	2,0	2,9*	< 2,0	<3	<3	<3	<3	< 1,0	< 1,0	Absence	Absence		
Tetragonisca angustula		3,5	2,3	4,1*	< 2,0	<3	<3	<3	<3	< 1,0	< 1,0	Absence	Absence		
Melipona nebulosa		4,0	1,7	< 1,0	< 1,0	<3	<3	<3	<3	< 1,0	< 1,0	Absence	Absence		
Lestrimelitta sp		2,5	2,3	< 1,0	< 1,0	<3	<3	<3	<3	< 1,0	< 1,0	Absence	Absence		

*It does not meet the maximum CFU recommended for honey by NMX-036-NORMEX-2006 NORMEX (Mexican Society for Standardization and Certification S.C) [78].

Table 7. Antioxidant capacity, total phenolic compounds and ascorbic acid content in honeys after applying heat treatment at 65°C, 3 minutes

Bee species	TEAC (mmol trolox/kg)		Total Phenolic Compounds (mg caffeic acid/100 g)		Ascorbic acid (mg/100g)		Color (Pfund scale)	
	Before	After	Before	After	Before	After	Before	After
Melipona grandis	0.3	0.4	6.4	8.1	241.7	233.7	38	45
Melipona grandis	0.4	0.4	3.7	20.2	221.8	225.7	19	22
Melipona grandis	0.4	0.3	18.3	18.7	210.3	230.5	24	24
Melipona eburnea	0.5	0.4	6.8	25.5	210.1	216.5	42	39
Melipona eburnea	0.5	0.4	20.6	18.9	213	208.1	11	10
Melipona eburnea	1.2	0.6	19.4	16.4	233.9	219.9	74	54
Melipona eburnea	0.8	0.8	20.1	13.6	215.9	223.8	41	42
Tetragonisca angustula	1.2	1.3	29.6	32.2	171.9	178.1	82	65
Melipona nebulosa	0.4	0.4	9.5	9.3	64.9	209.7	42	42
Melipona nebulosa	0.8	0.6	8.4	35.1	217.1	214.5	40	42
Tetragona sp	3.1	1.7	56.6	67.8	107.	91.4	136	128
Scaptotrigona sp	0.3	0.1	7.0	6.2	203.9	213.9	39	13

4. DISCUSSION

4.1 Physicochemical characterization

4.1.1 Moisture

The high moisture levels observed in the analyzed honey samples align with previous studies on Colombian honey from other regions [9,13] and honey from other countries such as Australia [28], Malaysia, Cuba, Nigeria, Brazil, Peru and Portugal [29,30]. These values differ markedly from those of honey produced by *Apis mellifera* and are characteristic of pot honey. This can be primarily explained by the smaller surface area to volume ratio available for water evaporation inside stingless bee hives. Honey pots in stingless bee hives present a surface area of 2.5 cm^2/cm^3, whereas *Apis mellifera* cells have 12 cm^2/cm^3 [31]. Additionally, the tropical rainforest climatic of the Colombian Amazon Piedmont, with relative humidity ranging from 80 to 98%, and temperatures above 25°C, creates a very low moisture transfer gradient, limiting water removal from nectar [3,8]. High moisture affects various characteristics of honey, especially its tendency to crystallize and its stability. Management throughout the entire production process must prevent deterioration; therefore, the transition from traditional to modern hives should be promoted, along with the implementation of good handling practices to avoid cross-contamination during honey extraction and packaging. Refrigeration is recommended for storage.

The current Codex Alimentarius [10] establishes 20% as the maximum limit for the moisture content of honey, a requirement that is not possible to meet for stingless bee honeys, therefore it is necessary to establish specific requirements. Only one of the samples presented an unusual humidity level, exceeding 40%, but this is consistent with reports for some Peruvian honeys that have shown values ranging between 40 and 45% [30]. Some researchers have proposed and evaluated the application of dehumidification processes in these cases [15].

4.1.2 Acidity and pH

The results obtained in this study show high levels of acidity and low pH values for all samples analyzed. This is consistent with previous studies on honeys from various stingless bee species in other regions of Colombia [9,13,32] and Mexico [31], but the concentrations are approximately 50% higher, which affects the noticeably acidic taste perceived. Honeys from *Melipona eburnea* and *Tetragonisca angustula* exhibit the highest average acidity values, with 55.39 ± 12.86 meq NaOH/kg and 64.60 ± 18.07 meq NaOH/kg, respectively. This fact indicates a different chemical and biological contribution from the bee's digestive tract depending on the species, triggering different reactions in the floral nectar. According to studies conducted by some

researchers [18], although palynological analysis shows the same floral origin for honey from *Apis mellifera* and stingless bees, the acidity and pH differ considerably between them. This provides a differentiating factor that suggests the acidic components are not only contributed by the nectar, but that the stingless bee also contributes acidity during the transport of nectar in its honey stomach to the hive, either through direct addition or through fermentative processes. The high acidity may also explain the antimicrobial activity commonly reported for stingless bee honeys [33], which is linked to the empirical and traditional therapeutic uses of these honeys. The flora of the Colombian Amazon Piedmont, influenced by both the Andes and the Amazon Basin, may contribute to a unique nectar composition, which could be explored as a sustainable marker of geographic identity in the future.

4.1.3 Diastase index

Despite the low values observed in this study, the presence of diastase is significant, as it serves as a measurable parameter for assessing honey authenticity. Most species displayed DN values below the *Apis mellifera* standard of 8, except for *Tetragonisca angustula,* which showed a mean DN value of 19.45, suggesting high intrinsic amylase production, which may indicate a capacity of these species to provide certain levels of this enzyme (alpha amylase) or probably the variability of the starch contents in the nectars collected by this species, considering that this enzyme is only provided by the bee, not by the nectar [32,34,35]. This is consistent with literature indicating that stingless bee honey naturally possesses low diastase activity, likely due to biological and ecological differences [13]. This suggests that any starches present in the nectar are being degraded, a process that can be further analyzed by measuring dextrin residues in honey samples. Bolivian pot honey from species *Tetragonisca angustula*, *Scaptotrigona polysticta*, and *Scaptotrigona depilis* showed DN values above 3 [36], while *Melipona eburnea* pot honey collected in Colombia by Zapata-Vahos et al. [37] had no detectable activity, contrasting with our findings.

4.1.4 Hydroxymethylfurfural (HMF)

The Codex Alimentarius establishes a maximum value of 40 mg/kg for *Apis mellifera* honey, but allows 80 mg/ for honeys produced in the tropics [10], recognizing that latitude, temperature, and ambient humidity affect this parameter, which indicates sugar degradation. Based on this, most samples assessed in this study had low HMF levels, indicating that the honey was fresh, not stored for extended periods, and not exposed to high temperatures. The differences between species and within the same species are due to the nature of the nectar collected in terms of the type and concentration of sugars present. These results are consistent with previous studies on Colombian pot honey [9,13,32], in the Peruvian Amazon [38], in Mexico [29,39], in Tanzania [40], and in Nigeria [41]. However, one sample exhibited high HMF content, which may indicate greater susceptibility to elevated ambient temperatures. Studies conducted in Brazil for Melipona subnitida and Melipona scutellaris and in Mexico for Melipona beecheii did not detected HMF for any sampled pot honey [31,42]. The high value obtained for *Lestrimelitta* sp. is very particular and it should be further revised to determine whether it is a species-specific characteristic, especially since Lico et al. [43] reported a mean HMF value of just 0.7 mg/kg for Brazilian pot honey samples from *Lestrimelitta limao*. However, the scarcity of reported data of this species in literature limited the availability of additional information for comparison.

4.1.5 Insoluble Solids

The average values found for insoluble solids in the honeys studied according to the species are quite high, with great variability within the same species, from values very similar to those reported by other researchers and those established for *Apis mellifera*, on the order of 0.01% [35], to values higher than 0.5%. For reference, Aroucha et al. [44] reported 0.62% insoluble solids in dehydrated *Melipona subnitida* pot honey from Brazil. Few studies have studied the insoluble solids content of stingless bees' honey; in order to make comparisons it is necessary to take into account the production method used, including the type of hive (rustic or technological) and the extraction technique applied (draining, syringe, compression). While it is normal for honey to contain traces of propolis and wax, elevated insoluble solids content can result from rustic hive practices and inadequate extraction, filtration, and packaging. To address this issue, it is essential to enhance training for producers to ensure honey quality in this regard. To avoid high levels of insoluble solids, it is also necessary to use technologically advanced hives if honey is intended for commercialization.

4.1.6 Sugar content

Pot honey is predominantly composed of reducing sugars; however, it exhibits high variability in non-reducing sugar content, mainly disaccharides, which exceeded the minimum levels established for *Apis mellifera* honey. This variability can be attributed to the diversity of floral sources, geographical origin, and the bee species included in this study. Fuenmayor et al. [9] described the monosaccharide and disaccharide content of pot honey from over 15 Colombian stingless bee species, with fructose levels between 17.1 and 40 g/100g, glucose between 9.3 and 38.5 g/100 g, and the combined content of sucrose and maltose between 0.9 and 12.1 g/100 g. Similarly, Vit et al. [45] found reducing sugar levels in Ecuadorian pot honey ranging from 16.24 to 63.38 g/100 g for the species *Trigona fuscipennis, Melipona mimetic* and *Scaptotrigona ederi*.

As this is an initial approach to assess the sugar content of pot-honey from the Colombian Amazon Piedmont, the Lane-Eynon method was used. This volumetric test employs Fehling's reagent to quantify reducing and total sugars, relying on the reducing capacity of monosaccharides before and after acid hydrolysis. Non-reducing sugars are then calculated by difference, assuming that primarily correspond to sucrose [11]. However, in this case, when total sugars are added to the moisture content, the sum does not approach 100%, as typically observed in *Apis mellifera* honey.

This discrepancy may be explained by the presence of oligosaccharides, higher molecular weight carbohydrates, within both the reducing and non-reducing fractions. Such compounds have already been reported in honey [46]. Some oligosaccharides are more resistant to acid and enzymatic hydrolysis and have been associated with the prebiotic potential of honey [47]. Additionally, the antioxidant properties of honey may interfere with the redox reaction on which the method is based. One of the limitations of the Lane-Eynon method is that the redox reaction (sugar–Fehling reagent) is not stoichiometric, which would require the development of specific calibration curves to improve accuracy [48]. Some authors opt for more specific methods such as high-performance liquid chromatography (HPLC) with prior solid-phase extraction, to characterize the sugar composition in honey [49,50]. Oligosaccharides found in honey have been linked to anti-diabetic properties [51,52] and demonstrated prebiotic effects [53,54]. Native bees in the Amazonian ecosystem forage from both floral and non-floral sources that remain largely unexplored regarding their carbohydrate profiles. Nonetheless, the presence of such carbohydrates is suspected by the wide range of viscosities and consistencies observed in these types of honey, despite similar moisture contents.

Future studies should apply advanced analytical methods, such as chromatography, to establish a detailed profile of monosaccharides, disaccharides, and oligosaccharides present. Chromatographic analysis could also detect the presence of enzymes and dextrins in this type of honey and investigate the relationship with perceived sweetness intensity.

4.1.7 Organic Acid Profile

No previous reports exist on the organic acid profile of stingless bee honey in Colombia, and reports from other countries are generally scarce. Organic acids are important in honey because they contribute to its identity, allowing differentiation based on botanical origin or bee species [18,55]. Although Colombia lacks reports on stingless bee honey organic acid profiles, in Australia over 30 non-aromatic organic acids have been identified in honey [55], contributing to its diverse flavors and aromas, and may suggest a strong connection to the botanical origin of the nectar. This aspect warrants further exploration through palynological studies and empirical information from producers regarding the floral routes followed by foraging bees. Similarly, acetic, gluconic, and lactic acids were found in Brazilian pot honey from the species *Melipona bicolor*, *Scaptotrigona bipunctata*, *Melipona quadrifasciata*, and *Melipona marginata* [56].

In the present study, citric acid was found in *Tetragonisca angustula* honey, with high variability, reaching levels of 409.9 mg/100g. This indicates that citric acid may serve as a marker of honey origin, potentially linked to bee species in the Colombian Amazon Piedmont. Additionally, this compound is associated with both the flavor and antimicrobial activity of honey.

Gluconic acid, the primary contributor to honey acidity, was found in all the honeys analyzed. Gluconic acid is produced by the action of glucose oxidase, an enzyme produced by bees. The results showed relatively high concentrations with significant variability, ranging from 94.7 mg/100g to 1324.6 mg/100g, with an average of 385.3 mg/100g ± 228.8 mg/100g. Similar analyzes conducted on various

stingless bees species in Australia also reported high variability in values, ranging from 159 to 4060 mg/100g. Specifically, *Austroplebeia australis* honey had an average gluconic acid content of 2500 mg/100g ± 870 mg/100g, *Tetragonula hockings*i 1290 mg/100g± 520 mg/100g, and *Tetragonula carbonaria* 1650 mg/100g ± 600 mg/100g [55].

Tartaric acid was not found in the honey samples of all the species studied and was only prominent in honeys from two species: *Melipona eburnea* (37.0 ± 36.3 mg/100g) and *Melipona grandis* (3.2 ± 5.2 mg/100g). Therefore, this acid may be a differentiating parameter linked to the bee species and the environment, giving these honeys from the Colombian Amazonian Piedmont their unique identity. On the other hand, another acid found as a marker in the honeys of five of the studied species is succinic acid, with significant variability among honeys of the same species, reaching concentrations exceeding 5000 mg/100g in some cases. Sensory aspects of these honeys are similar in their astringent taste, a consequence of the presence of succinic acid. This coincides with a study carried out in Malaysia in which this acid, along with gluconic acid, was identified as markers or components that give identity to honeys by their origin [18].

A particularly noteworthy finding in all the studied pot honey samples is the significant presence of ascorbic acid (vitamin C), with concentrations ranging from 47.1 to 233.7 mg/100g. This acid was found in all the analyzed samples and constitutes the first reports for honeys from different species of stingless bees in the Colombian Amazonian Piedmont. This finding allows us to relate it to antioxidant activity and explain the therapeutic properties traditionally attributed to these honeys. Additionally, it may reveal a characteristic related to geographic origin, regardless of the bee species, considering that this acid comes from the collected nectar. Reports of ascorbic acid content in stingless bee honeys from Latin America are scarce. A study conducted in Venezuela reports vitamin C contents of 12.74 and 40.13 mg/100g [57]. In other continents such as Africa, in western Tanzania, the composition of honeys from 6 species (*Dactylurina schmidti*, *Hypotrigona gribodoi*, *Meliponula beccarii*, *Meliponula ferruginea*, *Meliponula togoensis* and *Plebeia armata*) was evaluated and ascorbic acid contents were reported to vary between 7.42 and 60.50 mg/100 g [41], while in Nigeria average values of 161.69 ± 6.70 mg/kg were found [41]. Studies conducted in Asia stand out; for example, Indonesian honeys from *Tetragonula laeviceps* contain 6.49–13.58 mg/100g of ascorbic acid [58], which has been linked to anti-inflammatory effects in malnourished rats. These values are consistent with another study from the same country, using the same bee species, but in different regions, which revealed contents between 5.67 and 7.88 mg/100g [59]. Furthermore, honey from the West Coast of Malaysia has ascorbic acid contents ranging from 1.13 to 6.69 mg/kg [60]. This information demonstrates that the ascorbic acid content of honeys from the Colombian Amazon Piedmont is considerably higher than those previously reported for stingless bee honeys. Our finding, not previously reported in other studies, could help explain some of the therapeutic properties attributed to certain honeys by local farmers, such as anti-flu, anti-inflammatory, and anti-tumor effects. Additionally, it is worth noting that these levels are significantly higher than those found in tropical fruits known for their high vitamin C content, such as the Amazonian açai fruit (*Euterpe Oleracea Mart*), with values around 58.7 mg/100g [2,42], and other tropical fruits recognized as high sources of vitamin C and for their functional properties, which generally range between 40 and 70 mg/100g [61]. In contrast, most of the honeys evaluated in this study have values exceeding 100 mg/100g. Other studies, such as some conducted in Brazil, have not examined ascorbic acid and have not been able to fully explain the antioxidant properties of stingless bee honeys based on other physicochemical properties [62].

In this study, the organic acid profile revealed, for the first time, the presence of lactic acid in Colombian stingless bee honeys, at levels ranging from undetectable to 3119.9 mg/100g. The following average values stand out: *Melipona eburnea* 238.5 ± 250.6 mg/100g; *Melipona grandis* 173.6 ± 89.4 mg/100g; *Melipona nebulosa* 346.2 ± 227.1 mg/100g; and *Tetragonisca angustula* 265.9 ± 359.5 mg/100g. These values are approximately one-third of the lactic acid level found in yogurt, indicating high activity of lactic acid bacteria, as was indeed confirmed by evaluating the presence of these microorganisms in the honeys. This finding is consistent with similar studies conducted in Brazil for Melipona bees, in which, in addition to lactic acid, acetic and citric acids were also found [56]. These results are the first reported for honeys from stingless bees in the Colombian Amazonian Piedmont. The importance of this finding lies in the broad field of research it opens regarding the possibility that the bacteria present in the honeys are probiotic. Currently, there is only empirical

knowledge linking the consumption of certain honeys from the region to benefits for the digestive system, including combating gastritis.

Although many studies have been conducted on the search for lactic acid bacteria in different substrates, few efforts have been made to evaluate lactic acid bacteria in stingless bee products. *Lactobacillus plantarum* and *L. pentosus* have been isolated, their probiotic properties have been developed, and they have been used for encapsulation in the development of functional foods [63].

Acetic acid was not present in all samples, but there was at least one positive sample per studied species, and values ranged between 25.3 mg/100g (in *Melipona grandis*) and 1754.5 mg/100g (in *Lestrimelitta* sp). Similar variability has been reported in studies on stingless bee honey of genus *Geotrigona*, *Melipona* and *Scaptotrigona* from Ecuador, with values ranging from 121 mg/100g to 1960 mg/100g [44]. In Malaysia, honeys from *Heterotrigona itama* and *Tetrigona binghami* averaged 270 mg/100g ± 190 mg/kg and 680 mg/100g ± 340 mg/100g, respectively [64]. The origin of acetic acid can vary: it may come directly from the nectar or it results from honey deterioration caused by acetic bacteria. In the latter case, acetic acid is often associated with the presence of bubbles or foam in honey. This occurs when honey is contaminated with certain yeasts that initially produce ethanol, which is subsequently metabolized by acetic bacteria into acetic acid and carbon dioxide. As a result, foam may become visible inside the container, and over time, pressure can build up, potentially causing the container to burst.

Although organic acids typically constitute less than 1% of honey, they are believed to contribute significantly to its flavor and aroma, often more pronounced than in *Apis mellifera* honey.

4.1.8 Ash, Minerals, and Conductivity

In all honey types analyzed, potassium was the most abundant mineral, ranging from 6.13 to 192.6 mg/kg magnesium (0.2–11.9 mg/kg). Other minerals were found in very low concentrations: magnesium (0.2–11.9), manganese (0.65–10.88 mg/kg), sodium (<17.18 mg/100g) and zinc (<0.18 mg/kg). This mineral profile differs appreciably with findings in *Apis mellifera* honey [65]. As expected, honeys with higher ash content also exhibited elevated concentrations of mineral elements. The considerable variability observed suggests that ash content could serve as a useful criterion for differentiation, as it has been widely employed for *Apis mellifera* honey and other bee products.

Although low electrical conductivity values might be expected, the measured values for all honey samples were relatively high compared to the limit established for common honey (i.e. 0.8 mS/cm). This elevated conductivity is likely related to the high content of organic acids. Although not directly measured in this study, the presence of certain amino acids could also contribute to the higher conductivity levels.

Electrical conductivity has not been widely evaluated in stingless bee honey. In *Tetragonisca angustula*, the recorded values were high (4029 ± 2590 µS/cm), and were similar to those reported for pot honey from Paraná, Brazil [66]. In contrast, the only *Tetragona* sp . sample in this study showed a particularly low conductivity of 130 µS/cm, which is notably low for stingless bee honey. For comparison, the lowest previously reported conductivity for *Scaptotrigona xanthotricha* honey in Brazil was 0.62 µS/cm [66]. Interestingly, *Scaptotrigona* sp. and *Tetragona* sp. have low conductivity despite having high ash content, which may be an indicator of differences in ionic composition or matrix effects. On the other hand, Lestrimelitta sp. showed a distinct mineral and conductivity profile, therefore, further research is required to establish whether this is a species-specific fact.

4.19 Total phenolics and antioxidant activity

The total phenolic content in the analyzed honeys varied significantly among species. The highest concentrations were found in honeys from *Tetragonisca angustula* and *Tetragona* sp., which also exhibited stronger antioxidant activity. These high values are consistent with high ascorbic acid content. Studies conducted in Australia on honeys from *Tetragonula carbonaria* and *Tetragonula hockings* also showed great variability in total phenolic content, a fact that was related to the great variability of the floral sources [28]. Similarly, Brazilian pot honey from 10 different stingless bee species reported to contain relatively high levels of phenolic compounds, ranging from 10.3 to 98.0 mg GAE/ 100 g [67]. Furhermore, research in Indonesia has shown that fresh honey from *Tetragonula laeviceps* acts as an immunomodulator, stimulating lymphocyte proliferation and reducing the production of pro-inflammatory markers in both normal and malnourished rats [58]. These

findings suggest that stingless bee honey may have functional therapeutic applications particularly in addressing childhood malnutrition due to its bioactive and immunomodulatory properties.

Future research should explore the relationship between bioactive properties and mineral composition of stingless bee honey. As demonstrated in studies of *Apis mellifera* honey, the characterization of phenolic profiles and mineral content has proven valuable for botanical classification and geographical traceability [68–70]. Applying similar methodologies to stingless bee honey could provide deeper insights into its functional and medicinal potential while supporting quality control and standardization for efforts for commercial purposes.

In this study, the Trolox Equivalent Antioxidant Capacity (TEAC) method was used to evaluate the antioxidant potential of stingless bee honey from different species, revealing notable variability among samples. Such variation is expected, given that botanical origin, bee species, and environmental conditions significantly influence the accumulation of bioactive compounds in honey. The results indicated that *Tetragona sp.* exhibited the highest TEAC values, suggesting a greater concentration of phenolic compounds and other antioxidants. Likewise, *Tetragonisca angustula* honey demonstrated relatively high TEAC values, reinforcing the idea that bee species and nectar sources play crucial roles in determining antioxidant potential.

Conversely, pot honey from *Melipona grandis* and *Scaptotrigona* sp. displayed lower antioxidant activity, suggesting a reduced content of phenolic and flavonoid compounds in these samples. However, it is important to note that antioxidant capacity is not solely dependent by phenolic content, as other compounds such as ascorbic acid, organic acids, and specific enzymes can also contribute. In this study, high levels of ascorbic acid were detected in several samples, which may have contributed to the relatively high antioxidant activity observed, even when phenolic content was lower.

The impact of heat treatment (65°C for 3 minutes) on antioxidant capacity was also evaluated. For a strong treatment, at 80°C for 5 min, its antioxidant activity is considerably affected, consistent with an appreciable loss of ascorbic acid. But TEAC values remained largely unchanged when the thermal treatment is milder, 65°C for 3 minutes, indicating that the bioactive compounds responsible for antioxidant activity in stingless bee honey are relatively heat-stable under mild processing conditions. From a nutritional and functional perspective, these findings support the potential health benefits of stingless bee honey, particularly in terms of antioxidant defense.

4.2 Heavy metals

Most of the samples analyzed showed no heavy metal contamination, but some of the honeys collected by *Melipona grandis* and *Melipona eburnea* showed relatively high levels of copper, which could be explained by the prevalence of copper sulfate use as a fungicide in agriculture in the region. This aspect requires further investigation. Although studies evaluating heavy metals in native bee honeys are scarce, in Brazil, researchers have chosen to assess not only the specific concentration of each metal in the honey but also to calculate the risk associated with daily consumption of these honeys through trace element analysis, without finding any carcinogenic effects [71]. However, in the same country, the levels of Co, Cr, Mo, Ni, and Pb have been determined in relation to the limits recommended by the World Health Organization (WHO), and it has been found that Cr exceeds these limits. It has been concluded that the analysis of heavy metals in stingless bee honeys allows for the evaluation of a region's environmental health [66]. A review of honeys from urban and industrial areas in Malaysia has shown that honeys from hives located near roads or industrial plants have higher levels of Pb, Cd, and As [72]. The results obtained in this work are of great importance because they reflect the environmental health of the Colombian Amazon Piedmont, which is located near urban areas, rural areas and forests, but not industrial areas; monitoring the heavy metals in the honeys produced there can be a means of monitoring the degree of environmental contamination of the region, in order to take preventive actions.

Stingless bee honey can serve as a bioindicator of environmental contamination, as these bees forage nectar from diverse plants that may accumulate heavy metals from soil, water, and air. During nectar collection and honey production, these metals can be transferred to the final product. The presence and concentration of heavy metals in honey reflect the level of environmental pollution in the areas where the bees forage. Previous studies have demonstrated that stingless bee honey contains variable levels of metals depending on geographic location and environmental conditions, making it a

valuable tool for monitoring contamination across ecosystems [73].

Although the study area is predominantly forested, the region abundance of mineral resources -including oil, gold, copper, zinc, and molybdenum- has driven mining activities at various scales (legal, illegal, and alluvial), often leading to vegetation loss. These activities may contribute to environmental pollution, affecting air, soil, and water, with evident consequences on local flora [74]. Given these concerns, continued monitoring of heavy metals in stingless bee honey could provide important insights into the extent of environmental contamination in this ecologically sensitive region.

4.3 Microbiological quality and effect of heat treatments on microbial loads and bioactive compounds

In our study, from a safety microbiological perspective, all fresh samples collected were found to be free of pathogenic microorganisms, such as heat-resistant (fecal) coliforms, *Clostridium perfringens*, and *Salmonella*, unlike the results of previous studies conducted in Colombia in other geographic regions, which detected significant populations of sulfite-reducing *Clostridium* [21]. Although other studies on microbiological safety of stingless bee honeys are scarce, it has been reported that honeys from native Mexican bees produced at different times of the year *by Tetragonisca fiebrigi* have been affected by contamination with *Escherichia coli*, with a higher incidence in spring than in autumn [75]. Similarly, Mexican honeys from *Tetragonisca angustula* have been found to be 42.85% contaminated with sulfite-reducing *Clostridium* [76]. Food safety issues have also been found in a high percentage of honeys from the arid region of Brazil, where honeys from *Melipona subnitida* showed contamination with pathogenic microorganisms, especially *Clostridium perfringens*, *C. botulinum*, and *Salmonella* [77]. However, honeys from *Meliponula beccarii* in Ethiopia showed very low levels of contamination compared to those found in honeys from *Apis mellifera* [78].

The results of our study are of great importance because they demonstrate that neither in the field, from the sources used by the bees, nor through the handling by the beekeeper during extraction and packaging, has there been any cross-contamination of fecal origin. This can be explained by the fact that the group of producers who provided the samples have received specific training and use utensils and procedures that ensure the safety of the honeys at all stages. Considering that these honeys are empirically used for therapeutic purposes, these results support their suitability for consumption, noting that this is not a characteristic inherent to the bee, but rather a result of the application of good practices throughout the production process.

Regarding the aerobic mesophilic bacteria count, no regulations recommend or establish maximum permissible limits. There is only one non-mandatory standard in Mexico that recognizes that honey can have acceptable quality with a bacterial count of up to 10,000 CFU/g of aerobic mesophilic bacteria, provided it is free of fecal contamination (NMX-036-NORMEX-2006) [78]. This recommendation is consistent with the results obtained in our study. Furthermore, another non-mandatory standard in Mexico establishes that the presence of no more than 1,000 CFU/g of non-pathogenic bacteria and up to 100 CFU/g of yeasts and molds should be acceptable [78]. Based on this suggestion, the honeys evaluated in our study can be considered microbiologically safe. This is related to their high total acidity and low pH, which limit the survival of pathogenic microorganisms. Furthermore, the samples were collected directly from the hive using hygienic procedures, free from cross-contamination.

Considering the appreciable levels of aerobic mesophilic microorganisms found in some of the honeys evaluated, and the significant presence of lactic acid, the lactic acid bacteria population in the fresh honeys was found to be consistent with the other findings. Therefore, these bacteria cannot be considered contaminants but rather inherent to the type of honey, given that they are produced by the bee's digestive system. When the honeys were subjected to a strong heat treatment (80°C for 5 min), the aerobic mesophilic microorganisms, corresponding to lactic acid bacteria, were found to be completely degraded. Therefore, a milder heat treatment (65°C for 3 min) was examined. As observed in Table 6, this also had a negative effect on the lactic acid bacteria population, although to a lesser extent. This suggests that an even milder heat treatment is necessary to preserve these honeys, although, as shown in Table 7, it did not have a significant effect on color, antioxidant activity, total phenol content, or ascorbic acid. These results suggest that milder heat treatment better preserved the bioactive properties of the honey while still ensuring microbiological safety.

In honeys of certain bee species (*Melipona eburnea* and *Tetragonisca angustula*), the heat treatment did not significantly affect counts of aerobic mesophilic microorganisms and lactic acid bacteria. This could be explained by the thermotolerance of specific lactic acid bacteria, as well as to the protective effects of sugars and other solutes present in the honey, which can exert microbial resistance to heat based on their chemical nature. Although few studies have been conducted on honey, related work has modeled the inactivation behavior of lactic acid bacteria in other substrates under heat treatment and varying solute conditions [79].

Nevertheless, heat treatment significantly reduced lactic acid bacteria populations, which may be undesirable given their potential health benefits. Lactic acid bacteria in honey have been linked to probiotic effects, positively influencing digestive and immune health [80,81]. Furthermore, microbiota studies have identified these bacteria in honey, pollen, and propolis, where they play crucial roles in colony health, with potential implications for both human and animal well-being [80,82].

Lactic acid bacteria comprise multiple species with varying resistance to acidic conditions. In general, they can survive, grow, and function well at a pH between 5.5 and 6.0. However, as acid concentration increases, acidification can exert genotoxic effects on the cytosol and denature cellular proteins, with severity depending on the species involved [83,84]. In the honeys studied, both botanical and entomological origins result in considerable variation in microbial acid resistance. For instance, honeys from Tetragona species, despite having a lactic acid content nearly ten times higher than other species, showed very low counts of lactic acid bacteria, suggesting that excessive acid production may have led to microbial inactivation, potentially facilitated by solutes that enhanced this degradation.

Regarding the origin of lactic acid bacteria in honey, it is likely that bees themselves are the primary source. These bacteria are present in the digestive tracts of bees, where they metabolize nectar, pollen, and propolis, contributing to both hive nutrition and health [81]. In Mexican stingless bee honey, several species of lactic acid bacteria have been isolated [85]. Recent studies have identified new strains in fresh stingless bee honey from species such as *Melipona beecheii, Scaptotrigona pectoralis, Plebeia llorentei,* and *Plebeia jatiformis*. These bacteria preferentially metabolize fructose over glucose and may be responsible for some of the digestive health benefits attributed to honey [86]. Moreover, similar bacteria are also found in other sugar-rich sources, including flowers, fruits, fermented products, insects, and spices [87].

Although variations in antioxidant capacity were observed across samples of the same species, likely due to botanical origin, heat treatment did not significantly affect these properties. Similar findings have been reported in studies from Malaysia and Australia, where stingless bee honey treated at 45 °C, 55 °C and 65 °C for 60 minutes showed not significant alteration in phenolic content, flavonoid levels, or antioxidant activity, regardless of species or origin. However, antimicrobial activity was significantly reduced, suggesting that phenolic compounds and flavonoids are not the primary contributors to antimicrobial effects. Instead, organic acids or heat-sensitive enzymes may play a more crucial role [88].

In some cases, higher temperatures and prolonged treatments have been associated with enhanced antioxidant activity and phenolic content, due to the formation of new phenolic compounds or Maillard reaction products, a phenomenon also observed in foods like tomatoes and chili peppers [89].

The color of pot honey, measured on the Pfund scale before heat treatment, varied widely among samples. For example, *Melipona eburnea* and *Melipona nebulosa* exhibited extra light amber tones, *Melipona grandis* showed a white tone, and *Tetragonisca angustula* displayed a light amber color. Espinoza-Toledo et al. [90] studied Mexican pot honey from *Melipona solani*, *Scaptotrigona mexicana*, and *Melipona beecheii*, reporting color ranges from dark white to amber. Similarly, López-Garay et al. [91] found a mean Pfund value of 60 mm for *Scaptotrigona mexicana* honey, and Villacrés-Granda et al. [19] analyzed Ecuadorian pot honey from 12 stingless bee species and found average values ranging from 13 to 65 mm, further supporting the influence of bee species on honey color.

After heat treatment, most honeys maintained their light amber tones, suggesting that melanoidin formation via the Maillard reaction was minimal. This suggests limited degradation of amino acids and reducing sugars [92], thereby preserving the nutritional quality of the honey. Moreover, ascorbic acid and phenolic compound content, key contributors to antioxidant capacity, remained largely unchanged after heat treatment, as shown in Table 7. These results are consistent with findings from Thailand, where *Heterotrigona itamay* honey

was subjected to 37°C and 45°C for 24 and 48 hours showed no significant variations in phenolic, flavonoid or antioxidant levels [93].

5. CONCLUSIONS

This study provides the first comprehensive characterization of pot honey from seven stingless bee species in the Colombian Amazon Piedmont, highlighting its distinct physicochemical, microbiological, and bioactive properties. The honey exhibited significantly higher moisture content (26.6 ± 3.01%), a value explained by the high relative humidity of the region and the sugar composition of the honey, factors that limit water transfer from nectar to the environment and distinguish these honeys, while requiring careful application of good hygienic practices throughout the production process. Acidity levels were notably high, averaging 49.59 meq/kg, with pH values ranging from 3.22 to 4.63, creating an environment hostile to many pathogenic or spoilage-causing microorganisms and explaining the microbiological safety observed despite the high moisture content. A significant presence of ascorbic acid (vitamin C), derived from floral sources, was detected in all samples, with concentrations ranging from 47.1 to 233.7 mg/100 g, explaining the antioxidant activity and supporting therapeutic properties attributed by local farmers, such as flu-fighting, anti-inflammatory, and antitumor effects. Lactic acid was found in honeys of all analyzed species at average levels exceeding 150 mg/100 g, consistent with populations of lactic acid bacteria contributed by the bees digestive tract, in some cases reaching up to 5 log(CFU/g). This finding opens important research possibilities regarding potential probiotic properties and helps explain local empirical knowledge linking honey consumption to digestive benefits, including combating gastritis. In addition, gluconic acid was present at relatively high and variable concentrations, ranging from 94.7 to 1324.6 mg/100 g.

Additional aspects were identified concerning the relationship between specific organic acids and honey identity according to plant or entomological origin. Tartaric acid was not found in all species and was only prominent in *Melipona eburnea* (37.0 ± 36.3 mg/100 g) and *Melipona grandis* (3.2 ± 5.2 mg/100 g), suggesting its potential as a differentiating parameter linked to bee species and environment, contributing to the unique identity of honeys from the Colombian Amazonian Piedmont. Citric acid was detected exclusively in Tetragonisca angustula honeys, reaching 409.9 mg/100 g, indicating its possible role as a marker of honey origin associated with certain bee species. Succinic acid, associated with astringent flavors, was present in some honeys with high intra-species variability, in some cases exceeding 5000 mg/100 g. Further research on these acid–identity relationships could contribute to establishing a honey fingerprint. Although potassium was the most abundant mineral, overall mineral content was low, with sodium (<17.18 mg/100 g) and zinc (<0.18 mg/kg) being the most prominent among trace elements. Nevertheless, electrical conductivity was relatively high compared to common honeys, likely due to the abundant presence of organic acids and possibly certain amino acids, although these were not directly measured in this study.

Total phenolic content varied significantly among species, with the highest concentrations observed in *Tetragonisca angustula* and *Tetragona* sp. honeys, which also exhibited greater antioxidant activity. However, antioxidant capacity was not solely dependent on phenolic content, as other compounds such as ascorbic acid, organic acids, and specific enzymes likely contributed; indeed, high levels of ascorbic acid in several samples may explain the relatively high antioxidant activity even when phenolic content was lower. These findings suggest that stingless bee honey may have functional therapeutic applications, particularly in addressing childhood malnutrition due to its bioactive and immunomodulatory properties. All evaluated samples met food safety standards, with no detectable cadmium, lead, arsenic, or mercury, although traces of copper were detected in some samples, suggesting minimal environmental exposure possibly related to pesticide use and highlighting the need to monitor agricultural practices in the region. From a microbiological perspective, all fresh samples were free of pathogenic microorganisms, including heat-resistant coliforms (fecal), *Clostridium perfringens*, and *Salmonella*, demonstrating absence of cross-contamination during production, extraction, and packaging, while underscoring the importance of continued hygienic practices, training, and monitoring. Mold and yeast counts were acceptable, and although high counts of aerobic mesophilic microorganisms were observed, these corresponded to lactic acid bacteria, consistent with lactic acid concentrations and traditional digestive uses. Strong heat treatment

(80°C for 5 min) completely degraded these lactic acid bacteria, and a milder treatment (65°C for 3 min) also negatively affected their population without significantly altering color, antioxidant activity, total phenol content, or ascorbic acid. Further research is needed to develop even milder preservation treatments that maintain both functional properties and safety.

CONFLICTS OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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AUTHORS' CONTRIBUTIONS

Jonh Jairo Mueses-Cisneros: conceptualization, sample collection, supervision, writing - review & editing; Marta Cecilia Quicazán: conceptualization, methodology, data collection, writing - original draft; José Vicente Rueda-Almonacid: conceptualization, project administration, review & editing; Catalina Gutiérrez-Chacón: project administration, supervision, writing - review & editing; Carlos Alberto Fuenmayor: formal analysis, data collection, writing - review & editing; Carlos Mario Zuluaga-Domínguez: data curation; software, writing - original draft; Nicolás Ariza-Cruz: formal analysis; Golber Carvajal-Lavia: formal analysis.

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REFERENCES

[1] Londoño-Carvajal C, Cuellar-Nuñez J, Cely-Santos S, Nates-Parra G, Medina A. Abejas sin aguijón en Colombia. Estado de la meliponicultura en Colombia. Bogotá, Colombia: 2020.

[2] Quezada-Euán JJG, Nates-Parra G, Maués MM, Roubik DW, Imperatriz-Fonseca VL. The economic and cultural values of stingless bees (Hymenoptera: Meliponini) among ethnic groups of tropical America. Sociobiology 2018;65:534–57. DOI: https://doi.org/10.13102/sociobiology.v65i4.3447

[3] Real-Luna N, Rivera-Hernández JE, Alcántara-Salinas G, Rojas-Malavasi G, Morales-Vargas AP, Pérez-Sato JA. Las abejas sin aguijón (Tribu Meliponini) en los agroecosistemas de América Latina. Rev Mex De Cienc Agric 2022;13:331–44. DOI: https://doi.org/10.29312/remexca.v13i2.2866

[4] Requier F, Leyton MS, Morales CL, Garibaldi LA, Giacobino A, Porrini MP, et al. First large-scale study reveals important losses of managed honey bee and stingless bee colonies in Latin America. Sci Rep 2024;14:10079. DOI: https://doi.org/10.1038/s41598-024-59513-6

[5] Fischer B. Stingless Beekeeping (Meliponiculture) on Java. Youth With a Mission (YWAM). Kona, Hawaii: 2021.

[6] Rosso J, Nates-Parra G. Abejas sin aguijón (Tribu Meliponini). En: Iniciativa colombiana de polinizadores: abejas ICPA. Bogotá, Colombia: Universidad Nacional de Colombia; 2016.

[7] Engel MS, Rasmussen C, Ayala R, de Oliveira FF. Stingless bee classification and biology (Hymenoptera, Apidae): a review, with an updated key to genera and subgenera. Zookeys 2023;1172:239–312. DOI: https://doi.org/10.3897/zookeys.1172.104944

[8] Corpoamazonia. La meliponicultura en la Amazonia. 2022.

[9] Fuenmayor CA, Díaz-Moreno AC, Zuluaga-Domínguez CM, Quicazán MC. Honey of Colombian stingless bees: Nutritional characteristics and physicochemical quality indicators. In: Vit P, Pedro S, Roubik D, editors. Pot-Honey. New York: Springer; 2013. p. 383-94. DOI: https://doi.org/10.1007/978-1-4614-4960-7_27

[10] FAO. Codex Standards for Honey. Rome, Italy: Codex Alimentarius Commission Standards; 2001.

[11] IHC. Harmonized methods of the International Honey Commission. Switzerland: International Honey Commission; 2009.

[12] EEC. Council Directives 2001/110/EC relating to honey. Brussels: European Parliament of the Council; 2001.

[13] Fuenmayor CA, Zuluaga-Domínguez CM, Díaz-Moreno AC, Quicazán MC. Miel de angelita': nutritional composition and physicochemical properties of *Tetragonisca angustula* honey. Interciencia 2012;37.

[14] Gela A, Hora ZA, Kebebe D, Gebresilassie A. Physico-chemical characteristics of honey produced by stingless bees (*Meliponula beccarii*) from West Showa zone of Oromia Region, Ethiopia. Heliyon 2021;7. DOI: https://doi.org/10.1016/j.heliyon.2020.e05875

[15] Ismail NF, Maulidiani M, Omar S, Zulkifli MF, Radzi MNFM, Ismail N, et al. Classification of stingless bee honey based on species, dehumidification process and geographical origins using

physicochemical and ATR-FTIR chemometric approach. Journal of Food Composition and Analysis 2021;104:104126. DOI: https://doi.org/10.1016/j.jfca.2021.104126

[16] Maitip J, Polgate A, Promsart W, Butdee J, Rueangwong A, Sittisorn T, et al. Evaluation of stingless bee (*Tetragonula pagdeni*) honey properties and melissopalynological analysis from different geographical origins in Thailand. J Ecol Environ 2024;48:24–31. DOI: https://doi.org/10.5141/jee.23.062

[17] Sant'ana R da S, de Carvalho CAL, Oda-Souza M, Souza B de A, Dias F de S. Characterization of honey of stingless bees from the Brazilian semi-arid region. Food Chem 2020;327:127041. DOI: https://doi.org/10.1016/j.foodchem.2020.127041

[18] Shamsudin S, Selamat J, Sanny M, Abd. Razak S-B, Jambari NN, Mian Z, et al. Influence of origins and bee species on physicochemical, antioxidant properties and botanical discrimination of stingless bee honey. Int J Food Prop 2019;22:239–64. DOI: https://doi.org/10.1080/10942912.2019.1576730

[19] Villacrés-Granda I, Coello D, Proaño A, Ballesteros I, Roubik DW, Jijón G, et al. Honey quality parameters, chemical composition and antimicrobial activity in twelve Ecuadorian stingless bees (Apidae: Apinae: Meliponini) tested against multiresistant human pathogens. LWT 2021;140:110737. DOI: https://doi.org/10.1016/j.lwt.2020.110737

[20] Bijlsma L, de Bruijn LLM, Martens EP, Sommeijer MJ. Water content of stingless bee honeys (Apidae, Meliponini): interspecific variation and comparison with honey of *Apis mellifera*. Apidologie 2006;37:480–6. DOI: https://doi.org/10.1051/apido:2006028

[21] Correa-Mosquera AR, Quicazán MC, Zuluaga-Domínguez CM. Shelf-life prediction of pot-honey subjected to thermal treatments based on quality attributes at accelerated storage conditions. Food Control 2022;142:109237. DOI: https://doi.org/10.1016/j.foodcont.2022.109237

[22] Mustar S, Ibrahim N. A sweeter pill to swallow: A review of honey bees and honey as a source of probiotic and prebiotic products. Foods 2022;11:2102. DOI: https://doi.org/10.3390/foods11142102

[23] Chettri U, Kumari S. A review on prebiotic importance of stingless bee honey and its ethnomedicinal and therapeutic potential. Int J Pharm Sci Rev Res 2020;63:150–6.

[24] AOAC. Official Methods of Analysis. Rockville, MD: AOAC International; 2019.

[25] Bicudo de Almeida-Muradian L, Monika Barth O, Dietemann V, Eyer M, Freitas A da S de, Martel A-C, et al. Standard methods for *Apis mellifera* honey research. J Apic Res 2020;59:1–62. DOI: https://doi.org/10.1016/j.foodchem.2007.04.070

[26] Bertoncelj J, Dobersek U, Jamnik M, Golob T. Evaluation of the phenolic content, antioxidant activity and colour of Slovenian honey. Food Chem 2007;105:822–8. DOI: https://doi.org/10.1016/j.foodchem.2007.01.060

[27] Moon J-K, Shibamoto T. Antioxidant assays for plant and food components. J Agric Food Chem 2009;57:1655–66. DOI: https://doi.org/10.1021/jf803537k

[28] Mello dos Santos M, Khan N, Lim LY, Locher C. Antioxidant activity, physicochemical and sensory properties of stingless bee honey from Australia. Foods 2024;13:1657. DOI: https://doi.org/10.3390/foods13111657

[29] Zaldivar-Ortega AK, Cenobio-Galindo A de J, Morfin N, Aguirre-Álvarez G, Campos-Montiel RG, Esturau-Escofet N, et al. The physicochemical parameters, phenolic content, and antioxidant activity of honey from stingless bees and *Apis mellifera*: A systematic review and meta-analysis. Antioxidants 2024;13:1539. DOI: https://doi.org/10.3390/antiox13121539

[30] Rodríguez-Malaver AJ, Rasmussen C, Gutiérrez MG, Gil F, Nieves B, Vit P. Properties of honey from ten species of Peruvian stingless bees. Nat Prod Commun 2009;4(9):1221–6. DOI: https://doi.org/10.1177/1934578X0900400913

[31] Yah-Rosales LA, Chel-Guerrero LA, Sacramento-Rivero JC, Baz-Rodríguez SA. Physicochemical, rheological, and thermal properties of pot-honey from the stingless bee *Melipona beecheii*. Ingeniería e Investigación 2023;43:1. DOI: https://doi.org/10.15446/ing.investig.103232

[32] Cardona Y, Torres A, Hoffmann W. Colombian stingless bee honeys characterized by multivariate analysis of physicochemical properties. Apidologie 2019;50:881–92. DOI: https://doi.org/10.1007/s13592-019-00698-5

[33] Rosli FN, Hazemi MHF, Akbar MA, Basir S, Kassim H, Bunawan H. Stingless bee honey: Evaluating its antibacterial activity and bacterial diversity. Insects 2020;11:500. DOI: https://doi.org/10.3390/insects11080500

[34] Vit P, Medina M, Enriquez M. Quality standards for medicinal uses of meliponinae honey in Guatemala, Mexico and Venezuela. Bee World 2004;85:2–5.

[35] de Almeida-Muradian LB, Stramm KM, Horita A, Barth OM, da Silva de Freitas A, Estevinho LM. Comparative study of the physicochemical and palynological characteristics of honey from *Melipona subnitida* and *Apis mellifera*. Int J Food Sci Technol 2013;48:1698–706.

[36] Adler M, Zambrana-Camacho G, Flores-Prado L, Urquizo ON, Collao-Alvarado K, Pinto CF. Pollen composition and physicochemical properties of honey produced by three stingless bees species from a mesotropical subhumid region in Bolivia. Arthropod Plant Interact 2024;18:1013–32. DOI: https://doi.org/10.1007/s11829-024-10084-3

[37] Zapata-Vahos IC, Henao-Rojas JC, Yepes-Betancur DP, Marín-Henao D, Giraldo Sánchez CE, Calvo-Cardona SJ, et al. Physicochemical parameters, antioxidant capacity, and antimicrobial activity of honeys from tropical forests of Colombia: *Apis mellifera* and *Melipona eburnea*. Foods 2023;12:1001. DOI: https://doi.org/10.3390/foods12051001

[38] Delgado C, Espinoza R V. Physicochemical parameters and chemoprofiling of honey of two species of stingless bees in the Peruvian Amazon. Food and Humanity 2023;1:1145–56. DOI: https://doi.org/10.1016/j.foohum.2023.08.017

[39] Xolalpa-Aroche A, Hernández-Mena DI, Moguel-Chin WI, Contreras-Peruyero H, Rivero-Cruz BE, Ortiz-Vázquez E, et al. physicochemical properties of two Mexican stingless bee honeys to strengthen their biocultural value. Revista Brasileira de Farmacognosia 2024;34:1129–39. DOI: https://doi.org/10.1007/s43450-024-00566-z

[40] Mduda CA, Kalonga JS. Physicochemical properties, sugar analysis, antioxidants, and antibacterial activity of *Plebeina armata* honey from Western Tanzania. Discover Food 2025;5:335. DOI: https://doi.org/10.1007/s44187-025-00599-w

[41] Nweze JA, Okafor JI, Nweze EI, Nweze JE. Evaluation of physicochemical and antioxidant properties of two stingless bee honeys: a comparison with *Apis mellifera* honey from Nsukka, Nigeria. BMC Res Notes 2017;10:1–6. DOI: https://doi.org/10.1186/s13104-017-2884-2

[42] Sousa JMB de, Souza EL de, Marques G, Benassi M de T, Gullón B, Pintado MM, et al. Sugar profile, physicochemical and sensory aspects of monofloral honeys produced by different stingless bee species in Brazilian semi-arid region. LWT 2016;65:645–51. DOI: https://doi.org/10.1016/j.lwt.2015.08.058

[43] Lico JCT, Menezes Filho ACP de, Souza WC, Taques AS, Alves I, Castro CF de S, et al. Qualidade de méis de abelhas sem ferrão *Tetragonisca angustula* (Latreille 1811), *Trigona pallens* (Fabricius,

1798) e *Lestrimelitta limao* (Smith) em área urbana, Goiás, Brasil. Brazilian Journal of Science 2023;2:1–13. DOI: https://doi.org/10.14295/bjs.v2i7.347

[44] Aroucha EMM, Silva MCP, Leite RHL, Santos FK, Oliveira VRL, Araújo NO, et al. Physicochemical, antioxidants and sensorial properties of *Melipona subnitida* honey after dehumidifying. J Food Process Technol 2019;10:e1000781. DOI: https://doi.org/10.4172/2157-7110.1000781

[45] Patricia V, Silvia RMP, César Vargas J, Andino M, Maza F. Chemical composition of Ecuadorian commercial pot-honeys: *Trigona fuscipennis* "Abeja de tierra", *Melipona mimetica* "Bermejo" and *Scaptotrigona ederi* "Catiana." Pharm Anal Acta 2016;7:2. DOI: https://doi.org/10.4172/2153-2435.1000525

[46] Weston RJ, Brocklebank LK. The oligosaccharide composition of some New Zealand honeys. Food Chem 1999;64:33–7. DOI: https://doi.org/10.1016/S0308-8146(98)00099-5

[47] Schell KR, Fernandes KE, Shanahan E, Wilson I, Blair SE, Carter DA, et al. The potential of honey as a prebiotic food to re-engineer the gut microbiome toward a healthy state. Front Nutr 2022;9. DOI: https://doi.org/10.3389/fnut.2022.957932

[48] Dunsmore A, Mellet P, Wolff M. Some factors affecting the Lane and Eynon titration method for determining reducing sugars in sugar products. Proceedings of The South African Sugar Technologists' Association, 1980, p. 73.

[49] Da Costa Leite JM, Trugo LC, Costa LSM, Quinteiro LMC, Barth OM, Dutra VML, et al. Determination of oligosaccharides in Brazilian honeys of different botanical origin. Food Chem 2000;70:93–8. DOI: https://doi.org/10.1016/S0956-7135(99)00115-2

[50] Arias VC, Castells RC, Malacalza N, Lupano CE, Castells CB. Determination of Oligosaccharide Patterns in Honey by Solid-Phase Extraction and High-Performance Liquid Chromatography. Chromatographia 2003;58:797–801. DOI: https://doi.org/10.1365/s10337-003-0115-6

[51] Ali H, Abu Bakar MF, Majid M, Muhammad N, Lim SY. In vitro anti-diabetic activity of stingless bee honey from different botanical origins. Food Res 2020;4:1421–6. DOI: https://doi.org/10.26656/fr.2017.4(5).411

[52] Wani HA, Majid S, Khan MS, Bhat AA, Wani RA, Bhat SA, et al. Scope of honey in diabetes and metabolic disorders. Therapeutic applications of honey and its phytochemicals, Singapore: Springer Singapore; 2020, p. 195–217. DOI: https://doi.org/10.1007/978-981-15-7305-7_9

[53] Hajam Y. Honey as a potential prebiotic: A review of its effects on gut microbiota composition and functions. Honey, CRC Press; 2024, p. 86–108. DOI: https://doi.org/10.1201/9781003490180-6

[54] Susilowati F, Azkia MN. Prebiotic potential of oligosaccharides: *In vitro* study of Indonesian local honey from *Apis spp.* and *Trigona spp.* bees, 2022. DOI: https://doi.org/10.2991/absr.k.220101.025

[55] Hungerford NL, Yates HSA, Smith TJ, Fletcher MT. Organic acid profiles of Australian stingless bee honey samples determined by ion chromatography. J Food Compos Anal. 2023;122:105466. DOI: https://doi.org/10.1016/j.jfca.2023.105466

[56] Costa dos Santos A, Biluca FC, Brugnerotto P, Valdemiro Gonzaga L, Carolina Oliveira Costa A, Fett R. Brazilian stingless bee honey: physicochemical properties and aliphatic organic acids content. Food Res Int. 2022;158:111516. DOI: https://doi.org/10.1016/j.foodres.2022.111516

[57] Zago KI, García MY, Di Bernardo ML, Vit P, Luna JR, Gualtieri M. Determinación del contenido de vitamina C en miel de abejas venezolanas por volumetría de óxido-reducción. Revista Del Instituto Nacional de Higiene Rafael Rangel 2010;41:25–30.

[58] Umami N, Agus A. Stingless bee honey (*Tetragonula laeviceps*): Chemical composition and their potential roles as an immunomodulator in malnourished rats. Saudi J Biol Sci 2022;29:103404. DOI: https://doi.org/10.1016/j.sjbs.2022.103404

[59] Agus A, Agussalim N, Umami N, Budisatria IGS. Evaluation of antioxidant activity, phenolic, flavonoid and vitamin C content of several honeys produced by the Indonesian stingless bee: *Tetragonula laeviceps*. Livest Res Rural Dev 2019;31:2014–20.

[60] Selvaraju K, Vikram P, Soon JM, Krishnan KT, Mohammed A. Melissopalynological, physicochemical and antioxidant properties of honey from West Coast of Malaysia. J Food Sci Technol 2019;56:2508–21. DOI: https://doi.org/10.1007/s13197-019-03728-3

[61] Ellong EN, Billard C, Adenet S, Rochefort K. Polyphenols, carotenoids, vitamin C content in tropical fruits and vegetables and impact of processing methods. Food Nutr Sci 2015;06:299–313. DOI: https://doi.org/10.4236/fns.2015.63030

[62] Lavinas FC, Gomes BA, Silva MVT, Nunes RM, Leitão SG, Moura MRL, et al. Discriminant analysis of Brazilian stingless bee honey reveals an iron-based biogeographical origin. Foods 2023;12:180. DOI: https://doi.org/10.3390/foods12010180

[63] Goh CH, Heng PWS, Chan LW. Alginates as a useful natural polymer for microencapsulation and therapeutic applications. Carbohydr Polym 2012;88:1–12. DOI: https://doi.org/10.1016/j.carbpol.2011.11.012

[64] Vit P, van der Meulen J, Díaz M, Pedro SRM, Esperança I, Zakaria R, Beckh G, Maza F, Meccia G, Engel MS. Impact of genus (*Geotrigona*, *Melipona*, *Scaptotrigona*) in the ^1H-NMR organic profile and authenticity test of honey processed in cerumen pots by stingless bees in Ecuador. Curr Res Food Sci. 2023;6:100386. DOI: https://doi.org/10.1016/j.crfs.2022.11.005

[65] Zuluaga-Domínguez CM, Nieto-Veloza A, Quicazán-de-Cuenca M. Classification of Colombian honeys by electronic nose and physical-chemical parameters, using neural networks and genetic algorithms. J Apic Res 2018;57. DOI: https://doi.org/10.1080/00218839.2017.1339521

[66] Nascimento A, Marchini L, Carvalho C, Araújo D, Olinda R, Silveira T. Physical-chemical parameters of honey of stingless bee (Hymenoptera: Apidae). American Chemical Science Journal 2015;7:139–49. DOI: https://doi.org/10.9734/ACSJ/2015/17547

[67] Biluca FC, Braghini F, Gonzaga LV, Costa ACO, Fett R. Physicochemical profiles, minerals and bioactive compounds of stingless bee honey (Meliponinae). Journal of Food Compositi on and Analysis 2016;50:61–9. DOI: https://doi.org/10.1016/j.jfca.2016.05.007

[68] Scripcă LA, Norocel L, Amariei S. Comparison of physicochemical, microbiological properties and bioactive compounds content of grassland honey and other floral origin honeys. Molecules 2019;24:2932. DOI: https://doi.org/10.3390/molecules24162932

[69] Terrab A, Díez MJ, Heredia FJ. Palynological, physico-chemical and colour characterization of Moroccan honeys: I. River red gum (*Eucalyptus camaldulensis* Dehnh) honey. Int J Food Sci Technol 2003;38:379–86. DOI: https://doi.org/10.1046/j.1365-2621.2003.00715.x

[70] Terrab A, González-Miret L, Heredia FJ. Color characterization of thyme and avocado honeys by diffuse reflectance spectrophotometry and spectroradiometry. European Food Research and Technology 2004;218:488–92. DOI: https://doi.org/10.1007/s00217-004-0890-9

[71] de Oliveira DF, Braga DJN, Júnior WAC, Holanda GHA, Ronqui L, Parpinelli RS, et al. Health risk due to the presence of trace elements in stingless bee honey consumed in the Amazon and Southern Brazil. Journal of Food Composition and Analysis 2025;147:108073. DOI: https://doi.org/10.1016/j.jfca.2025.108073

[72] Salman NH, Mok Sam L, Ador K, Binjamin B, Johny-Hasbulah MIJ, Benedick S. Linking measure of the tropical stingless bee

(Apidae, Meliponini, and *Heterotrigona itama*) honey quality with hives distance to the source of heavy metal pollution in urban and industrial areas in Sabah, Borneo. J Toxicol 2022;2022:1–7. DOI: https://doi.org/10.1155/2022/4478082

[73] Okeola FO, Oluade O, Liad MT. Stingless bee honey as bio-indicator of heavy metals pollution in and around the university of Ilorin Environ kwara state, Nigeria. Journal of Applied Sciences and Environmental Management 2020;24:773–8. DOI: https://doi.org/10.4314/jasem.v24i5.7

[74] ANM. Departamento de Putumayo. Caracterización de la actividad minera departamental. Agencia Nacional de Mineria; 2017.

[75] Schvezov N, Pucciarelli AB, Valdes B, Dallagnol AM. Characterization of yateí (*Tetragonisca fiebrigi*) honey and preservation treatments: Dehumidification, pasteurization and refrigeration. Food Control 2020;111:107080. DOI: https://doi.org/10.1016/j.foodcont.2019.107080

[76] Pucciarelli A, Schapovaloff M, Kummritz S, Señuk I, Brumovsky L, Dallagnol A. Microbiological and physicochemical analysis of yateí (*Tetragonisca angustula*) honey for assessing quality standards and commercialization. Rev Argent Microbiol 2014;46:325–32. DOI: https://doi.org/10.1016/S0325-7541(14)70091-4

[77] Pinheiro C de GM da E, Abrantes MR, Silva ROS, Oliveira Junior CA, Lobato FCF, Silva JBA da. Microbiological quality of honey from stingless bee, jandaíra (*Melipona subnitida*), from the semiarid region of Brazil. Ciência Rural 2018;48. DOI: https://doi.org/10.1590/0103-8478cr20180151

[78] Tesfaye O, Desalegn A, Muleta D. Melissopalynological analysis and microbiological safety of fresh and market honey (*Apis mellifera* L. and *Meliponula beccarii* L.) from Western Oromia, Ethiopia. Heliyon 2024;10:e28185. DOI: https://doi.org/10.1016/j.heliyon.2024.e28185

[79] Huerta-González L, López-Valdez F, Luna-Suárez S. Estabilidad térmica de las bacterias ácido-lácticas bajo la influencia de un emulsificante a base de monoacilgliceroles. Investigación y Desarrollo En Ciencia y Tecnología de Alimentos 2023;8:151–6. DOI: https://doi.org/10.29105/idcyta.v8i1.23

[80] Abadi MEGM, Hosseini-Safa A, Habibi S, Dehghan M, Forouzani-Moghaddam MJ, Oshaghi M. Isolation and characterization of the lactobacillus strain from honey and its probiotic properties. Iran J Microbiol 2023;15:439. DOI: https://doi.org/10.18502/ijm.v15i3.12905

[81] Iorizzo M, Letizia F, Ganassi S, Testa B, Petrarca S, Albanese G, et al. Functional properties and antimicrobial activity from lactic acid bacteria as resources to improve the health and welfare of honey bees. Insects 2022;13:308. DOI: https://doi.org/10.3390/insects13030308

[82] Ramos OY, Basualdo M, Libonatti C, Vega MF. Current status and application of lactic acid bacteria in animal production systems with a focus on bacteria from honey bee colonies. J Appl Microbiol 2020;128:1248–60. DOI: https://doi.org/10.1111/jam.14469

[83] Sionek B, Szydłowska A, Trząskowska M, Kołożyn-Krajewska D. The impact of physicochemical conditions on lactic acid bacteria survival in food products. Fermentation 2024;10:298. DOI: https://doi.org/10.3390/fermentation10060298

[84] Wang C, Cui Y, Qu X. Mechanisms and improvement of acid resistance in lactic acid bacteria. Arch Microbiol 2018;200:195–201. DOI: https://doi.org/10.1007/s00203-017-1446-2

[85] Torres-Moreno R, Humberto SH-S, Méndez-Tenorio A, Palmeros-Sánchez B, Melgar-Lalanne G. Characterization and identification of lactic acid bacteria from Mexican stingless bees (Apidae: Meliponini). IOP Conf. Ser. Earth Environ. Sci., vol. 858, IOP Publishing; 2021, p. 12010. DOI: https://doi.org/10.1088/1755-1315/858/1/012010

[86] Andrade-Velásquez A, Hernández Sánchez H, Dorantes-Álvarez L, Palmeros-Sánchez B, Torres-Moreno R, Hernández-Rodríguez D, et al. Honey characterization and identification of fructophilic lactic acid bacteria of fresh samples from *Melipona beecheii*, *Scaptotrigona pectoralis*, *Plebeia llorentei*, and *Plebeia jatiformis* hives. Front Sustain Food Syst 2023;7:1113920. DOI: https://doi.org/10.3389/fsufs.2023.1113920

[87] Takatani N, Endo A. Viable fructophilic lactic acid bacteria present in honeybee-based food products. FEMS Microbiol Lett 2021;368:fnab150. DOI: https://doi.org/10.1093/femsle/fnab150

[88] Mat Ramlan NAF, Md Zin AS, Safari NF, Chan KW, Zawawi N. Application of heating on the antioxidant and antibacterial properties of Malaysian and Australian stingless bee honey. Antibiotics 2021;10:1365. DOI: https://doi.org/10.3390/antibiotics10111365

[89] Shaimaa GA, Mahmoud MS, Mohamed MR, Emam AA. Effect of heat treatment on phenolic and flavonoid compounds and antioxidant activities of some Egyptian sweet and chilli pepper. Nat Prod Chem Res 2016;4:1000218. DOI: 10.4172/2329-6836.1000218

[90] Espinoza-Toledo C, Vázquez-Ovando A, Santos RT de los, López-García A, Albores-Flores V, Grajales-Conesa J. Stingless bee honeys from Soconusco, Chiapas: a complementary approach. Rev Biol Trop 2018;66:1536–46. DOI: https://doi.org/10.15517/rbt.v66i4.32181

[91] López-Garay LA, Trejo-Téllez LI, Gómez-Merino FC, Contreras-Oliva A, Pérez-Sato JA, Salinas-Ruiz J. Physicochemical properties of *Scaptotrigona mexicana* honey from the highlands of Veracruz, Mexico. Ecosistemas y Recursos Agropecuarios 2023;10. DOI: https://doi.org/10.19136/era.a10n1.3380

[92] Brudzynski K, Miotto D. Honey melanoidins: analysis of the compositions of the high molecular weight melanoidins exhibiting radical-scavenging activity. Food Chem 2011;127:1023–30. DOI: https://doi.org/10.1016/j.foodchem.2011.01.075

[93] Wongsa K, Meemongkolkiat T, Duangphakdee O, Prasongsuk S, Rattanawannee A. Physicochemical properties, phenolic, flavonoid contents and antioxidant potential of stingless bee (*Heterotrigona itama*) honey from Thailand. Current Research in Nutrition & Food Science 2023;11. DOI: https://doi.org/10.12944/CRNFSJ.11.1.18