PHYSICOCHEMICAL AND MELISSOPALYNOLOGICAL CHARACTERIZATION OF HONEY PRODUCED BY *APIS MELLIFERA* IN MONGUÍ, BOYACÁ, AS A BASIS FOR IDENTIFYING QUALITY ATTRIBUTES AND TERRITORIAL DIFFERENTIATION: A RESEARCH PROTOCOL

CARACTERIZACIÓN FISICOQUÍMICA Y MELISOPALINOLÓGICA DE LA MIEL DE *APIS MELLIFERA* PRODUCIDA EN MONGUÍ, BOYACÁ, COMO BASE PARA IDENTIFICAR LOS ATRIBUTOS DE CALIDAD Y LA DIFERENCIACIÓN TERRITORIAL: UN PROTOCOLO DE INVESTIGACIÓN

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ABSTRACT

Honey produced by *Apis mellifera* is a food product of economic importance whose quality characteristics may be influenced by environmental and floral factors, as well as by practices used during production, extraction, and storage. Honey from Monguí, Boyacá, has potential for differentiation associated with local environmental conditions and floral resources. However, integrated information linking physicochemical and melissopalynological characteristics to production conditions is needed.

This paper presents a research protocol for characterizing *Apis mellifera* honey from eight selected apiaries in Monguí, Boyacá, as a basis for identifying quality attributes and informing future research on territorial differentiation. The prospective, observational, and descriptive study will follow 32 colonies (four per apiary) across eight apiaries during two flowering periods expected in October 2026 and mid-January 2027. Up to 16 composite samples are expected to be collected across the two flowering periods.

The planned assessments include physicochemical measurements and the identification and relative quantification of pollen types to explore potential botanical origins. Environmental conditions, flowering periods, beekeeping management practices, extraction, storage, and sample traceability will also be documented.

From an engineering perspective, integrating these indicators is intended to establish a technical baseline to guide future quality control and production management decisions. As the project is at an early stage, no experimental results are presented. Conclusions will be limited to the selected apiaries and will not establish attributes unique to the municipality.

Keywords: honey, *Apis mellifera*, physicochemical characterization, melissopalynology, quality, botanical origin, territorial differentiation

RESUMEN

La miel de *Apis mellifera* es un producto alimenticio de importancia económica cuyas características de calidad pueden verse influidas por factores ambientales y florales, así como por las prácticas aplicadas durante la producción, la extracción y el almacenamiento. La miel de Monguí, Boyacá, presenta un potencial de diferenciación asociado a las condiciones ambientales locales y a los recursos florales. Sin embargo, se necesita información integrada que relacione las características fisicoquímicas y melisopalinológicas con las condiciones de producción.

Este artículo presenta un protocolo de investigación para caracterizar la miel de *Apis mellifera* procedente de ocho colmenares seleccionados en Monguí, Boyacá, como base para identificar los atributos de calidad y orientar futuras investigaciones sobre la diferenciación territorial. El estudio, de diseño prospectivo, observacional y descriptivo, comprenderá ocho colmenares con 32 colonias (cuatro por colmenar), a los que se hará seguimiento durante dos períodos de floración previstos para octubre de 2026 y mediados de enero de 2027. Se prevé obtener hasta 16 muestras compuestas en total, una por colmenar y período.

Las evaluaciones previstas abarcan parámetros fisicoquímicos, así como la identificación y cuantificación relativa de los tipos de polen para explorar posibles orígenes botánicos. También se documentarán las condiciones ambientales, los períodos de floración, la gestión apícola, la extracción, el almacenamiento y la trazabilidad de las muestras.

Desde una perspectiva de ingeniería, la integración de estos indicadores tiene por objeto desarrollar una base de referencia técnica que sirva de guía para futuras decisiones en materia de control de calidad y gestión de la producción. Dado que el proyecto se encuentra en una fase inicial, no se presentan resultados experimentales; las conclusiones se referirán únicamente a los colmenares seleccionados y no establecerán atributos exclusivos del municipio.

Palabras clave: miel; *Apis mellifera*; caracterización fisicoquímica; melisopalinología; calidad; origen botánico; diferenciación territorial

1. INTRODUCTION

Honey is a natural sweet substance produced by bees from plant nectar, secretions of living plant parts, or excretions of plant-sucking insects. Bees collect these materials and transport them to the hive, where they transform them by adding substances of their own, reducing the water content, and subsequently storing and ripening the honey in combs (Codex Alimentarius Commission, 2022; Machado De-Melo et al., 2018).

During this process, honeybees act as biological engineers, converting collected raw materials into a food with distinctive physical, chemical, and sensory characteristics (Machado De-Melo et al., 2018). Honey can thus be described as a substance obtained through nectar collection and processing, followed by deposition and ripening in honeycombs (Haider et al., 2024).

Honey has been part of the human diet since ancient times. In addition to being consumed directly, it is used in food preparation as a sweetener, flavoring agent, and ingredient in a variety of products. Its importance extends beyond its taste and role as an energy source, as it also contains bioactive compounds associated with antioxidant properties (da Silva et al., 2016; Khalil et al., 2010).

The nutritional value of honey is primarily associated with its high carbohydrate content. Its composition includes fructose, glucose, sucrose, maltose, oligosaccharides, and polysaccharides, as well as smaller quantities of enzymes, pigments, aromatic compounds, pollen grains, minerals, vitamins, waxes, and organic acids (Conti et al., 2018; Haouam et al., 2016; Machado De-Melo et al., 2018).

Because simple sugars predominate, honey provides a readily available source of energy. It also contains constituents associated with antioxidant activity, including organic and aromatic acids, ascorbic acid, flavonoids, carotenoids, and enzymes such as catalase and glucose oxidase (Khalil et al., 2010). These components contribute to its antioxidant potential and may help protect against oxidative damage.

Honeybees belong to the order Hymenoptera, family Apidae, and genus *Apis*. Within this genus, *Apis mellifera* is the principal species used in beekeeping, and most honey intended for human consumption originates from its colonies (Michener, 2007; Papa et al., 2022).

Beyond its importance in beekeeping, *A. mellifera* plays a fundamental role as a pollinator. Its activity contributes to biodiversity conservation, the sustainability of agricultural systems, and crop productivity (Papa et al., 2022). This species is therefore important both as a source of commercially valuable bee products and as a provider of an essential ecosystem service.

Pollen provides a starting point for understanding the relationship between bees and plants in honey production. Each pollen grain has specific structural features, including its exine, size, and aperture type, that support microscopic identification

(Halbritter et al., 2018). These features make it possible to explore links between the palynological composition of honey and a region's biogeographic and ecological setting (Louveaux et al., 1978; von der Ohe et al., 2004).

Beekeeping is an agricultural activity of substantial nutritional, economic, social, and environmental importance. It yields honey, beeswax, propolis, pollen, royal jelly, and bee venom, which can provide income and support economic diversification in rural communities. In addition to generating marketable products, beekeeping contributes to agricultural sustainability and ecosystem functioning (Papa et al., 2022).

Beekeeping depends directly on the floral biodiversity available to honeybees. Identifying pollen types in honey can provide evidence of the plants visited by bees and, consequently, support inferences about potential botanical and geographic origins (Pospiech et al., 2021; Ricciardelli D'Albore, 1997; Santos de Novais et al., 2010).

Melissopalynology, the microscopic examination of pollen grains in honey, is a conventional approach to investigating botanical origin (Louveaux et al., 1978; von der Ohe et al., 2004). It helps distinguish monofloral honeys, in which pollen from one plant species predominates but is not the only type present (Tulu et al., 2023), from multifloral honeys, which contain pollen from several species without a marked predominance (Soares et al., 2017). The resulting profiles offer information on the plant sources of nectar and pollen used by bees (Pospiech et al., 2021).

Botanical classification has economic and sensory implications: monofloral honeys often command higher market prices and exhibit distinctive volatile compound profiles that influence their organoleptic properties (Aliferis et al., 2010; Mădaş et al., 2019; Soares et al., 2017). Honeydew honey may also be identified by considering the ratio of honeydew elements to pollen grains (HDE/PG); values greater than 3 have been used as evidence of predominantly honeydew origin (Louveaux et al., 1978; von der Ohe et al., 2004).

Honey quality and authenticity are essential to regulatory compliance and the economic integrity of the beekeeping sector. Honey is vulnerable to adulteration through the addition of sugar syrups or undeclared additives and the mislabeling of botanical or geographic origin, potentially undermining its nutritional and commercial value. Reliable indicators and analytical methods are therefore necessary to distinguish authentic, high-quality honey from adulterated or lower-quality products (Kiran & Riyaz, 2026).

Honey quality is commonly evaluated using physicochemical parameters, including moisture, pH, free acidity, electrical conductivity, hydroxymethylfurfural (HMF), diastase activity, color, and sugar composition. These measurements provide complementary information about freshness, composition, and stability and can be compared with relevant international standards (Kiran & Riyaz, 2026; Tsagkaris et al., 2021).

The commercial value of honey depends on its botanical and geographic origin, color, aroma, flavor, moisture, physicochemical

composition, presentation, and production method. Extraction, processing, and storage conditions, whether artisanal or industrial, may also modify its characteristics and market acceptance (Campo Barrera & Hincapié Llanos, 2023).

Differentiating honey by origin and quality may therefore add value, strengthen producers' livelihoods, and facilitate access to specialized markets. Such potential nevertheless depends on meeting applicable requirements for authenticity, composition, and quality.

In this context, the physicochemical and melissopalynological characterization of honey produced in Monguí, Boyacá, offers an opportunity to generate scientific information about a regionally relevant bee product and its relationships with floral resources and production conditions. For the eight selected apiaries operated by Vibee La Apicultura S.A.S. BIC, there is a particular need to systematically integrate data on honey composition, pollen types, floral phenology, and practices associated with harvesting, extraction, and storage.

From an engineering perspective, this integration will establish a technical baseline to help explain variability in honey characteristics and inform future decisions about quality control, traceability, and product handling. Although the characterization of the selected apiaries cannot demonstrate attributes exclusive to the municipality, the findings may underpin subsequent research into the potential for territorial differentiation.

Accordingly, this study aims to characterize the physicochemical and melissopalynological properties of *Apis mellifera* honey produced in eight selected apiaries in Monguí, Boyacá, over two flowering periods, integrating environmental, floral, and production information as a basis for identifying quality attributes and developing future territorial differentiation strategies.

2. MATERIALS AND METHODS

The research will use a quantitative, prospective, observational, and descriptive design with exploratory comparisons. The physicochemical and melissopalynological properties of *Apis mellifera* honey will be characterized in eight apiaries selected from those operated by Vibee La Apicultura S.A.S. BIC, with four colonies per apiary (32 in total), during two planned flowering periods: October 2026 and mid-January 2027. This manuscript describes the project's initial phase and proposed design; no experimental findings are reported.

Each apiary will be the longitudinal observational unit, whereas each apiary–period composite sample will be the unit of measurement. Up to 16 composite samples (eight apiaries × two periods) are expected, provided eligible honey is available during both periods. Laboratory analytical replicates will not be treated as independent biological samples.

Sampling will be nonprobabilistic and restricted to the participating company's selected apiaries. The findings will therefore

describe these production systems and allow exploratory assessment of changes between periods but will not be generalizable to all honey produced in Monguí or independently establish unique territorial differentiation. Between-period comparisons will account for repeated observations of each apiary, as specified in Section 2.5.

2.1. Study Area and Sample Collection

The study will be conducted at apiaries operated by Vibee La Apicultura S.A.S. BIC in Docuaza, a rural locality in the municipality of Monguí, Boyacá, Colombia. The landscape consists of a transition between Andean forest, subpáramo, and modified rural areas near the Ocetá páramo. Apiary coordinates and elevations will be recorded in the field; location within a protected area or buffer zone will not be assumed without cartographic evidence.

The eight apiaries will be selected according to criteria established before results are available: company affiliation, location within the study area, individually identifiable colonies, access for visits during both periods, and availability of basic management and harvest information. These eight apiaries will constitute the nonprobability sample. Four *Apis mellifera* colonies will be selected per apiary, for a total of 32 colonies.

The two planned flowering and sampling periods, designated P1 and P2, will occur in October 2026 and mid-January 2027, respectively. The exact start and end dates of the honey accumulation and harvest windows will be documented using floral observations, management records, and actual harvest dates. Hive management around harvest time will be coordinated to support attribution of the harvested honey to the flowering period under study.

One composite sample per apiary is planned for each period, using honey from the four selected colonies, provided the combs are capped, brood-free, and sufficiently mature. The same 32 identified colonies will be followed in P1 and P2 whenever possible. The actual contributing colonies and any colony loss, relocation, replacement, or failure to harvest will be documented, along with dates and reasons. Replacement colonies will receive new identifiers; when an apiary yields no eligible honey, the observation will be recorded as missing rather than replaced with honey from another apiary.

Each composite sample will have a target total mass of approximately 500 g. When all four selected colonies in an apiary provide eligible honey, 125 g will be collected from each colony to form an equal-mass blend. If one or more colonies do not meet the sampling criteria and the remaining colonies are used, 500/k g of honey will be collected from each eligible colony, where k is the actual number of contributing colonies (k = 1, 2, 3, or 4). Actual contribution masses and any deviations from the four-colony scheme will be recorded. The resulting 500-g composite represents equal contributions from the contributing colonies rather than contributions proportional to their honey yields and does not permit estimation of between-colony variability.

Honey from each contributing colony will be collected separately by mechanical uncapping and centrifugal extraction using clean, dry, food-contact equipment, without adding water or deliberately applying heat. Each portion will be homogenized before weighing, and the specified masses will be gently combined to produce the composite without excessive air incorporation. Fine filtration that could remove pollen will not be performed; removal of coarse wax particles and any protocol deviations will be documented.

Each composite sample will be placed in a clean, dry, airtight amber-glass container. Identification will include the apiary code (A01–A08), period (P1 or P2), and collection date, linked to a record of contributing colonies. For example, A01-P1 will identify the sample from the first apiary during the first period, with the collection date documented in the corresponding record. Honey from different apiaries or periods will not be pooled.

During transport and storage, the honey will be kept away from light, moisture, and heat sources at a target temperature of 20–25 °C, with actual temperatures and elapsed times recorded. Operational targets are laboratory receipt within 48 h of collection and analysis within 7 calendar days. Deviations and their potential effects on measurements will be documented. These time limits do not constitute a validated shelf-life claim.

Before analysis, each sample will undergo careful homogenization. Unheated aliquots will be reserved for HMF and applicable colorimetric assays. When crystallization impedes representative subsampling, the condition will be documented, and a conditioning procedure suited to the specific determination will be established beforehand. Where conditioning of other aliquots is justified, temperatures will not exceed 40 °C, and treatment duration will be recorded; preanalytical conditioning will be distinguished from heating inherent to an analytical method.

2.2. Floral Resource Survey

Floral resources surrounding the eight selected apiaries of Vibee La Apicultura S.A.S. BIC will be surveyed during the flowering periods planned for October 2026 and mid-January 2027. The aim will be to document the composition and phenology of flowering vegetation, identify resources potentially used by *Apis mellifera*, and contextualize melissopalynological profiles. Observations will cover the honey accumulation period associated with each harvest rather than only the extraction date. The beginning of each period will be determined from the previous harvest date, management records, and dated information from beekeepers; any uncertainty will be documented. The researcher responsible for the floral survey will personally visit all eight apiaries and complete the field records.

A survey area extending 1 km from each apiary will be defined, within which four fixed, accessible, 250-m transects will be

established to represent the main vegetation cover types. The starting and ending coordinates, land cover, accessibility, and unsurveyed sectors will be recorded. Overlapping areas between apiaries will be identified so that shared observations are not treated as independent evidence. The 1-km radius defines the botanical survey area, not the maximum foraging distance of honeybees.

Visits will occur at 14-day intervals from the documented start of monitoring through harvest, including an observation at the time of honey collection. The same transects will be used, and effective observation time will be recorded to maintain comparable sampling effort. If honey accumulation began before field visits, the discrepancy will be documented. Historical information from beekeepers will be used for context but will not be presented as direct observations.

For each transect, a field sheet will identify the apiary, period (P1 or P2), date, and visit code. Recorded variables will include coordinates, vegetation cover, survey duration (min), plant taxon or morphotype, flowering stage (absent, bud, open flowers, or senescence), approximate abundance of flowering individuals when they can be counted, observed bee visitation (yes/no), observed resource (nectar, pollen, or undetermined), photographic evidence, and field notes. Direct observations will be distinguished from beekeeper reports and documentary sources. The mere presence of flowering plants will not be considered evidence that bees use them.

Plant identification will use taxonomic keys, Colombian flora databases, herbaria, and reference collections. Where relevant permits are available, voucher specimens may be collected for taxonomic identification and preparation of reference pollen material. When species-level identification is not reliable, observations will be retained at the genus or morphotype level, with photographs and diagnostic characteristics, without making unsupported taxonomic assignments.

Phenological records will be linked to each composite sample by apiary code, flowering period, and honey accumulation and harvest dates. Interpretation of potential botanical origin will integrate pollen-type frequencies, flowering observations, and bee visitation evidence, recognizing that pollen abundance is not a direct measure of nectar contribution and that certain taxa may be underrepresented or overrepresented in pollen spectra. Thus, the survey can support hypotheses regarding floral sources but cannot independently demonstrate an exclusive botanical origin or distinctive territorial identity.

2.3. Physicochemical Characterization of Honey

Physicochemical characterization of the composite samples will follow the Harmonised Methods of the International Honey Commission (IHC), official AOAC methods, and the specific procedures cited for each determination (Bogdanov, 2009; Latimer,

2023). Each sample will be homogenized before aliquots are taken. Analyses will be performed in duplicate using two independently prepared analytical portions. Individual values will be retained and, once acceptance criteria are met, the mean and standard deviation will be reported. These determinations do not constitute additional biological replicates.

Analytical Quality Control

Instrumentation and traceability. An analytical balance, a refractometer with temperature compensation or control at 20 °C, a pH meter, a conductivity meter, a UV–visible spectrophotometer with suitable cuvettes, calibrated volumetric glassware, a centrifuge, and temperature-control devices will be used. The instruments must have measurement capability, resolution, and calibration or verification status appropriate to each analytical method. To ensure instrumental traceability, the manufacturer, model, equipment identifier, resolution, calibration or verification date, and analyst will be recorded.

Quality-control and duplicate acceptance criteria. Two independently prepared analytical portions will be analyzed per sample, and both results will be retained. For each IHC method, the absolute difference between duplicate results will be compared with the repeatability value (r) corresponding to the concentration level documented in the original collaborative study (Table 1), taking into account the method, the matrix, and the specific source of the precision study. These values will be applied only under comparable conditions and concentration levels and will not be interpreted as legal honey-quality limits. For adapted procedures without directly applicable collaborative precision data (color, °Brix, Folin–Ciocalteu, and flavonoid assays), acceptance criteria will be established through analytical performance verification using blanks, control samples, and spiked samples, as well as assessments of repeatability across independent preparations.

Each analytical run will include a reagent blank, calibration verification, and, where feasible, a homogeneous honey control sample. Colorimetric assays will include sample blanks and spike-recovery controls; a recovery range of 80–120% will be established as an operational criterion, subject to verification of method performance in the honey matrix under study. Discordant duplicate results will be investigated, and the determination will be repeated using a new independent pair, with all records retained. Samples will be received by the laboratory within 48 h after harvest, and analyses will be performed within 7 calendar days after collection. Samples will be protected from light and moisture at 20–25 °C, with temperature and elapsed time recorded; aliquots intended for HMF determination will not be preheated.

Table 1. Published Repeatability Values (r) From Collaborative Studies Included in the Harmonised Methods of the International Honey Commission (Bogdanov, 2009)

Parameter/ method	Concentration or study level	Published repeatability (*r*)	Specific source
Moisture, refractometry	Different moisture levels in the collaborative study (four levels corresponding to repeatability values of 0.13, 0.15, 0.15, and 0.11 percentage points); see the method's precision table for the exact level-by-level correspondence	0.13; 0.15; 0.15; 0.11 percentage points, respectively	Bogdanov (2009), IHC method 1, p. 12
pH	Different pH levels in the collaborative study; see the method's precision table for the exact level-by-level correspondence	0.07; 0.07; 0.06; 0.04–0.06; 0.06 pH units, depending on the reported level	Bogdanov (2009), IHC method 4.1, p. 22
Free acidity	Different free-acidity levels in the collaborative study; see the method's precision table for the exact level-by-level correspondence	0.9; 1.2; 1.5; 0.7; 0.9; 1.0; 1.7 mEq/kg, depending on the reported level	Bogdanov (2009), IHC method 4.1, p. 22
Electrical conductivity	Different conductivity levels in the DIN study; see the method's precision table for the exact level-by-level correspondence	0.002; 0.005; 0.020 mS/cm, respectively	Bogdanov (2009), IHC method 2, p. 18
HMF, White method	3.8; 22.3; 42.1 mg/kg	0.9; 1.2; 2.2 mg/kg, respectively	Bogdanov (2009), IHC method 5.2, pp. 30–31

Note. Compiled by the authors based on data reported in Bogdanov (2009).

Table 2. Reference Criteria for Honey Composition and Quality Under Codex CXS 12-1981 (Applicability Varies by Honey Category)

Parameter	Codex reference criterion	Interpretation
Moisture	Generally ≤20% w/w; *Calluna* honey ≤23%	Apply the criterion appropriate to the honey category.
Free acidity	≤50 mEq kg^{-1} (Annex)	Additional quality reference in the Annex; does not constitute comprehensive certification.

Parameter	Codex reference criterion	Interpretation
HMF	≤40 mg kg^{-1}; exception: ≤80 mg kg^{-1} for honey declared to originate in tropical areas and relevant blends (Annex)	Do not automatically apply the exception based solely on location in Colombia.
Electrical conductivity	Generally ≤0.8 mS cm^{-1} for common blossom honeys; ≥0.8 for honeydew/chestnut honey, with botanical exceptions (Annex)	Classification depends on botanical honey type.
Apparent reducing sugars (Lane–Eynon)	Not directly equivalent to the Codex fructose-plus-glucose criterion	Do not claim compliance with individual-sugar criteria.
pH, °Brix, Pfund color, phenolic and flavonoid equivalents	No general Codex limit in this comparison	Report descriptively; do not claim regulatory compliance.
Diastase, insoluble solids, individual sugars	Not measured in this study	Not assessed; avoid compliance claims.

Note. Based on Codex Alimentarius Commission (2022), CXS 12-1981 (revised in 2001; amended in 2019 and 2022). The 2022 amendment concerns the labeling of nonretail containers and does not change the physicochemical values compared here. Annex provisions are presented as additional voluntary reference criteria.

Moisture

Moisture will be determined by refractometry at 20 °C using a refractometer calibrated with water or an appropriate reference material. Refractive index will be converted to moisture (% w/w) using the honey-specific IHC tables (Bogdanov, 2009). The actual measurement temperature will be recorded.

pH and Free Acidity

Ten grams of honey will be dissolved in 75 mL of carbon dioxide-free water. The pH meter will be calibrated using buffers at pH 4.00, 7.00, and 9.00, and the pH of the solution will be recorded. Free acidity will be determined by titration to pH 8.30 with standardized NaOH (approximately 0.1 mol L^{-1}) according to the IHC method (Bogdanov, 2009). The blank-corrected result will be expressed in mEq kg^{-1} and calculated as follows: free acidity = $[(V - Vb) \times c \times 1000] / m$, where V and Vb are NaOH volumes for the sample and blank (mL), c is NaOH concentration (mol L^{-1}), and m is honey mass (g).

Electrical Conductivity

Conductivity will be measured at 20 °C in a solution containing 20 g of honey dry matter per 100 mL of solution, using a cell calibrated with an appropriate conductivity standard (Bogdanov, 2009). The required mass of wet honey will be calculated as $m = 20$ /

(1 - *H* / 100) *g*, where H is measured moisture (% w/w). Results will be expressed in mS cm^{-1} as a complementary indicator, without claiming direct quantification of individual minerals.

Apparent Soluble Solids

Apparent soluble solids will be determined by refractometry at 20 °C and reported as °Brix. This sucrose-equivalent reading is descriptive and does not quantify individual sugars; it will be interpreted alongside moisture because both measurements share a refractometric basis.

Hydroxymethylfurfural (HMF)

HMF will be determined using the White spectrophotometric method adopted by the IHC (Bogdanov, 2009; White, 1979). Approximately 5 g of honey will be dissolved in water; 0.5 mL of Carrez I and 0.5 mL of Carrez II will be added, followed by dilution to 50 mL and filtration, discarding the first 10 mL. The test solution will consist of 5 mL of filtrate and 5 mL of water, while the reference solution will contain 5 mL of the same filtrate and 5 mL of freshly prepared 0.2% w/v sodium bisulfite. Absorbance will be measured against the reference at 284 and 336 nm in 1-cm quartz cuvettes within the time interval specified by the method.

HMF content will be calculated as HMF(mg kg^{-1}) = (*A284* - *A336*) × 149.7 × 5/*m* × *D*, where A284 and A336 are the absorbances measured against the bisulfite reference, m is the actual honey mass in grams, and D is any additional dilution factor (D = 1 when no additional dilution is made). Interference and dilution controls prescribed in the full method will be applied. Aliquots will not be preheated.

Color

Color will be estimated by UV–visible spectrophotometry using a 50% w/v honey solution, water as the blank, and a 1-cm optical path. Absorbance will be recorded at 635 nm following the approach described in the original publication (Bianchi, 1990). Estimated Pfund values will be calculated as mm Pfund = 371.39 × *A*635 - 38.70. Turbidity will be recorded, and the suitability of the conversion will be checked against an appropriate standard or comparator. Out-of-range values will not be assigned to color categories without verification.

Apparent Reducing Sugars

Apparent reducing sugars will be determined by Lane–Eynon titration using standardized Fehling solutions according to the full IHC procedure for honey and applicable AOAC references (Bogdanov, 2009; Latimer, 2023). Dilutions, Fehling factor, indicator, endpoint, and calculations will be documented. Results will be expressed as % w/w. This assay does not separately quantify fructose, glucose, or sucrose and therefore will not be used to infer compliance with limits specified for individual sugars.

Folin–Ciocalteu Reactivity (Estimated Total Phenolic Content)

The low-alkalinity method adapted for honey by Lawag et al. (2023) will be followed. A 0.20 g mL^{-1} honey solution will be prepared, together with a working Folin reagent made

by diluting 1 mL of commercial 2 *N* reagent with 30 mL of water. One milliliter of working reagent will be added to 200 µL of honey solution or standard. After 5 min, 800 µL of 0.75% w/v Na_2CO_3 will be added. The mixture will be incubated in the dark for 2 h at a controlled temperature of 20–25 °C, and absorbance will be measured at 760 nm. The final reaction pH will be checked to confirm that it is close to 7.9, in accordance with the method's rationale.

The initial gallic acid calibration range will be 3–100 µg mL^{-1} before reaction; only the range demonstrating satisfactory analytical performance will be used for calculations. Reagent blanks, sample-color blanks, sugar-matrix controls, and recovery checks will be used to assess interference. Results will be expressed as mg gallic acid equivalents per kg of honey (mg GAE kg^{-1}) and interpreted as assay reactivity rather than selective quantification of all phenolic compounds or a direct measure of antioxidant capacity (Granato et al., 2016; Lawag et al., 2023).

Estimated Total Flavonoids

An aluminum-complexation colorimetric assay adapted from Cianciosi et al. (2020) will be used. A honey solution with a concentration of 0.20 g mL^{-1} will be prepared. To 125 µL of sample or standard, 625 µL of water and 37.5 µL of 5% w/v $NaNO_2$ will be added. After 6 min, 75 µL of 10% w/v $AlCl_3 \cdot 6H_2O$ will be added; after another 5 min, 250 µL of 4% w/v NaOH will be added, followed by water to a final volume of 1,250 µL (1.25 mL). Absorbance will be recorded at 510 nm, 1 min after final mixing at 20–25 °C. Catechin will serve as the reference standard, with an initial calibration range of 0.0008–0.05 mg mL^{-1} before reaction.

The final volume of 1,250 µL has been adopted to account for a possible unit misprint in the cited source: the component volumes total 1,112.5 µL, so 137.5 µL of water will be added to reach the final volume. This is an operational adaptation made for the present protocol rather than a verbatim reproduction of the published procedure. The use of µL as the unit for the final volume will be explicitly documented in the laboratory procedure.

Reagent blanks, sample blanks, and recovery checks will be performed. Results will be reported as mg catechin equivalents per kg of honey (mg CE kg^{-1}), recognizing that different flavonoids do not necessarily exhibit identical color responses. For both colorimetric assays, calculations will account for honey mass, initial solution volume, and any predilution without double-counting the reaction dilution already accounted for by identically treated calibration standards.

2.4. Melissopalynological Characterization

Melissopalynological characterization will follow the methods described by Louveaux et al. (1978) and the harmonized procedures of von der Ohe et al. (2004). The relative

composition of pollen types in each composite honey sample will be determined; this procedure will not quantify the absolute number of pollen grains per gram of honey.

Sample Preparation

For each sample, 10 g of honey will be dissolved in 20 mL of distilled water at 20–40 °C. The suspension will be centrifuged at 1,000 × *g* for 10 min; the supernatant will be carefully removed, the sediment will be washed with 20 mL of distilled water, and the preparation will be centrifuged again at 1,000 × *g* for 5 min. The recovered sediment will be mounted in glycerinated gelatin on clean microscope slides. Sample-specific materials will be labeled, and cross-contamination and airborne pollen contamination during mounting will be prevented.

Microscopic Examination and Identification

Slides will be examined under a light microscope using 40×–100× objectives, corresponding to total magnifications of 400×–1,000×. Pollen types will be identified on the basis of size, shape, symmetry, apertures, and exine ornamentation, supported by pollen atlases, reference collections, and plant records from the field surveys (Halbritter et al., 2018). Taxonomic assignments will be reported at the level supported by the evidence (species, genus, family, or reproducible morphotype), without making unsupported identifications.

Microscopic Counting

An initial exploratory count of 100 pollen grains per preparation will be performed across five evenly spaced, parallel transects, avoiding repeated examination of the same microscopic fields. Counts by pollen type, the number of unidentified grains, and the total number counted will be recorded. Because of the limited count size, the resulting frequencies will be interpreted as preliminary descriptive estimates; rare pollen types may be missed, and a conclusive monofloral classification will not be established on the basis of this count alone.

Frequency Calculations and Treatment of Unidentified Grains

The observed relative frequency of each type will be calculated using the actual number of grains counted (N = 100 when the initial count is completed), as follows:

$$F_i\,(\%) = \left(\frac{n_i}{N}\right) \times 100 \qquad (1)$$

where n_i is the number of grains of type i and N is the total pollen count, including unidentified grains. Unidentified grains will be reported separately and will not automat-

ically be treated as pollen from nectarless plants. When botanical interpretation justifies excluding confirmed nectarless taxa, both original and adjusted frequencies will be reported:

$$F_i\ adjusted\ (\%) = \left[\frac{n_i}{(N - E)}\right] \times 100 \quad (2)$$

where E is the number of grains excluded, with documented justification. Adjusted frequencies will be calculated only when N – E is greater than zero.

For classification and interpretation, conventional frequency classes will be used: predominant pollen, >45%; secondary pollen, >15% to 45%; important minor pollen, 3% to 15%; and minor pollen, <3% (Louveaux et al., 1978; von der Ohe et al., 2004). Percentages will be reported with sufficient precision to apply these boundaries unambiguously. These categories describe pollen abundance but do not independently establish monofloral status or prove exclusive geographic origin.

Interpretation will integrate pollen spectra, phenological records, observations of plants around the eight apiaries, and the physicochemical characteristics of each sample, taking into account differences in pollen production and dispersal, as well as possible contributions from nectarless plants. Spectra that do not support sufficiently robust botanical attribution will be reported as inconclusive. Apiary and harvest-period identifiers will be maintained to ensure traceability of the interpretations.

2.5. Statistical Analysis

The study will be descriptive and exploratory. Each apiary will constitute the longitudinal observational unit, and each apiary–period composite will be the measurement unit. Eight apiaries will be observed in P1 (October 2026) and P2 (mid-January 2027), yielding at most 16 composite samples and up to eight complete pairs. Four colonies per apiary will contribute to one composite and will therefore not be treated as independent analytical replicates or statistical units. Likewise, repeated observations of a given apiary will not be considered independent.

For each physicochemical parameter, two independent analytical determinations will be performed on the composite sample. Both results will be recorded and, when they meet the acceptance criteria described in Section 2.3, their mean will be used as the apiary–period value. Analytical duplicates will not increase the biological sample size. Pollen composition will be analyzed using the relative frequencies of identified types and, descriptively, pollen-type richness per sample.

Variables will be summarized separately for P1 and P2 using the number of available observations (n), median, interquartile range, minimum, and maximum. Mean and standard deviation will be provided as complementary information when interpretable. Changes will be visualized by plotting individual values and connecting observations from the same apiary, and complete pairs will be summarized by the paired difference $\Delta = P2\text{-}P1$ in each variable's original units. The number of complete pairs, the distribution of differences, and any changes in contributing-colony composition will be reported. No paired difference will be calculated for apiaries lacking valid measurements in either period.

Physicochemical associations will be explored using Spearman's rank correlation coefficient (ρ), calculated separately for each period and accompanied by the number of valid observations for each variable pair. Exploratory associations of primary interest will include HMF–color, moisture–free acidity, conductivity–color, and Folin–Ciocalteu reactivity–aluminum-complexation response. The 16 composite samples will not be pooled as independent observations for a single correlation analysis. Associations will not be interpreted causally. Analysis of pollen types will consider relative abundance, frequency of occurrence, and the constraints inherent in compositional data.

The feasibility of using principal component analysis (PCA) solely as an exploratory graphical tool for physicochemical variables will be assessed. Variables will be centered and scaled where measurement units differ; invariant variables will be excluded, and the number of complete observations will be documented. Moisture and °Brix will not be included simultaneously because they share a refractometric basis. The two periods will be distinguished, and observations from the same apiary will be connected in plots.

As a conservative operational rule, PCA will be omitted if fewer than eight complete observations or fewer than three suitable variables are available. Even when undertaken, loadings and groupings will be interpreted cautiously because of the small sample size; PCA will not be presented as proof of geographic differentiation or a validated classification.

3. EXPECTED OUTCOMES

As part of the preparatory documentation developed before experimental implementation, the study framework has been defined to include eight apiaries with four selected colonies each (32 total), two planned sampling windows (October 2026 and mid-January 2027), and up to 16 composite samples. IHC analytical methods and the floral survey and melissopalynological strategies have also been selected, and their interpretive limitations have been identified. These are protocol-development and literature-review outputs, not measured physicochemical or melissopalynological results. No sample values, pollen profiles, or experimental comparisons are reported in the absence of preliminary data.

The study is expected to produce a traceable baseline of the physicochemical and melissopalynological characteristics of *Apis mellifera* honey from eight apiaries operated by Vibee La Apicultura S.A.S. BIC during the two planned periods (P1 and P2), subject to the availability of eligible honey.

The first planned deliverable will be a dataset linking each sample to its apiary, actual contributing colonies, harvest dates and conditions, extraction, transport, storage, and floral survey records. These data will allow production conditions to be described and changes in composite composition to be identified when different colonies contribute in the two periods.

Physicochemical characterization will provide sample-level results for moisture, pH, free acidity, electrical conductivity, apparent soluble solids, HMF, color, apparent reducing sugars, and colorimetric responses expressed as gallic acid and catechin equivalents. Results will be accompanied by both analytical readings, quality-control checks, and flags identifying nonquantifiable or unacceptable measurements. Colorimetric responses will be interpreted as operational estimates rather than as chemically specific identifications of phenolics or flavonoids or direct measurements of antioxidant activity.

The melissopalynological component is expected to yield relative-frequency spectra of pollen types, with explicit reporting of unidentified grains and any criteria used to adjust frequencies. Interpreting these spectra alongside phenological surveys will support hypotheses regarding potential botanical sources, as well as descriptions of multifloral profiles or the possible predominance of particular pollen types. The abundance of a single pollen type will not, by itself, be used to certify monofloral origin.

Between-period comparisons will be presented as separate descriptive summaries, apiary-level plots, and paired P2 – P1 differences only for apiaries with observations in both periods. Spearman correlations and, if feasible, PCA will be exploratory, without claims of causality, statistical significance, or territorial validation. Missing data, changes in contributing colonies, and verified atypical values will be explicitly addressed in the interpretation.

Regulatory comparisons will be conducted parameter by parameter, documenting the method, units, reference value, honey category, and scope of assessment according to Table 2. Codex criteria will be applied only where the category and analytical method are appropriate. Comparison with NTC 1273 would require an authenticated official edition and verification of its applicability; compliance will not be claimed without such verification. Apparent reducing sugar values obtained using the Lane–Eynon method will not be equated with the sum of individually measured fructose and glucose. Unmeasured attributes, including diastase activity, individual sugars, contaminants, and authenticity, will be designated as not assessed.

Overall, the anticipated findings may provide technical evidence for future harvesting, extraction, storage, traceability, and quality-

control decisions and support the associated degree project and subsequent research on Boyacá honey. Because the design covers eight apiaries belonging to one enterprise over two periods, the baseline will neither identify properties exclusive to Monguí nor support generalization to all honey produced in the municipality. Territorial differentiation will remain a subject for future research requiring comparisons with other origins and independent validation.

4. CONCLUSIONS

This proposal presents a research protocol for the physicochemical and melissopalynological characterization of *Apis mellifera* honey produced in eight selected apiaries in Monguí, Boyacá. Its principal methodological contribution is the integration of laboratory measurements, floral resource surveys, and production, handling, and traceability records across two flowering periods. This approach is intended to provide a documented baseline and to inform future quality-control decisions for the beekeeping system under study.

Monitoring four colonies per apiary (32 total) and obtaining up to 16 composite samples will make it possible to describe the properties of the honey blends and to explore within-apiary changes between October 2026 and January 2027. However, pooling individual contributions will prevent assessment of between-colony variability, while the small sample size, nonprobability selection, and restriction to a single enterprise will limit generalization and causal inference.

Integrating pollen profiles with phenological observations will provide evidence for interpreting potential botanical sources without assuming that the predominance of a pollen type alone establishes monofloral origin. Likewise, comparisons with reference criteria will be limited to the parameters and methods assessed; no inference will be made regarding comprehensive regulatory compliance, authenticity, functional properties, or health effects that have not been measured.

Implementation of the protocol will require documented sampling during both designated periods, sample traceability, and application of the specified analytical quality controls. The evidence generated may support recommendations on harvesting, extraction, and storage and help highlight the productive value of honey from Monguí. Demonstrating exclusive characteristics or territorial differentiation would require subsequent studies incorporating honey from other origins and independent validation; such differentiation remains a future research objective rather than a predetermined outcome.

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