

Floral offer, honey and wax quality from hybrid and European bees in Nariño-Colombia

Oferta floral y calidad de miel y cera de abejas híbridas y europeas en Nariño-Colombia

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ABSTRACT

Beekeeping is one of the most significant agricultural activities in Colombia, despite the lack of technological and industrial advancements in this field. Therefore, the floral offer and the quality of both honey and beeswax from hybrid and European bees were evaluated. The study was conducted at the University of Nariño's apiary (Colombia). Colonies of hybrid and European bees were used to determine the floral offer and its apicultural potential, as well as the production and quality of honey and beeswax during the period from May 2022 to February 2023. Seventeen nectariferous and polliniferous plant species were found, with the Asteraceae and Fabaceae families standing out. Differences were observed in honey production, with values of 6206 g/colony for European bees and 3971 g/colony for hybrid bees. Brix degrees of 81.0 were found for hybrid bee honey and 80.0 for European bee honey, with a pH of 4.15 in hybrid bees and 4.52 in European bees. Differences were observed in beeswax production and a good physicochemical composition (moisture <2 g/100g and 2.30 g/100g in hybrid and European bees, respectively). Microbiological results showed the absence of *Salmonella* and *Escherichia coli* <10 CFU/100g in the beeswax of both types.

Keywords: apiarian flora; *apis mellifera*; nectariferous; operculum; polliniferous; wax glands

RESUMEN

La apicultura es una de las actividades agropecuarias más importantes en Colombia, a pesar de la falta de avances tecnológicos e industriales en este campo. Por ello, se evaluó la oferta floral y la calidad tanto de la miel como de la cera de abejas híbridas y europeas. El estudio se llevó a cabo en el Apiario de la Universidad de Nariño (Colombia). Se usaron colmenas de abejas híbridas y europeas para determinar la oferta floral y su potencial apícola, así como la producción y calidad de la miel y la cera de abejas durante el periodo comprendido entre mayo de 2022 a febrero de 2023. Se encontraron diecisiete especies vegetales nectaríferas y poliníferas, destacándose las familias Asteraceae y Fabaceae. Se observaron diferencias en la producción de miel, con valores de 6206 g/colmena para las abejas europeas y 3971 g/colmena para las abejas híbridas. Se encontraron grados brix de 81,0 para la miel de abejas híbridas y 80,0 para la de abejas europeas, con un pH de 4,15 en abejas híbridas y 4,52, en las de tipo europeo. De igual forma, se observaron diferencias en la producción de cera de abejas y una buena composición fisicoquímica (humedad <2 g/100g y 2,30 g/100g en abejas híbridas y europeas respectivamente). Los resultados microbiológicos presentaron ausencia de *Salmonella* y *Escherichia coli* <10 UFC/100g en la cera de abejas de ambos tipos.

Palabras clave: *apis mellifera*; flora apícola; glándulas de cera; nectarífera; opérculo; polinífera

INTRODUCTION

In Colombia, beekeeping is one of the longest-standing agricultural activities, yet it has not experienced significant technological and industrial advancements to improve its production processes. Most Colombian beekeepers are farmers who rely on traditional knowledge and dedicate only a portion of their time to apitary work (Correa *et al.*, 2019).

Despite global concerns about a potential bee extinction crisis, the Colombian beekeeping sector has shown significant growth in recent years, evidenced by increases in hive numbers and honey production. Bees are highly valued for their role in crop pollination, and preserving them requires favorable environmental conditions. The country's geographical diversity and floral richness position it as a potential regional leader in beekeeping. Ninety percent (90%) of Colombian beekeepers are small-scale producers managing, on average, up to 20 hives. This highlights the importance of supporting small-scale beekeeping initiatives and, if possible, scaling them to a mid-level (Baena-Díaz *et al.*, 2022).

The beekeeping potential in Nariño, Colombia, suggests a promising future for the industry due to recent collaborations among beekeeping farmers to formalizing associative processes. While there are opportunities for strengthening beekeeping, addressing the lack of knowledge and practices regarding production, bee species, bee-friendly flora, and other factors influencing the productivity and quality products like honey and beeswax remains crucial (Caicedo-Terán, 2022).

Colombian beekeeping primarily focuses on hybrid bees, with limited opportunities to work with pure European species. The latter are known for their management advantages, particularly their lower aggressiveness compared to Africanized and hybrid bees. However, hybrid bees are favored for their higher honey production, which leads to better overall hive productivity (Gonzalez-Delgado *et al.*, 2022).

The aforementioned reasons have allowed us to investigate not only the contributions of plant diversity to bees but also how different bee types influence honey and wax production and quality, aiming to optimize these parameters. To achieve this, we evaluated the floral supply, production, and quality of honey and beeswax from *Apis mellifera* hybrid and *Apis mellifera* European bee types.

MATERIAL AND METHODS

The study was conducted at the “Botana” Experimental Farm in Catambuco, a small town near Pasto, Nariño, Colombia. This town is located at an altitude of 2800 to 3200 meters above sea level. The average climatic conditions in Catambuco are as follows: approximately 32.4 mm of rainfall per month, a temperature of 13.11°C, and a relative humidity of 91% (IDEAM, 2021).

Floral evaluation and bee ethology

A characterization of the bee floral resources was conducted using the methodology described by Muñoz-Galíndez (2022) and Cardona-Reyes *et al.* (2022). The following plant covers were considered for the study: meadows, agricultural crops, living fences, forests, and ornamental plants, among others. To identify and classify the plants, the radial transect methodology was employed, using hives placed at a distance of 100 m and a width of 4 m (Pérez & Matus, 2010).

The floral inventory and the type of food resource offered (pollen, nectar, or both) were recorded. Pollen was assessed by observing pollen loads in the corbiculae or on the bee's body, while nectar was indicated by the dilation and contraction of the bee's abdomen during foraging (Aguilar Sierra & Smith Pardo, 2009). Additionally, the preference of *Apis mellifera* bees for flower coloration was recorded by noting the time spent on each flower in a 1 m² area, measured with a stopwatch. The frequency of visits was measured from 8 to 11 AM and 2 to 5 PM, with each observation lasting 5 min (Velandia *et al.*, 2012). The results were categorized using the following scale: Low Frequency (less than 3 bees/5 min/m²); Medium Frequency (4 to 8 bees/5 min/m²); High Frequency (more than 9 bees/5 min/m²).

In addition to the previously mentioned data, a floral calendar for the area was created through biweekly one-day tours for 10 months. During these tours, individuals of each plant species were randomly observed to establish their flowering phases. The investigation was further enriched with information provided by the farm's technical staff.

Production and bee honey analysis

The research team established honey production from hives of both hybrid *Apis mellifera* and European *Apis mellifera* bees. For the selection process, hives were chosen based on similar characteristics, including the number of populated frames, frames with honey and pollen reserves, and the age of the queen bee. The hives were inspected biweekly to monitor population growth, queen behavior, frame expansion, and overall health. These periodic assessments facilitated productive diagnoses and the identification of optimal honey harvest times.

Bee honey. Six frames were extracted from each hive and weighed when full of honey. After removing the honey, the operculum wax was weighed, and the frames were centrifuged to extract the remaining honey. Finally, the empty frames were weighed. Samples of 300 g of honey were collected in sterile glass jars and stored for subsequent analysis. To calculate the honey production per hive, the weights of the empty frames and operculum wax were subtracted from the weight of the full frames. The collected honey underwent laboratory analysis to determine its color, scent, appearance, flavor, and physicochemical properties, including Brix degrees, pH, moisture content, solids content, sugar composition (glucose and sucrose), and mineral content. Microbiological analyses for total coliforms, *Salmonella*, and yeasts were also performed.

Production and quality of beeswax

Measurement and monitoring techniques were employed to collect the necessary data during monthly technical visits to the apiary. Additionally, staff members and researchers from the beekeeping unit provided support and participated in various activities.

Beeswax production. Wireframes with stamped wax were weighed before being placed in the hive. After honey harvesting, the frames were reweighed, and the weight of the extracted operculum wax was added to the final weight. Six frames of honeycombs were extracted from each hive and weighed on a digital scale. The collected wax was purified in the laboratory using the method described by Al-Shehri *et al.* (2022). The wax was melted in a water bath at 65°C and then filtered through filter paper.

Beeswax quality. Analyses of color, appearance, and smell were conducted. Additionally, physicochemical parameters such as moisture content, ash content, protein content, fat content, and pH were determined. Microbiological analysis was performed to assess the presence of total coliforms, *Salmonella*, yeasts, aerobic spores, and enterobacteria in the beeswax.

Statistical analysis

Data were collected and organized in an Excel spreadsheet. Statistical analyses were performed using R 4.3.2 with the agricolae 1.0.3 package. A Student's t-test was used to compare honey and wax production between the different bee breeds at a 95% significance level. Other parameters were evaluated using descriptive statistics and graphical representations.

RESULTS AND DISCUSSION

Characterization of the Beekeeping Floral Supply of nectariferous and polliniferous Species.

Seventeen honey plant species were identified on the farm. Table 1 presents the most important characteristics of the species found.

Table 1. Characteristics of the beekeeping flora present on the farm.

Genus and species	Common name	Family	Growth habit	Vegetable cover	Relative abundance		
					Nectar	Pollen	Nectar and Pollen
<i>Abutilon pictum</i> (Gillies ex Hook. & Arn.) Walp	Abutilon	Malvaceae	Bush	Living fence	-	-	x
<i>Acacia decurrens</i> Willd	Acacia negra	Fabaceae	Tree	Forest	-	x	x
<i>Acacia melanoxylon</i> R. Br.	Acacia	Fabaceae	Tree	Forest	-	x	-
<i>Bidens andicola</i> L.	Gallinazo	Asteraceae	Herbaceous	Weed	-	-	-
<i>Brassica rapa</i> L.	Nabo amarillo	Brassicaceae	Herbaceous	Weed	-	-	x
<i>Cirsium vulgare</i> (Savi) Ten.	Cardo	Asteraceae	Bush	Weed	-	-	x
<i>Dahlia imperialis</i>	Margarita	Asteraceae	Bush	Crop	-	-	x
<i>Eucalyptus globulus</i> Labill.	Eucalipto	Myrtaceae	Tree	Forest	-	-	x
<i>Fuchsia dependens</i> Hook	Bailarina	Onagraceae	Bush	Weed	-	-	x
<i>Hypochaeris radicata</i> L.	Achicoria amarilla	Asteraceae	Herbaceous	Weed	-	-	x

Genus and species	Common name	Family	Growth habit	Vegetable cover	Relative abundance		
					Nectar	Pollen	Nectar and Pollen
<i>Pisum sativum</i> L	Arveja	Fabaceae	Herbaceous	Crop	-	-	x
<i>Rubus bogotensis</i> Kunth	Mora silvestre	Rosaceae	Bush	Weed	x	-	x
<i>Sambucus nigra</i> L.	Sauco	Adoxaceae	Bush	Living fence	-	-	x
<i>Taraxacum officinale</i> L.	Diente de León	Asteraceae	Herbaceous	Weed	-	-	x
<i>Trifolium pratense</i> L.	Trébol rojo	Fabaceae	Herbaceous	Weed	-	-	x
<i>Trifolium repens</i> L.	Trébol blanco	Fabaceae	Herbaceous	Weed	-	-	x
<i>Zea mays</i> L:	Maíz	Poaceae	Herbaceous	Crop	-	x	x

The results indicate that Asteraceae and Fabaceae were the most abundant families, each comprising 29% of total floral composition. This finding aligns with previous studies that highlight the high availability of these families in apicultural significant regions, particularly in southwestern Colombia. The predominance of these families is attributed to their diversity and their ability to provide continuous floral resources to bees throughout the year (Cardona-Reyes *et al.*, 2022).

In contrast, Poaceae, Rosaceae, Brassicaceae, and Malvaceae each accounted for 6% of the total composition. Regarding growth habits, herbaceous plants comprised 47%, followed by shrubs at 35% and trees at 18%. The vegetation cover demonstrated values of 47% for weedy species, 18% for forests, 18% for crops, and 17% for living fences. This indicates a considerable variety of species in the system, resulting from the high availability of melliferous resources. The management of apicultural floral resources, alongside the extensive agricultural activities on the farm, has improved the nutritional supply for bees, thereby increasing both floral availability and hive food.

The predominance of herbaceous plants (47%) is understandable, as this type of vegetation provides continuous and accessible flowering for bees throughout much of the year, particularly in open areas. This finding aligns with previous research in southwestern Colombia, which identified herbaceous plants as pioneer species in disturbed areas, providing valuable resources during the regeneration of other vegetation types (Sardi Saavedra *et al.*, 2024). Shrubs, comprising 35% of the flora, contribute to stability and a prolonged food source, while trees, although representing only 18%, are crucial for the apicultural landscape, offering shelter and high-quality pollen during specific flowering periods (Jarvis *et al.*, 2007).

In terms of relative resource frequency, 76.5% of the species provided both pollen and nectar, 17.6% provided only pollen, and 5.9% offered only nectar. These findings are consistent with previous research by Potosí-Criollo & Yopez-Moncayo (2015) in the same area, which identified 19.44% of species as pollen

sources, 19.44% as nectar sources, and 61.11% as poly-nectariferous species. These results emphasize the importance of maintaining a diverse range of apicultural floral species to ensure a continuous food supply throughout the year.

This study underscores the importance of planning diversified apicultural landscapes that provide bees with a year-round supply of diverse floral resources. It is crucial to continue promoting sustainable management practices that integrate the preservation of native flora with the responsible utilization of agricultural ecosystems.

Duration of *Apis mellifera* Visits and Flower Color. Figure 1 illustrates the average durations of bee visits to flowers of various evaluated plant species, categorized by five distinct flower colors: yellow (seven species), white (six species), violet (two species), red (one species), and salmon (one species). These analyses are crucial for understanding how bees select flowers based on visual signals, with implications for both beekeeping and biodiversity conservation.

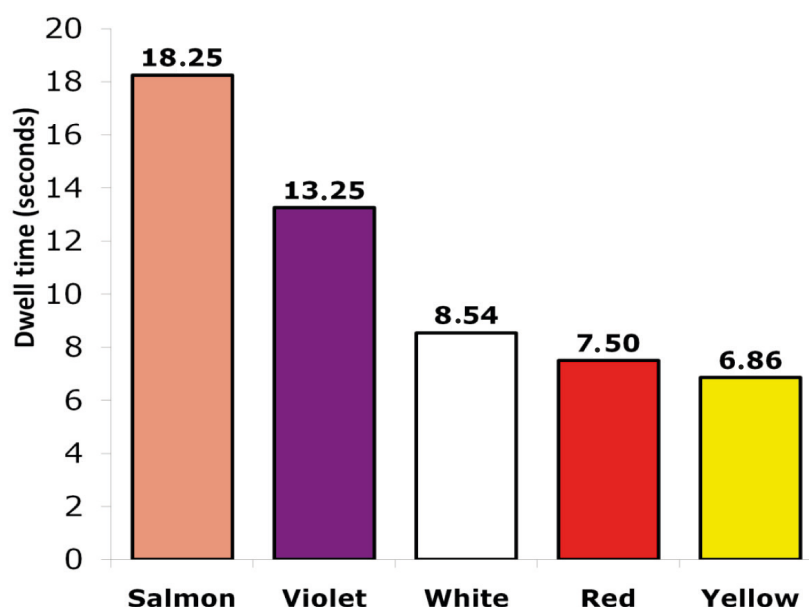


Figure 1. Flower color and bees Stay Time (seconds), salmon, violet, white, red, yellow *Apis mellifera* visit frequency.

Yellow, represented by seven species, was the most prominent color in the studied ecosystem. Yellow flowers tend to attract more bees due to their high visibility in the bee's visible light spectrum and their association with high nectar yields (Sardi Saavedra *et al.*, 2024). White flowers, comprising six species, are also highly attractive to bees due to their UV light reflection, which is invisible to humans but strongly attracts these pollinators (Jarvis *et al.*, 2007).

The violet color, present in only two species, may be less abundant in the evaluated apicultural ecosystem. However, previous studies have shown that when present, violet flowers can be significant nectar sources. Red and salmon flowers, each represented by one species, generally attract fewer bees, as red falls outside their visible spectrum. However, some red flowers may

produce more abundant nectar, which is visible in the UV spectrum, facilitating pollination (Jarvis *et al.*, 2007).

These results demonstrate a clear correlation between nectar availability and flower visibility in the spectrum perceived by bees. In ecosystems where yellow and white flowers predominate, a higher level of apicultural activity are likely observed. However, it is important to continue promoting the conservation of less common flower colors to ensure floral diversity that supports the health and productivity of bee colonies.

It was observed that violet and salmon-colored flowers, specifically *Cirsium vulgare* (Savi) Ten and *Trifolium pratense*, retained bees for longer durations. However, comparable residence times were observed among white, red, and yellow-colored flowers.

The limited number of bees observed for each flower color, except for yellow, prevented conclusive findings. Amaya-Marquez (2009) suggests that bees perceive color differently than humans, with color perception changing as they approach a flower. Brunet *et al.* (2021) found that bees associate color with the benefits they derive from a flower, making the color less relevant until it is correlated with the flower's nutritional value.

Table 2 presents the average frequency of bee visits, both quantitatively and qualitatively.

Table 2. *Apis mellifera* visit frequency

Botanical Name	Visits number	Frequency
<i>Sambucus nigra</i> L.	3.25	*
<i>Bidens andicola</i> L.	3.50	*
<i>Pisum sativum</i> L.	3.50	*
<i>Hypochaeris radicata</i> L.	4.00	**
<i>Trifolium pratense</i> L.	4.25	**
<i>Cirsium vulgare</i> (Savi) Ten.	4.50	**
<i>Dahlia imperialis</i> Roetzl	4.50	**
<i>Acacia melanoxydon</i> R.Br.	4.75	**
<i>Eucalyptus globulus</i> Labill.	5.25	**
<i>Fuchsia dependens</i> Hook	5.25	**
<i>Taraxacum officinale</i> L.	5.25	**
<i>Acacia decurrens</i> (J.C.Wendl.) Willd.	5.50	**
<i>Abutilon pictum</i> (Gillies ex Hook. & Arn.) Walp.	6.75	**
<i>Trifolium repens</i> L.	7.25	**
<i>Zea mays</i> L.	8.75	**
<i>Brassica rapa</i> L.	11.50	***
<i>Rubus bogotensis</i> Kunth	12.25	***

*Low frequency **Mid frequency *** High frequency

Species such as *Bidens andicola*, *Pisum sativum*, and *Sambucus nigra*, comprising 17.64% of the evaluated specimens, received fewer bee visits. According to Potosí-Criollo & Yepez-Moncayo (2015), the number of bee visits increases with a greater number of open flowers. Additionally, species such as *Abutilon pictum*, *Acacia decurrens*, *Acacia melanoxylon*, *Bidens andicola*, *Carduus canthoides*, *Eucalyptus globulus*, *Fuchsia dependens*, *Hypochaeris radicata*, *Taraxacum officinale*, *Trifolium repens*, and *Zea mays* exhibited an average frequency of 5 to 10 bees per 5 minutes per plant.

These species comprise 64.7% of the total number of plants analyzed. Their presence on the farm is highly significant, likely influencing a greater frequency of visits by *Apis mellifera*. Rodríguez *et al.* (2011) report that dominant plant species in ecosystems often receive the highest frequency of bee visits, possibly due to bee communication regarding food source locations.

Only *Brassica rapa* and *Rubus bogotensis* species exhibited a high frequency of visits, exceeding 9 bees per 5 minutes per plant. This suggests a significant presence of these species in the area, likely influencing the high frequency of *Apis mellifera* visits. Barazani *et al.* (2019) noted that the frequency of bee visits increases with a greater number of open flowers, especially during clear weather, and decreases during cloudy conditions or rainfall.

Blossoming Periods. According to Table 3, blossoming occurred throughout the entire evaluation period, with at least eight species showing open corollas, except for the month of November, when only five flowering plant species were observed. This phenomenon suggests that plant diversity contributes to a sustained availability of blossoming and food resources for bees throughout the year in a given region.

Table 3. Blossoming periods of the selected plant species.

Botanical name	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb
<i>Abutilon pictum</i>										
<i>Acacia decurrens</i>										
<i>Acacia melanoxylon</i>										
<i>Bidens andicola</i>										
<i>Brassica rapa</i>										
<i>Cirsium vulgare</i> (Savi) Ten.										
<i>Dahlia imperialis</i>										
<i>Eucalyptus globules</i>										
<i>Fuchsia dependens</i>										
<i>Hypochaeris radicata</i>										
<i>Pisum sativum</i>										
<i>Rubus bogotensis</i>										
<i>Sambucus nigra</i>										

Botanical name	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb
<i>Taraxacum officinale</i>										
<i>Trifolium pretense</i>										
<i>Trifolium repens</i>										
<i>Zea mays</i>										

Knowledge of plant blossoming periods in areas with beehives can improve beekeeping management by allowing for the planning of periods of resource abundance and scarcity. As Insuasty-Santacruz *et al.* (2016) noted, understanding the plant species visited by *Apis mellifera* and their floral phenology is crucial for optimizing beekeeping operations. Months with abundant flowering can provide favorable conditions for pollen and nectar collection. However, environmental factors like rainfall can limit bee foraging activities and flight time, as highlighted by Ngo *et al.* (2021).

Some plants, such as *Trifolium repens*, *Taraxacum officinale*, and *Bidens andicola*, displayed continuous flowering throughout the study period and were categorized as weed plants. Aguilar *et al.* (2021) noted that while these weed plants exhibit a high level of self-pollination, they also rely on cross-pollination for genetic diversity.

Bee honey production

Information about honey production is presented in Table 4. One European-type colony encountered challenges, including *Varroa* mite infestation and reduced queen-laying activity, which hindered the growth of the bee nuclei and consequently impacted resource collection by foraging bees.

Table 4. Production and quality of honey and beeswax

Item	Characteristic	Hybrids	Europeans	EEM	p-value
Honeybee	Production (g)	6226	3972	12.56	0.025
	Color	Amber	Amber	---	---
	Aspect		Acceptable	---	---
	Smell	Acceptable	Acceptable	---	---
	Flavor	Acceptable	Acceptable	---	---

Furthermore, the queen's replacement, a natural process, further delayed hive development, limiting population growth and preventing honey production during the experimental period. As Tenczar *et al.* (2014) noted, bees adjust their work activity based on external factors like food availability and internal factors such as hive demographics.

The statistical test revealed significant differences in honey production ($p < 0.05$), potentially attributable to various bee characteristics. Sereno *et al.* (2004) noted that while European and Africanized bees exhibit similar morphologies,

they display distinct behavioral patterns. Hybrid bees, resulting from the genetic intermingling of *Apis mellifera* scutellata and European bee races in South America, demonstrate remarkable adaptability.

Giray *et al.* (1999) observed that Africanized bees initiate work earlier, typically between 12 and 14 days after birth, compared to European bees, which start working between 14 and 16 days. Valderrama (2010) noted that the hybridization of Africanized bees with European subspecies, combined with selective breeding practices, has led to improvements in honey production.

Bee honey quality. The interplay of environmental factors, hive health, and genetics is crucial for implementing production practices that enhance the quality of hive products and bolster the competitiveness of the beekeeping industry, benefiting both beekeepers and associations.

According to honey analysis criteria, the organoleptic characteristics were within acceptable limits, classifying the product as fit for consumption. The honey exhibited an amber color, while Colombian Technical Standard NTC 1273 (ICONTEC, 2007) does not explicitly define quality levels for these characteristics, it mandates that honey must be free from unacceptable flavors, aromas, and contaminants acquired during production, processing, or storage. Additionally, the standard stipulates that honey must not show signs of fermentation or effervescence.

Table 5. Nutritional and physical-chemical variables of bee honey

Variable	Unit	Hybrid bees	NTC 1273:2007
Hum	g/100 g	18.6	Max 20
Attal	meq/1000 g	36.33	50 meq/1000 g
Cenz	g/100 g	0.15	Max 0,6
AREg	g/100 g	64.44	Min 65 %
AREs	g/100 g	0.92	Max 10 %
Pbh	g/100 g	0.64	N.D
pH	Und	4.15	N.D
	°C	21.4	
Gbrix	° Brix	81	N.D
	T°C	20	
HMF	mg/kg	0.72	Max 80 mg/kg
Ca Ttal	mg Ca/100 g	8.14	N.D
K Ttal	Mg k/100g	82.38	N.D
Mag Ttal	mg Mg/100g	1.96	N.D
Fe Ttal	mg Fe/100g	0.55	N.D
P Ttal	mg P/100 g	0.22	N.D

Hum: humidity, Attal: total acidity, Cenz: ash, AREg: reducing sugars expressed in glucose, AREs: reducing sugars expressed in sucrose, Pbh: protein on a wet basis, Gbrix: degrees Brix, HMF: hydroxymethyl furfural, Ca ttal: calcium total, K Ttal: total potassium, Mag Ttal: total magnesium, Fe Ttal: total iron, P Ttal: total phosphorus.

The humidity value obtained in this research falls within the standard humidity limit range, indicating that the sampled honey meets this compositional requirement and is suitable for human consumption. Moisture content is a crucial physicochemical parameter that influences honey's stability and resistance to microbial degradation, particularly fermentation caused by yeasts (Khalil *et al.*, 2012).

The acidity value indicates that the honey has not undergone any quality alterations or unwanted fermentation. Acidity is a factor that helps identify the degree of deterioration and the development of undesirable fermentation processes, which can negatively impact the texture, stability, and shelf life of the product (Dias *et al.*, 2017).

The ash content exceeds the criteria established by NTC 1273, classifying the honey as having significant mineral content. According to Vásquez-Romero *et al.* (2012), ash content can differentiate honey types and identify potential adulteration. The authors suggest that ash content is typically lower in floral honey compared to honeydew honey. Adulteration with molasses, for example, can significantly increase ash content to around 10%. The determined sugar content values comply with the physicochemical requirements specified by the Colombian standard, which establishes recommended glucose levels of $\geq 60\%$ for floral honeys and sucrose levels of $\leq 10\%$ for tropical honey (Ministerio de la Protección Social, 2010).

According to Insuasty-Santacruz *et al.* (2016), the protein content typically ranges around 0.5%. They found a protein content of 0.41% in honey from bees analyzed in the same study area, a value similar to that found in the present investigation and indicating that the honey is within the normal range for an unadulterated product.

Regarding pH, Ribeiro *et al.* (2014) mention that normal honey pH values range between 3.2 and 4.5, allowing natural acidity to inhibit the growth of pathogenic microorganisms and ensure honey preservation. The pH values of the honey samples from hybrid bees (4.15) and European bees (4.52) fall within this range.

Carbohydrates are the primary component of honey. The main sugars are the monosaccharides fructose and glucose, which constitute approximately 85% of its solid content. Honey is essentially a highly concentrated sugar solution in water (Ulloa *et al.*, 2010).

The Brix degrees measured for hybrid bees (81.0) and European bees (80.0) indicate similar sugar content between these bee types. Insuasty-Santacruz *et al.* (2017) reported a Brix value of 77.85 in honey from similar bee species, while Torres & Alves Junior (2023) determined a Brix value of 85.60 in *Apis mellifera* honey.

The concentration of hydroxymethylfurfural (HMF) can increase naturally over time, serving as an indicator of honey freshness (Urrego-Ramírez, 2017). The Colombian standard sets a maximum HMF value of 0.60 mg/kg (ICONTEC, 2007), which exceeds the value found in honey from hybrid bees (0.72 mg/kg). According to the same technical standard, HMF is a derivative of sugar degradation, primarily fructose. Fructose, a "noble" sugar in honey, is susceptible to decomposition into HMF when exposed to high temperatures in an acidic medium.

The calcium content found in hybrid bee honey was 8.14 mg Ca/100 g, which aligns with the range of 4-10 mg Ca/100 g reported by García-Chaviano *et al.* (2022) for *Apis mellifera* honey. Zandamela (2008) reported a calcium content of 4.8 mg Ca/100 g, while Insuasty-Santacruz *et al.* (2016) found values ranging from 2.5 to 7.5 mg Ca/100 g with an average of 5.53 mg Ca/100 g in hybrid bee honey, similar to the values observed in this study.

García-Chaviano *et al.* (2022) note that potassium is the predominant mineral in honey, significantly exceeding sodium, calcium, and magnesium. This aligns with the findings of this study, where potassium accounted for 88% of the ash content. Periago-Castón *et al.* (2019) reported significant differences in potassium content among honey from various botanical origins, with values ranging from 6 to 160 mg K/100 g, suggesting that the mineral content is influenced by the type of flowering plants.

The magnesium content in hybrid bee honey was 1.96 mg/100 g, similar to the value of 2.00 mg/100 g reported by Álvarez-Mesías & Sanchez-Casamen (2016). Insuasty-Santacruz *et al.* (2016) determined a magnesium content of 3.16 mg/100 g in honey from bees in the same region. These findings suggest that the analyzed honey provides a typical mineral profile of pure honey.

Regarding iron, Insuasty-Santacruz *et al.* (2016) concluded that iron is one of the least abundant minerals in bee honey and found a value of 0.28 mg/100 g, close to the 0.55 mg/100 g determined in the sample evaluated. Bradbear (2005) reported that the iron content in bee honey can range from 0.03 to 4 mg/100 g, depending on mineral absorption from the soil by plants. Additionally, harvesting and beekeeping extraction techniques can influence the iron content of honey (Arung *et al.*, 2021), potentially contributing to the iron content of hybrid bee honey.

Microbiological analysis. The microbiological analysis of honey from hybrid bees, including the count of aerobic mesophilic microorganisms, total coliforms, *Escherichia coli*, *Salmonella*, and mold count, yielded optimal results that indicate good microbiological quality, meeting the requirements established by NTC 1273. (Table 6).

The observed results may be attributed to various antimicrobial factors present in bee honey. As noted by Cárdenas *et al.* (2008), “Honey is a very stable product concerning microorganisms due to its low water activity (aw). The aw values of bee honey range between 0.56 and 0.62, which inhibits the growth of most microorganisms except for certain yeasts and osmophilic bacteria”. The Enterobacteriaceae count was less than 10 CFU/g, consistent with the quantification of Coliforms, which are bacterial species belonging to the Enterobacteriaceae family.

No *Salmonella* was detected in the honey. *Salmonella* is a genus of pathogenic bacteria that poses a significant risk to public health (OMS, 2018). The absence of *Clostridium* Sulfite-Reducing spores is also important for honey quality, as their presence indicates contamination (Collins *et al.*, 2004). While molds from the genera *Penicillium* and *Mucor*, as well as contamination with *Bettsya alvei*, can be found in honey in spore form, they generally do not pose a significant risk as long as honey maintains low humidity. However, under certain conditions, these molds can develop and negatively impact the quality of the product (Cárdenas *et al.*, 2008).

Table 6. Microbiological variables of honey from *Apis mellifera* Hybrid bees.

Variable	Units	Result	NTC 1273:2007
Count of aerobic Mesophilic Microorganisms	U.F.C/G	<10	100
Enterobacteriaceae	U.F.C/G	<10	N.R
Total Coliforms	U.F.C/G	<10	<10

Variable	Units	Result	NTC 1273:2007
<i>Escherichia coli</i>	U.F.C/G	<10	<10
Clostridium Spores Sulfite Reducer	U.F.C/G	<10	N.R
Mesophyllous Aerobic Spores	U.F.C/G	<10	N.R
<i>Bacillus cereus</i>	U.F.C/G	<10	N.R
<i>Salmonella</i> in 25 g. of food	absence / Presence	<10	Absent
Mold	U.F.C/G	<10	10
Yeast count	U.F.C/G	<10	10

Another aspect evaluated was the presence of *Bacillus cereus*, which was found below 10 CFU/g, meeting the limit value criterion suggested by the Colombian standard for other microorganisms. Cárdenas *et al.* (2008) confirm that *Bacillus* species are present in honey as spores and do not pose a significant health risk to consumers.

Beeswax production

Table 7. Production and organoleptic characteristics of beeswax

Product	Characteristic	Hybrid	European	EEM	p-value
Beeswax	Production (g)	984.0	757.5	11.45	0.02
	Color	Yellow	Yellow	---	---
	Appearance	Acceptable	Acceptable	---	---
	Smell	Acceptable	Acceptable	---	---
	Flavor	Acceptable	Acceptable	---	---

The Student's t-test revealed statistically significant differences in wax production between hybrid and European bee hives ($p < 0.05$). This difference is evident in both total wax production (comb wax + operculum wax) and purified wax production. However, when comparing the percentage of purified wax relative to total wax, no significant differences were found between the two bee types. This suggests that both hybrid and European bees produce similar proportions of purified wax from the total wax produced.

These findings align with the concept proposed by Gómez Otálora & Chavarro Miranda (2022), who explain that bees produce wax from honey. While both bee types produce wax from honey, the specific factors influencing the overall wax production and the proportion of purified wax remain to be further investigated.

Beeswax quality. Table 8 indicates that the physicochemical characteristics of both hybrid and European-type beeswax were acceptable in appearance, smell, and flavor. Both wax types exhibited a characteristic yellow color (Figure 2).

Table 8. Physicochemical properties of beeswax.

Variable	Units	Hybrid	European
Humidity	g/100 g	<2.0	2.30
Total ashes	g/100 g	<0.1	<2.0

Variable	Units	Hybrid	European
Protein (on wet base)	g/100 g	0.78	0.324
Ph	Units (°C)	8.61 (24.0°C)	7.6 (21.5°C)
Fats and/or oils	mg/100 g	78.43	61.24

The yellow color observed in the wax is considered a light color since there are also dark brown colors. This is probably because the wax collected belongs to young honeycombs, meaning they were not in the honeycomb for long after being made by bees. Portela-Márquez *et al.*, (2022) suggest that the dark color in the wax is probably due to the accumulation of bee waste found in the honeycomb cells.

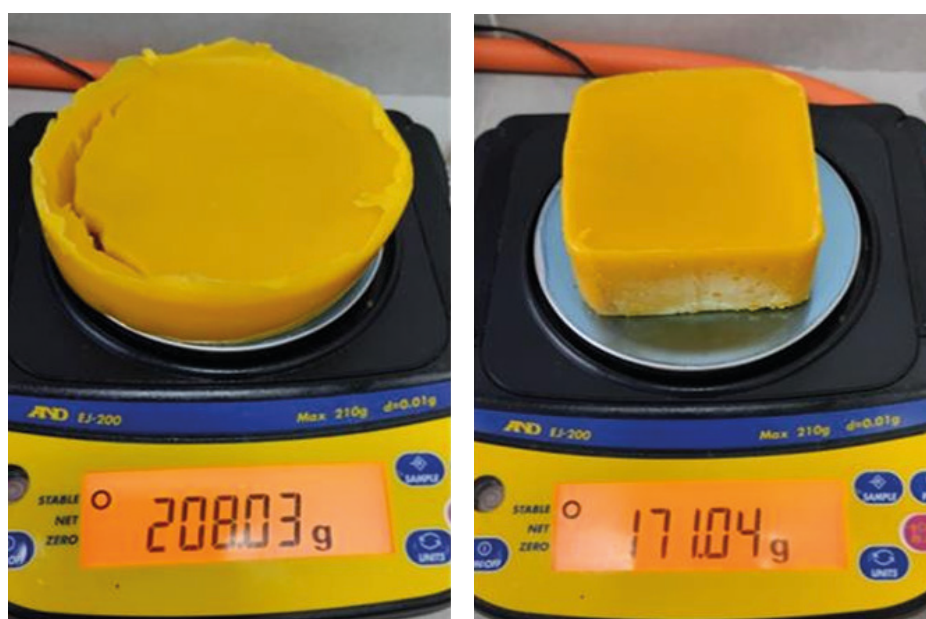


Figure 2. Purified wax. Left side, hybrid beeswax; right side European beeswax

The physicochemical quality of the wax is presented in (Table 9). The moisture content differed between the two types of beeswax. According to the standard (ICONTEC, 2007), the moisture content of European-type beeswax may not meet the quality parameter. However, it is important to note that only one sample was evaluated, which is insufficient to definitively conclude that European-type beeswax generally exceeds the humidity limit compared to hybrid beeswax.

The protein content of hybrid beeswax was 0.78 g/100g, while European-type beeswax had a protein content of 0.324 g/100g. This protein fraction in beeswax likely originates from residual pollen after the purification process, as bees often mix pollen with wax during comb construction (Dardón & Enríquez, 2008).

The fat and oil content differed slightly between hybrid and European-type bees, with values of 78.43 mg/100g and 61.24 mg/100g, respectively. Additionally, the total ash content was less than 0.1 g/100g for hybrid bees and less than 2 g/100g for European-type bees.

The microbiological quality of the wax, as shown in Table 9, was generally acceptable for both types of beeswax, with low levels of bacteria, molds, and yeasts. However, European-type beeswax exhibited a higher count of mesophilic

aerobic spores compared to hybrid beeswax. This discrepancy may be related to the higher moisture content found in European-type beeswax. The growth of these microorganisms in beeswax can be attributed to improper handling during extraction, purification, or storage processes.

Table 9. Microbiological quality of beeswax

Variable	Units	Hybrid	European
Aerobic mesophilic microorganisms	UFC / g	<10	<10
Enterobacteriaceae	UFC / g	<10	<10
Total coliforms	UFC / g	<10	<10
<i>Escherichia coli</i>	UFC / g	<10	<10
<i>Clostridium</i> Spores Sulfite Reducer	UFC / g	<10	<10
Aerobic Mesophilic Spores	UFC / g	<10	9.1x10 ¹
<i>Bacillus cereus</i>	UFC / g	<10	<10
<i>Salmonella</i> in 25 g of food	Absence / Presence	Absence	Absence
Molds	UFC / 100 g	<10	<10
Yeast count	UFC / 100 g	<10	<10

The above results demonstrate the potential for broad market opportunities for beeswax due to its favorable microbiological quality. Therefore, it is essential to implement technical and scientific monitoring of the production and handling processes within beekeeping systems.

CONCLUSIONS

It was observed that bees exhibit a preference for yellow and white flowers, although maintaining floral diversity is crucial. The visitation frequency of *Apis mellifera* is influenced by flower abundance, the dominance of specific plant species, and climatic conditions, emphasizing the importance of managing ecosystems with appropriate floral diversity. Species such as *Trifolium repens* and *Bidens andicola* are significant. Finally, hybrid bees demonstrated superior performance in honey and beeswax production compared to pure European bees, indicating greater adaptation to the regional environment of Nariño.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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