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PHYSICOCHEMICAL CHARACTERIZATION AND DETERMINATION OF THE BIOLOGICAL ACTIVITY OF MONOFLORAL ENCENILLO HONEY FROM THE HIGH ANDEAN ZONE OF COLOMBIA

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REMARKS

The botanical and geographical origin of honey remains difficult to determine; therefore, it is increasingly necessary to standardize analytical protocols that contribute to verifying the quality of this important hive product, to establish its floral origin, and to reduce the market of fraudulent honeys adulterated with glucose syrups, which, by their lower prices, undermine its consumption. In Colombia, studies related to the quality of *Apis mellifera* honeys, according to their nature, have not been extensive, despite the country being considered a honey producer. Given Colombia's location in the intertropical convergence zone, honey can be harvested at different times of the year depending on local climatic conditions and microclimates.

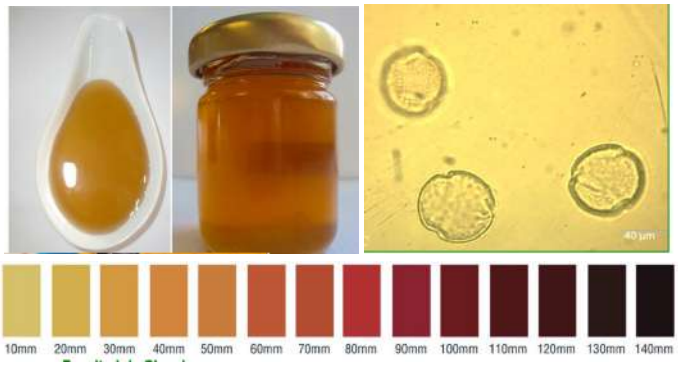
The Encenillo (*Weinmannia tomentosa* Don) is an endemic tree species, associated with cliserie forests in páramo and subpáramo ecosystems of Colombia and Venezuela, growing between 2600 and 3700 m a.s.l. Its inflorescences, arranged in spikes of creamy to white tones, are a source of nectar, pollen, and resinous materials collected by *Apis mellifera* to meet brood requirements and colony protection. This forest species is of apicultural interest due to its nectar flows, which allow the production of monofloral honey as a result of the

MATERIALS AND METHODS

The objective of this study has focused on the analysis of the physicochemical, viscoelastic, and volatile components of Encenillo honey obtained from cliserie forest ecosystems in the high Andean zone of Boyacá. Fifteen samples of freshly centrifuged honey were collected in the high Andean cloud forests of Sotaquirá, Boyacá (5°46′25.5"N; 73°15′24.8"). The site corresponds to the biogeographic association of lower montane humid forest (*lm-HF*)-MB: 2800–3000 m a.s.l.; rainfall 1000–2000 mm/year; 12–16 ºC), following the intense flowering and nectar flow periods of the species. Analytical parametres: Moisture. Soluble solids (°Brix). pH and Acidity. Electrical conductivity. Reducing sugars. HMF. Diastase index. Color. Viscosity. Thermal properties: Cp and k.Optical rotation. GC/MS

RESULTS

Encenillo honey is a light amber, floral honey with: Good maturity indicators (high proline, adequate diastase activity, moderate HMF). High sugar concentration with a balanced fructose/glucose ratio, suggesting a moderate crystallization tendency. Notable antioxidant potential due to high phenolic and flavonoid contents. Stable physicochemical profile comparable to other premium unifloral honeys such as Manuka or Ulmo. The honey exhibited Newtonian behavior.



Compound	Peak	Retention Time (RT)	Kovats Index (KI)	µg kg⁻¹ (± SD)	Identification
Alcohols					
Ethanol	12	5.04–6.62	878–926	185.3 (1.91)	Sc; MS; KI
1-Butanol	19	7.90–9.40	990–1020	201 (0.40)	MS; KI
2-Butanol	15	7.84–9.36	990–1020	450 (0.73)	Sc; MS; KI
2-Methyl-1-propanol	18	10.3–11.9	1067–1120	17.1 (1.90)	Sc; MS; KI
2-Methyl-2-propanol	10	3.33–5.59	1124–1260	12.3 (0.23)	MS; KI
2-Methyl-1-butanol / 3-Methyl-1-butanol	23	13.4–15.4	1175–1268	22.9 (4.03)	MS; KI
2-Propanol	11	4.76–6.40	862–916	5.10 (0.24)	Sc; MS; KI
Aldehydes					
Acetaldehyde	2	5.90–5.94	715–761	450 (30.1)	MS; KI
2-Methylpropanal	5	1.50–2.20	752–794	0.47 (0.10)	MS; KI
Benzaldehyde	25	20.8–22.7	1450–1516	12.1 (0.23)	MS; KI
Benzeneacetaldehyde	26	24.5–25.7	1570–1630	0.80 (0.05)	MS; KI
Ketones					
2-Butanone	9	4.31–5.72	836–891	20.5 (0.40)	MS; KI
2-Propanone (Acetone)	6	1.72–2.41	755–797	38.1 (10.6)	MS; KI
3-Hydroxy-2-butanone	22	14.1–15.8	1244–1271	15.8 (0.77)	Sc; MS; KI
2-Pentanone	13	6.41–7.92	937–975	3.20 (0.50)	MS; KI
Hydrocarbons					
n-Heptane	1	1.23–2.42	736–757	0.91 (0.06)	Sc; MS; KI
n-Octane	4	1.38–3.36	737–757	0.46 (0.08)	Sc; MS; KI
Toluene	17	8.63–10.0	1017–1042	7.40 (1.16)	MS; KI
Esters					
Ethyl acetate	7	3.53–5.29	796–868	3.35 (0.06)	MS; KI
Furans					
Furfural	4	20.2–22.0	1379–1436	67.3 (1.28)	Sc; MS; KI
2-Methylfuran	8	3.72–5.49	802–8979	86.7 (1.65)	Sc; MS; KI
Nitriles					
2-Methylpropanenitrile	14	7.35–8.30	976–1002	0.08 (0.02)	MS; KI
Sulfur compounds					
Dimethyl disulfide	3	0.80–1.30	727–772	3.75 (1.60)	MS; KI
Terpenes					
D-Limonene	20	12.3–13.9	1156–1201	4.87 (0.35)	MS; KI
Linalool oxide	23	16.8–18.5	1497–1563	0.57 (0.07)	MS; KI

Parameter	Units	Mean ± SD
Refractive Index	–	1.4864 ± 0.0010
°Brix	g 100 g⁻¹	78.3 ± 0.44
Density	g mL⁻¹	1.4129 ± 0.0020
Specific Rotation	–	−20.8 ± 0.16
E. Conductivity (EC)	mS cm⁻¹	0.525 ± 0.004
Ash	g 100 g⁻¹	0.198 ± 0.002
Total C. (TCD)	ppm	0.261 ± 0.003
Thermal Conductivity (k)	W m⁻¹ °C⁻¹	0.349 ± 0.007
Heat Capacity (Cp)	kJ kg⁻¹ °C⁻¹	2.10 ± 0.05
Water Activity (aw)	–	0.604 ± 0.050
Moisture	g 100 g⁻¹	20.1 ± 0.43
pH	units	3.87 ± 0.04
Free Acidity	meq kg⁻¹	29.7 ± 1.30
Lactonic Acidity	meq kg⁻¹	2.20 ± 0.15
Total Acidity	meq kg⁻¹	31.8 ± 1.40
Viscosity (25 °C)	Pa·s	5.61 ± 0.50
Thixotropy	Pa/s	11,410 ± 1,671
Reducing Sugars	g 100 g⁻¹	69.7 ± 0.17
Fructose	g 100 g⁻¹	40.1 ± 0.30
Glucose	g 100 g⁻¹	29.6 ± 0.54
Fructose/Glucose		1.35 ± 0.03
Phenols	mg kg⁻¹ (Gallic Acid equiv.)	1570 ± 14
Flavonoids	mg kg⁻¹ (Quercetin equiv.)	129.4 ± 1.20
Proline	mg kg⁻¹	368 ± 37.2
HMF	mg kg⁻¹	43.8 ± 0.67
Diastase Index	Shade	11.8 ± 0.30
Optical Density (420 nm)	Absorbance	0.597 ± 0.025
Optical Density (635 nm)	Absorbance	0.117 ± 0.015
Tuboouet Index	–	3.75 ± 0.06
Pfund Color	mm	69.0 ± 1.00
Luminance (L)	–	59.6 ± 1.48
Chromaticity a*	–	17.4 ± 0.94
Chromaticity b*	–	79.8 ± 1.90
Chroma (C)	–	81.7 ± 2.10

Encenillo honey shows a strongly viscous and temperature-dependent rheological profile, dominated by G″ over G′, with little elastic response. This honey can be classified as a viscous-dominant Newtonian fluid under the tested conditions, with minimal elastic contributions. Its flow properties make it comparable to other unifloral honeys of tropical origin, which generally show similar Newtonian behavior at moderate temperatures.

Its antimicrobial potential is attributed to multiple factors: High osmotic pressure (≈78% sugars, aw ≈0.60) → inhibits microbial growth by dehydration. Low pH (≈3.9) and acidity creates unfavorable conditions for microorganisms. Phenolic and flavonoid compounds (≈1570 mg/kg GAE, 129 mg/kg QE) provide antioxidant and antimicrobial effects by damaging bacterial membranes and proteins. Enzymatic activity (diastase, H_2O_2 production) contributes to oxidative stress in bacteria. Volatile compounds (furfural, benzaldehyde, limonene, linalool oxide) → act as additional antibacterial agents.

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Physicochemical characterization and determination of the biological activity of monofloral encenillo honey from the high Andean zone of Colombia

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Honey is the best-known natural product with sweetening properties. It is produced by bees from flower nectar and plant secretions. Encenillo honey (Weinmannia tomentosa L. f; Cunoniaceae) is the only certified monofloral honey in Colombia. It comes from native plants free of human intervention, from high Andean zones located between 2400 and 3700 meters above sea level. The objective of this study has been focused on the melissopalynological characterization, determination of sensory attributes, physicochemical properties and volatile components, as well as the determination of total phenol and flavonoid contents, their reducing capacity, antiradical potential, and antibacterial activity. Pollen grains in the samples were greater than 45%, conferring a monofloral quality. Pfund color was 69.0 ± 1.00, pH and total acidity were 3.87 ± 0.04 and 31.8 ± 1.40 meq kg ^{1}, respectively, still within standard limits. Electrical conductivity was 0.525 ± 0.004 mS cm ^{1}, moisture content was 20.1 ± 0.43 g 100 g ^{1}, ash content was 0.198 ± 0.002%, reducing sugars were 69.7 ± 0.17 g 100 g ^{1}, total phenols were 1570 ± 14 mg kg ^{1}, and flavonoids were 129.4 ± 1.20 mg kg ^{1}. Analytical determinations for FRAP and ABTS showed significant levels for these parameters that correlate with the observed phenol levels. Tabouret's index was 3.75 ± 0.06, based on a fructose/glucose ratio of 1.35 ± 0.03 and a water activity of 0.604 ± 0.050. The honey exhibited Newtonian behavior. GC/MS analysis identified 25 volatile compounds, including alcohols, aldehydes, ketones, esters, hydrocarbons, furans, nitriles, sulfides, and terpenes. Phenolic extracts showed biological activity against Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli.