Date of report: **2023-10-23**

UE-S-CROMATOGRAFIA-NAPL2023-S4-67/2023

NUTRIMENTAL, FUNCTIONAL AND PESTICIDE ANALYSIS OF HONEY AND PROTEIN FROM NATIVE BEE

TECHNICAL REPORT

PRESENTED TO:

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Date of report: **2023-10-23** **UE-S-CROMATOGRAFIA-NAPL2023-S4-67/2023**

Sample

Identification: A honey sample
Description: Sample in a glass jar labeled as “Miel “mieltitlán” *Scaptotrigona Mexican”*
Sampling: Performed by the client
Reception date: August 2023
Determination: Brix grade, density, color, moisture, minerals, sugars, and presence of coliforms/*E. coli*.
Methods: CIATEJ internal methods according to normativity.
Date of testing: August 14th to August 24th, September, 2023.

REPORT CONTENT:

Objective

To determine the nutrimental content of the honey according to the stablished by the FDA in a honey and native bee protein sample, pesticide analysis R&D (166 molecules), flavonoid content and phenolic compounds determination.

Material and methods

pH, color, density, and Brix grade determination

For the pH determination a potentiometer was used, according to the methodology stablished in the NMX-317-S-1978. The color was determined using a color table by visual comparison using a color scale based on Pfund range (Appendix 1). The density measurements were performed in an analytic balance, comparing the corresponding weight to the measured volume. The Brix grade was registered using a field reflectometer CVW-4013 0-90% °Brix. Four measurements were performed on each sample.

Moisture determination

For the moisture content determination an Ohaus thermobalance was used, following the procedure described in NMX-F428-1982.

Determination of crude protein by the Kjeldahl method

The method was based on the determination of the amount of organic Nitrogen contained in the sample, passing through digestion, distillation, and titration. 1.5 g of each pollen was used. The protein percentage was calculated by multiplying the amount of nitrogen obtained by the conversion factor (6.25).

Determination of crude fat by the Soxhlet method

The methodology consisted of continuous extraction with ethyl ether to 2g of dry sample, heated to boiling and the condensed liquid reached the siphoning back to the boiling flask. The amount of fat was determined by weight difference.

Determination of crude fiber

The defatted and ground samples were used. They were analyzed in the Fibertec equipment, which consisted of the sequential treatment of the pollen with hot H_2SO_4 (1.25%) and NaOH (1.25%). The total fiber percentage was determined by weight difference.

Determination of total ashes (total minerals) by muffle

The method was based on the carbonization of the dry sample on a grill, then placed in the muffle for carbonization at 550°C. The total amount of ash was calculated by difference in weights; the inorganic matter that was not volatilized is what is known as ash.

Chromatographic determinations (sugars and HMF)

Determination of sugars by chromatography: A Thermo Scientific Finnigan Surveyor high resolution chromatograph (HPLC) was used. The separation was carried out using the 300 x 7.8 mm Phenomenex Rezex RPM-carbohydrates Na+2 (8%) column. The column temperature was 80° C with a flow rate of 0.4 mL/min and the detector temperature was 37° C. The sample was previously diluted and filtered before injection. The concentrations of sugars in the samples were determined from calibration curves prepared with a mixture of analytical grade standards (Sigma-Aldrich).

Microbiologic analysis

The samples were processed in a laminar flow hood (Horizontal Clean Bench - Labconco®), the honey was poured into sterile glass bottles and 1 mL was taken with the help of a micropipette (Pipetman 200-1000 µL - Gilson) which was deposited in a tube with 9 mL of sterile physiological solution (NaCl 0.9% w/v) (Dilution factor 1:10). The mixture was homogenized in a vortex (MX-S – ScienceMED) for 30 s. 1 mL of sample was taken and poured onto a plate of selective medium for E. coli/coliforms (RIDA®COUNT *E.coli* / Coliform – r-biopharm). The plates were incubated for 48 h at 37°C.

Pesticide Analysis

Internal method from CIATEJ INS-SM/NE-01

Results and discussion

Table 1 presents the results obtained from the physicochemical determinations and the coliform count of the honey, where it is observed that the soluble solids correspond to around 71° Brix, likewise the moisture was 29.86%, this value is higher as generally reported for honey from stingless bees, the color according to the Pfund scale is described as light amber, as for the general mineral content it was 0.34% and the product does not include the presence of coliform bacteria or *E. coli*.

Subsequently, Table 2 shows the results of the nutritional analysis that allow us to identify the content of the main nutrients in the sample: sugars, protein, fiber, and fats. The values are within the parameters established in the Codex Alimentarius for the content of sugar; in terms of moisture the value is higher, however there is no specific standard for honey from stingless bees.

Table 1. Physicochemical analysis and microbiological counting of coliforms in honey sample labeled as "mieltitlán" *Scaptotrigona mexican.*

Parameter	Result
°Brix	71 ± 0.00
Density (g/mL)	1.34 ± 0.02
Color	Light amber
***E. coli* / Coliforms**	0 ± 0.00

Table 2. Nutrimental analysis of the honey

Parameter	Result
Moisture (%)	29.86 ± 0.49
Minerals (%)	0.34 ± 0.13
Total sugars (g/100mL)	68.61 ± 0.28
Saccharose	5.84 ± 0.08
Glucose	32.85 ± 0.6
Fructose	29.90 ± 0.16
Total fat	0 ± 0.00
Trans fat	0 ± 0.00
Saturated fat	0 ± 0.00
Protein	0 ± 0.20
Fiber	0 ± 0.20

The quantification of pesticides in honey sample is presented in table 3.

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Table 3. Pesticides content

Determination	Result	Limit of Quantification (L.O.Q)	Unit	Method	Analyzed by
2,4'-D 2-ethylhexyl ester	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
2,4'-D isopropyl ester	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
2,4'-DDT	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
2-Phenylphenol	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Acrinathrine	<L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Aldrin	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Azoxystrobin	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Bifenthrin	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Boscalid	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Bromopropylate	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Carfentrazone-ethyl	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Cyhalotrin, gamma-	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Cyhalotrin, lambda-	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Chlorfenapyr	<L.C.	0,010	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Chlorpyrifos-ethyl	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Chlorpyrifos-methyl	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM

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Determination	Result	Limit of Quantification (L.O.Q)	Unit	Method	Analyzed by
Coumaphos	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Deltamethrin	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Diazinon	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Diclofenthion	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Dichloran	< L.C.	0,010	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Dicofol	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Dieldrin	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Diphenylamine	< L.C.	0,026	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Endosulfan I	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Endosulfan II	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Spiromesifen	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Ethion	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Ethoxazol	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Fenchlorphos	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Phenitrotion	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Fention	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Fentoate	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Fenvalerate I	< L.C.	0,004	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Fenvalerate II	< L.C.	0,001	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Fipronil	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Fipronil -sulfone	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Fipronil -sulfide	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM

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Determination	Result	Limit of Quantification (L.O.Q)	Unit	Method	Analyzed by
Fipronil-desulfinyl	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Flubendiamide	<L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Folpet	< L.C.	0,049	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Fonofos	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Phorato	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Formotion	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Phtalimide	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
HCH (alfa isomer)	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Heptachlor epoxide (beta isomer)	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Hexachlorobenzene	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Malathion	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Oxyfluorfen	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Paclobutrazol	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Parathion-ethyl	< L.C.	0,010	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Pendimethalin	< L.C.	0,010	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Pentachloroaniline	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Pentachloroanisole	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Pentachlorophenol	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Permethrin,cis	< L.C.	0,003	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Permethrin,trans	< L.C.	0,004	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Pertan	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Pyridaben	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM

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Determination	Result	Limit of Quantification (L.O.Q)	Unit	Method	Analyzed by
Pyridaphention	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Pyrimethanil	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Pirimiphos-methyl	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Pyriproxyfen	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Profenofos	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Propetamphos	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Quinoxyfen	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Sulfalate	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Tebuconazole	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Technacene	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Terbufos	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Tetradifon	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Tetrahydrophtalimide, cis-1,2,3,6-	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Triazophos	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM
Trifloxystrobin	< L.C.	0,005	(mg/kg)	Internal method INS-SM/NE-01	BGRM

Observations:
The results reported only concern the analyzed sample.

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Signatures

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Appendix 1

Honey colors according to the P_{FUND} American Scale

0 mm	Water white	8 mm
8 mm	Extra white	17 mm
17 mm	White	34 mm
34 mm	Extra light amber	50 mm
50 mm	Light amber	85 mm
85 mm	Amber	114 mm
114 mm	Dark amber	140 mm

Extracted from Chapter IV, "Características fisicoquímicas, sensoriales y técnicas analíticas en la calidad de la miel" Pacheco-López Neith, Ayora-Talavera Teresa del R, García-Cruz Norberto, González-Flores Tania, Patrón-Vázquez Jesús, Sánchez-Contreras Ángeles, Ramos-Díaz Ana. From the book "Producción y comercialización de miel y sus derivados en México: Desafíos y oportunidades para la exportación Mérida", Yucatan, Mexico. 2016.