ORGANIC PROPOLIS FROM URUGUAY: DEVELOPING A METHODOLOGY TO ANALYZE ITS VOLATILE COMPONENTS

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INTRODUCTION

Propolis Volatile Components (PVCs) Not only contribute to the product's pleasant aroma but also exhibit a diverse range of biological activities, including antimicrobial, immunomodulatory, and cytotoxic effects against human cancer cells [1]. Furthermore, PVCs serve as key indicators of propolis's botanical and geographical origin, making them essential for quality control [1]. To date, only one previous study has reported PVCs from Uruguayan samples, without specifying the origin or production conditions [2]. This study identified 38 compounds, mainly monoterpenes and short-chain alcohols and acids [2]. In this ongoing project, we studied the PVCs of organic samples from Uruguay (Figure 1), using SPME/GC-MS protocols.

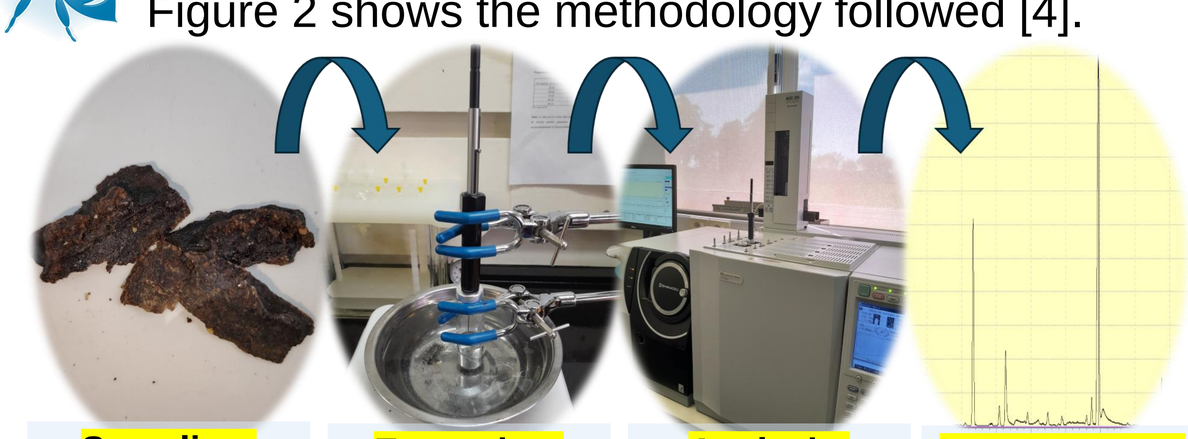
Figure 1: Area of study in Southeastern Uruguay (Cerro Negro, Rocha Department). The propolis sampling best practices were in accordance with the official Uruguayan recommendations [3].

AIM

To outline the initial steps in developing an analytical methodology to study PVCs of organic samples from Uruguay.

METHODS

Figure 2 shows the methodology followed [4].



Sampling:	**Extraction:**	**Analysis:**	**Interpretation:**
0.03 g of ground propolis	SPME with two sorbents* (50°C, 30 min)	GC-MS using three stationary phases**	LRI calculation, MS spectra comparison***

Figure 2: Methodology employed por the PVCs extraction and analysis. (*) sorbents **1.** DVB/PDMS/Carboxen, **2.** Polyacrylate (PA); (**) stationary phases: Rxi-5MS, Stabilwax-MS, and Rt-*β*DEXsm (chiral selector of modified *β*-cyclodextrin; (***) Linear Retention Index (LRI) calculation using a C_8-C_{20} alkane solution (Sigma-Aldrich) and a Terpene Mega-Mix (Supelco) standards.

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RESULTS

Different PVC profiles were obtained using both SPME sorbents. As expected, PA extracted more polar compounds, being the hydrocarbons negligible extracted (Figure 3). The structures of the main PVCs are shown in Figure 4, while Figure 5 shows the enantiomeric separation of chiral monoterpenes.

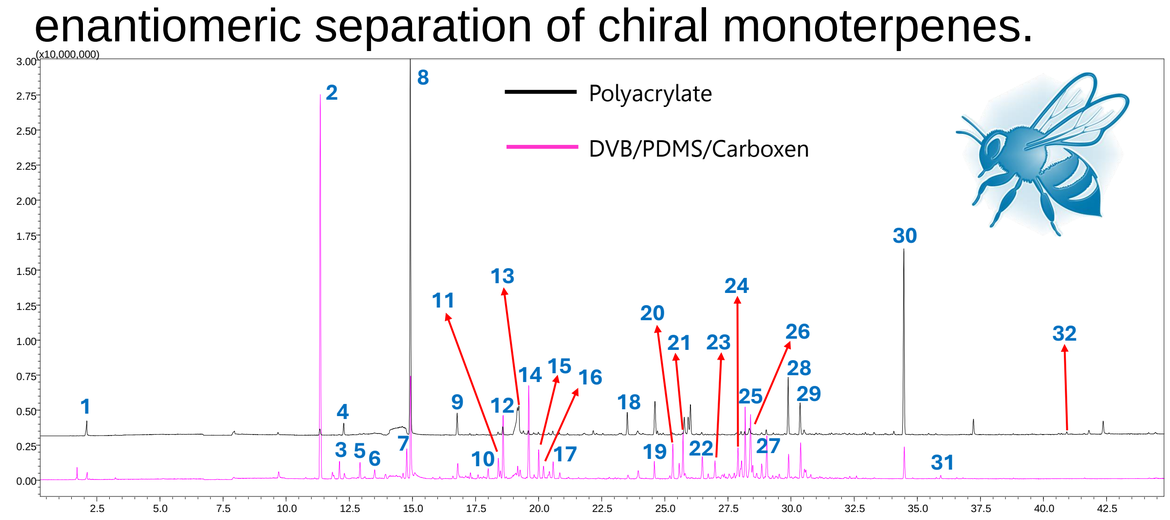

Figure 3: PVCs GC-MS profile of the organic Uruguayan propolis simples analyzed: **1.** acetic acid, **2.** *α*-pinene, **3.** thuja-2,4(10)-diene, **4.** benzaldehyde, **5.** *β*-pinene, **6.** myrcene, **7.** limonene, **8.** benzyl alcohol, **9.** *ortho*-guaiacol, **10.** *α*-campholenal, **11.** *trans*-pinocarveol, **12.** *trans*-verbenol, **13.** benzoic acid, **14.** terpinen-4-ol, **15.** *α*-terpineol, **16.** myrtenol, **17.** verbenone, **18.** *para*-vinyl guaiacol, **19.** *α*-cubebene, **20.** *α*-copaene, **21.** *β*-elemene, **22.** *trans*-caryophyllene, **23.** aromadendrene, **24.** *γ*-muurolene, **25.** *β*-selinene, **26.** *α*-selinene, **27.** *γ*-cadinene, **28.** *trans*-nerolidol, **29.** spathulenol, **30.** benzyl benzoate, **31.** neophytadiene, **32.** benzyl cinnamate. Analytical conditions: column: Rxi-5MS (30 m × 0.25 mm × 0.25 µm). Oven program: 40°C (5 min) - 5°C.min^{-1} – 235°C (2 min). Gas Carrier: He (1.0 mL/min).

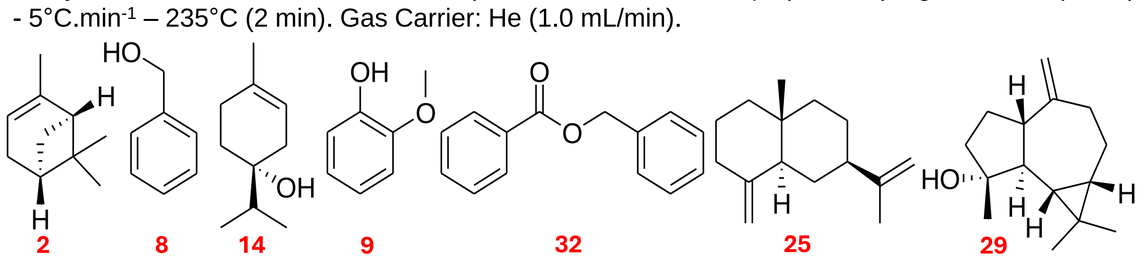

Figure 4: Main PVCs determined in this study. Numeration according to Figure 3.

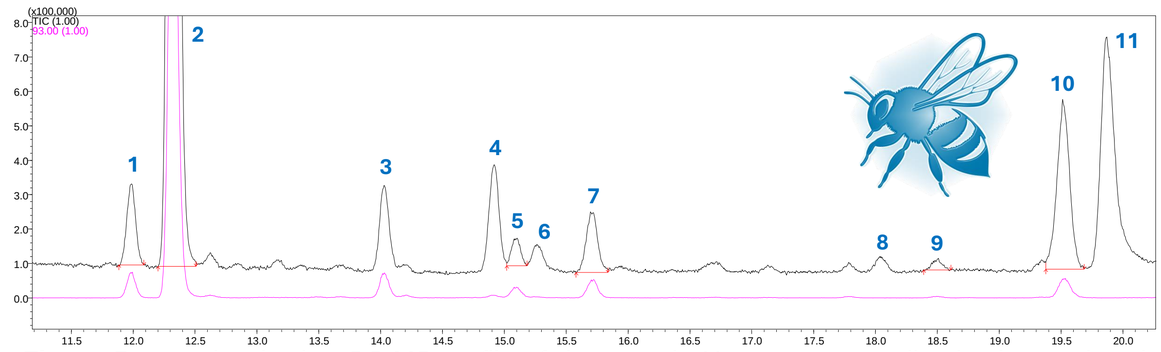

Figure 5: enantioselective GC-MS profile of the organic Uruguayan propolis simples analyzed (partial view): **1.** 1*S*-(-)-*α*-pinene, **2.** 1*R*-(+)-*α*-pinene, **3.** myrcene, **4.** thuja-2,4(10)-diene, **5.** 1*R*-(+)-*β*-pinene, **6.** not identified, **7.** 1*S*-(-)-*β*-pinene, **8.** not identified, **9.** 4*R*-(+)-limonene, **10.** 4*S*-(-)-limonene, **11.** benzaldehyde. Enantiomeric elution order according to Liberto *et al.*, ***J. Chromatogr. A 2008***, 1195: 117–126. Analytical conditions: column: Rt-*β*DEX (30 m × 0.25 mm × 0.25 µm). Oven program: 65°C (1 min) - 1°C.min^{-1} – 100°C (1 min) - 2°C.min^{-1} – 150°C-10°C.min^{-1} – 220°C (3 min). Gas Carrier: He (1.0 mL/min).

CONCLUSIONS

Over 100 PVCs were detected, including phenylpropanoids, benzenoids and terpenes, most of them not previously described for Uruguayan Propolis samples. *trans*-Nerolidol suggested *Baccharis dracunculifolia* as the botanical origin of the samples, like Green Propolis [5]. Further studies including sampling in different seasons and geographical sites, as well as studies on the chiral patterns of selected PVCs could contribute to define chemomarkers and support origin certification of this product.

References:

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