

Geographical characterization of monofloral citrus honeys by nuclear magnetic resonance and chemometrics

Tamaño, Gabriela^{5,6} ; López, Andrés¹; Bonini, Ana²; Daners, Gloria³; Fagundez Guillermina⁴; Godoy, Ana²; Moyna, Guillermo¹; Fariña, Laura²; Dellacassa, Eduardo²

¹ Departamento de Química del Litoral, CENUR Litoral Norte, UdelaR, Ruta 3 Km 363, Paysandú 60000, Uruguay

² Laboratorio de Biotecnología de Aromas-DQO-CYTAL, Facultad de Química-UdelaR, Gral. Flores 2124, 11800-Montevideo, Uruguay

³ Laboratorio de Evolución de -cuencas, Departamento de Paleontología, Instituto de Ciencias Geológicas, Facultad de Ciencias, UdelaR, Montevideo, Uruguay

⁴ Laboratorio de Actuopalinología. CICyTTP-CONICET/FCyT-UADER. Entre Ríos, Argentina

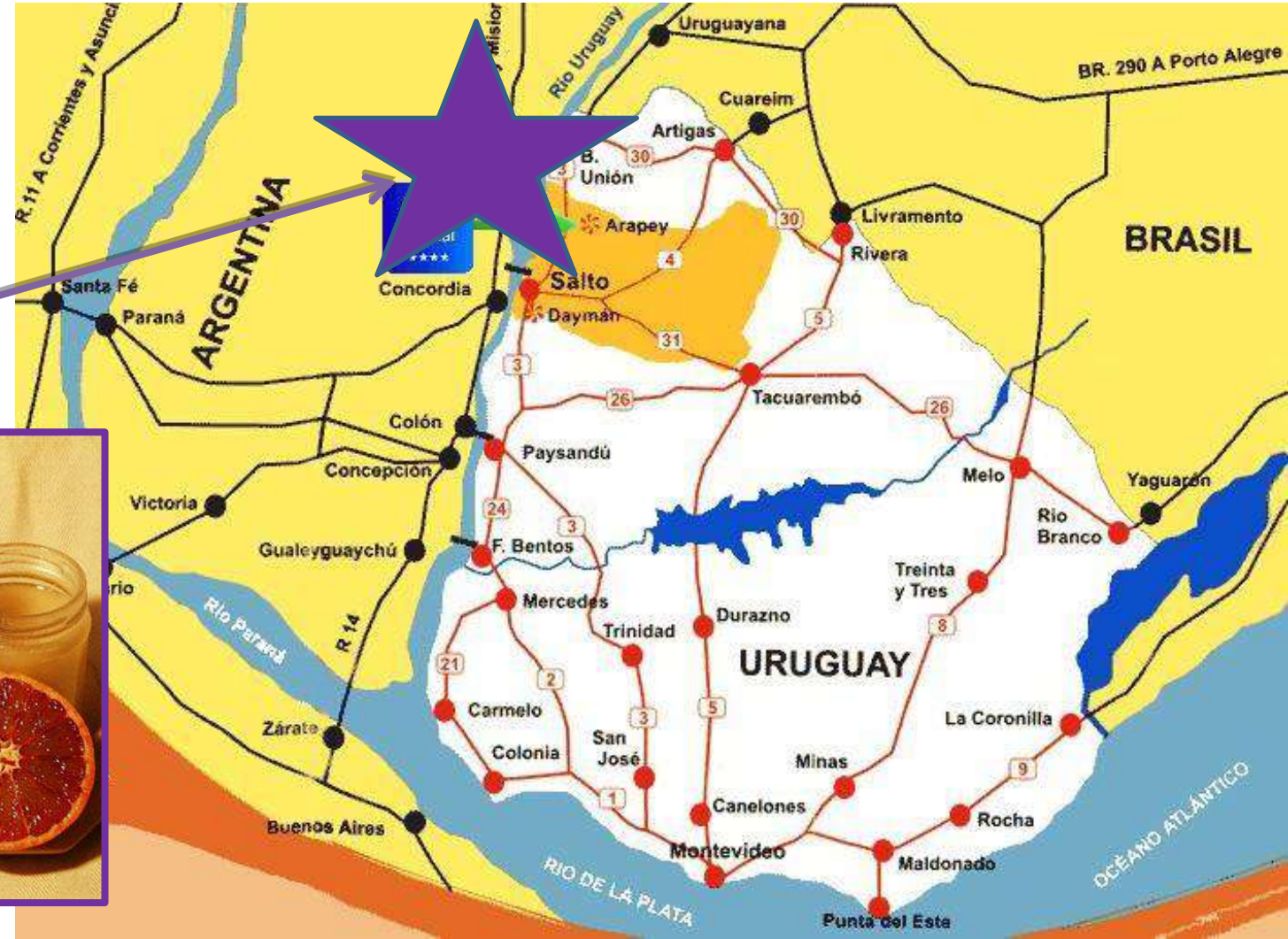
⁵ Laboratorio Análisis de Miel y Productos de la Colmena, Fa. De Cs. De la Alimentación, Universidad Nacional de Entre Ríos, Mons. Tavella 1450,3200-Concordia ,Entre Ríos, Argentina.

⁶ Tecnología de la Miel y Derivados, ITR Suroeste, Universidad Tecnológica, Polo Tecnológico Paysandú, Paysandú 60000, Uruguay

HONEY... a particular food

- BY DEFINITION ITS ORIGIN: VEGETABLE - ANIMAL
 - COMPLEXITY OF HONEY COMPOSITION
- SPECIFIC COMPONENTS FROM THE VEGETABLE SPECIES OF ORIGIN

URUGUAY - ARGENTINA BORDER REGION



Usually the **floral origin** of honey is determined by pollen analysis (melyssopalynology), since it can reflect the source of nectar. However, this approach has the limitation of needing specialists.

In addition, **some pollen grains are underrepresented** in honey pollen, such as those of ***Citrus* species**, where a minimum of 10% of *Citrus* sp. in pollen is enough in order to consider a citrus honey as monofloral.

In this study we present the investigation of a combined NMR and chemometric data analysis approach to describe the variability in the composition of citrus honey samples and to discriminate samples from different geographic (Argentina and Uruguay) and botanical origins (native flora).

- Sixty-honey samples obtained from flowers of citrus and different other plants were studied.
- All samples were provided by the beekeepers and stored at room temperature (18-23 °C) from the time of acquisition to the different analyzes (max. six months).

HONEYS WITH DEFINED BOTANICAL ORIGIN

PHYSICO-CHEMICAL AND POLLEN ANALYSIS

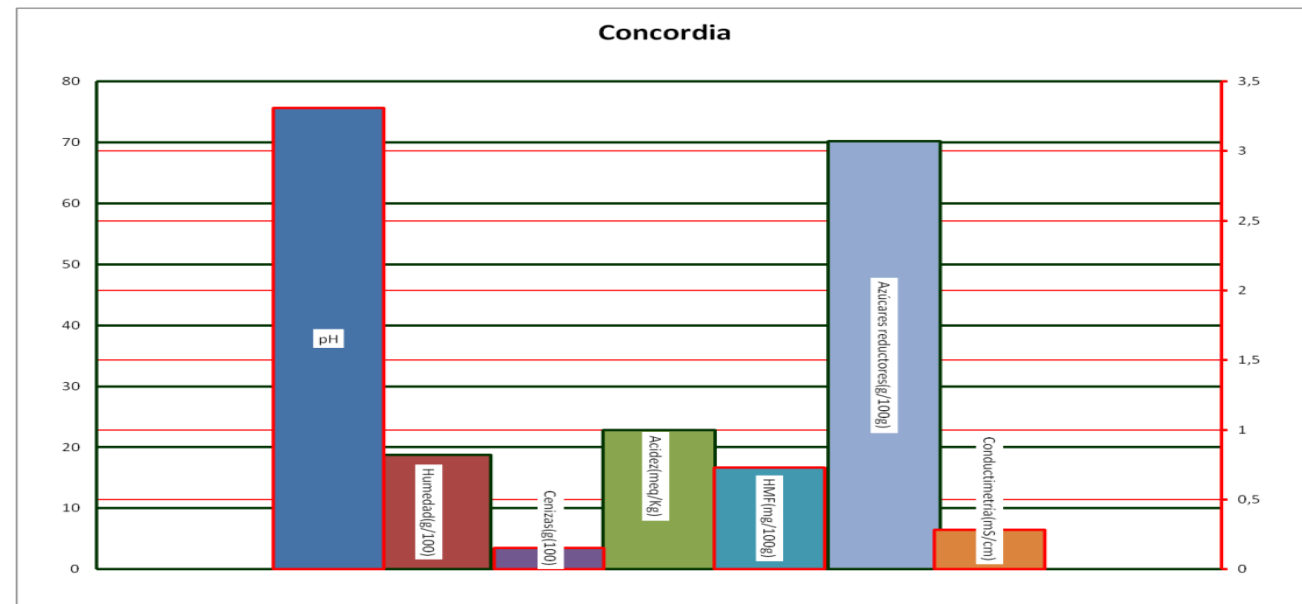
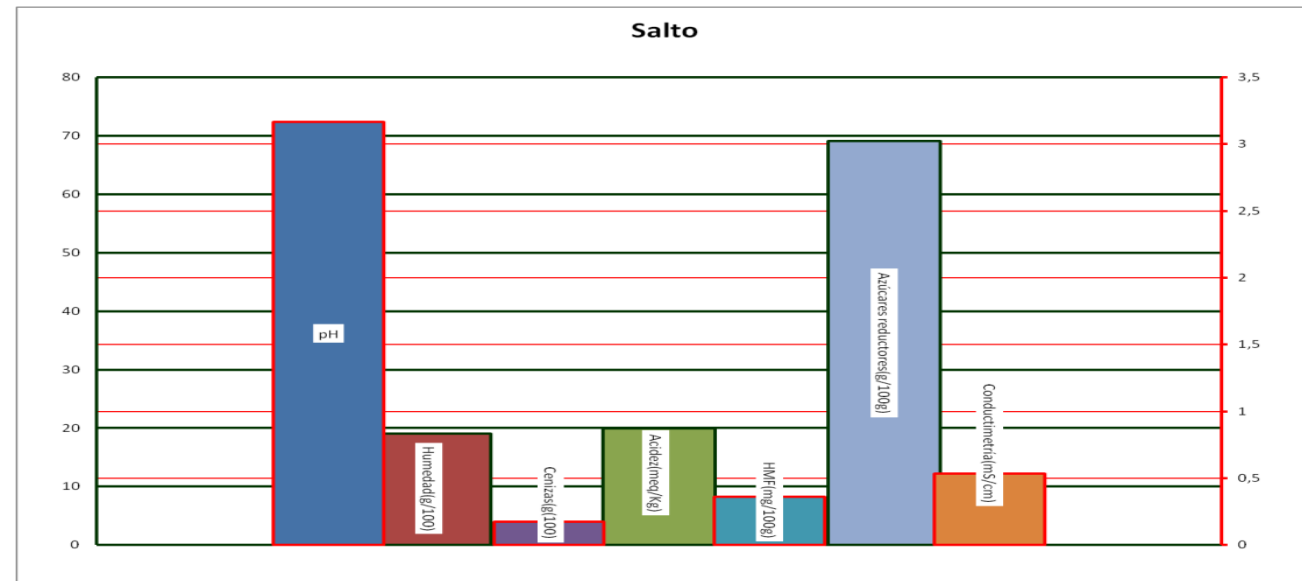
VOLATILE COMPOUNDS ANALYSIS

Espectro 1H NMR

PHYSICOCHEMICAL ANALYSIS

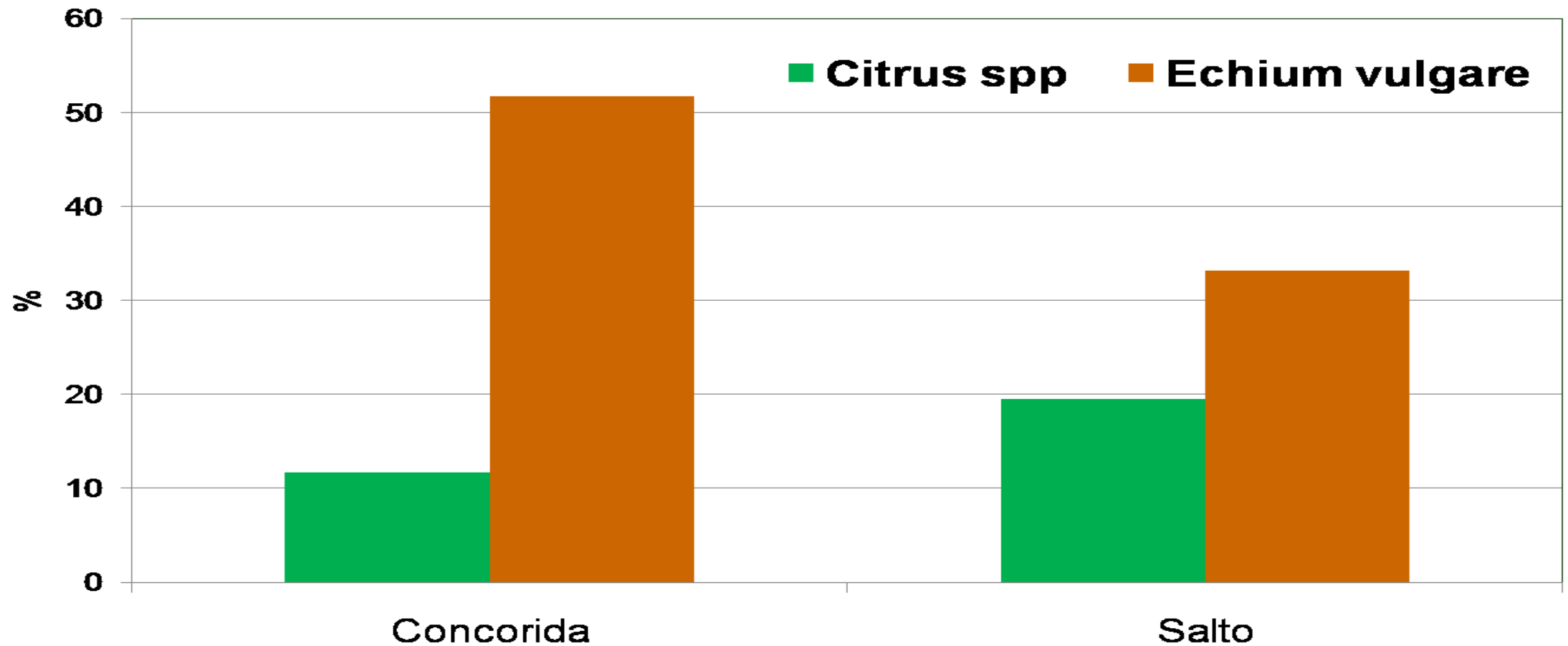
- pH
- COLOUR
- MOISTURE
- HMF
- FREE ACIDITY
- ELECTRICAL CONDUCTIVITY
- REDUCING SUGARS
- ASHES
- DIASTASE ACTIVITY

Association of Official Analytical Chemists. Official Methods of Analysis of the A.O.A.C. 1990 15th. Vol.2. Ed. Arlington

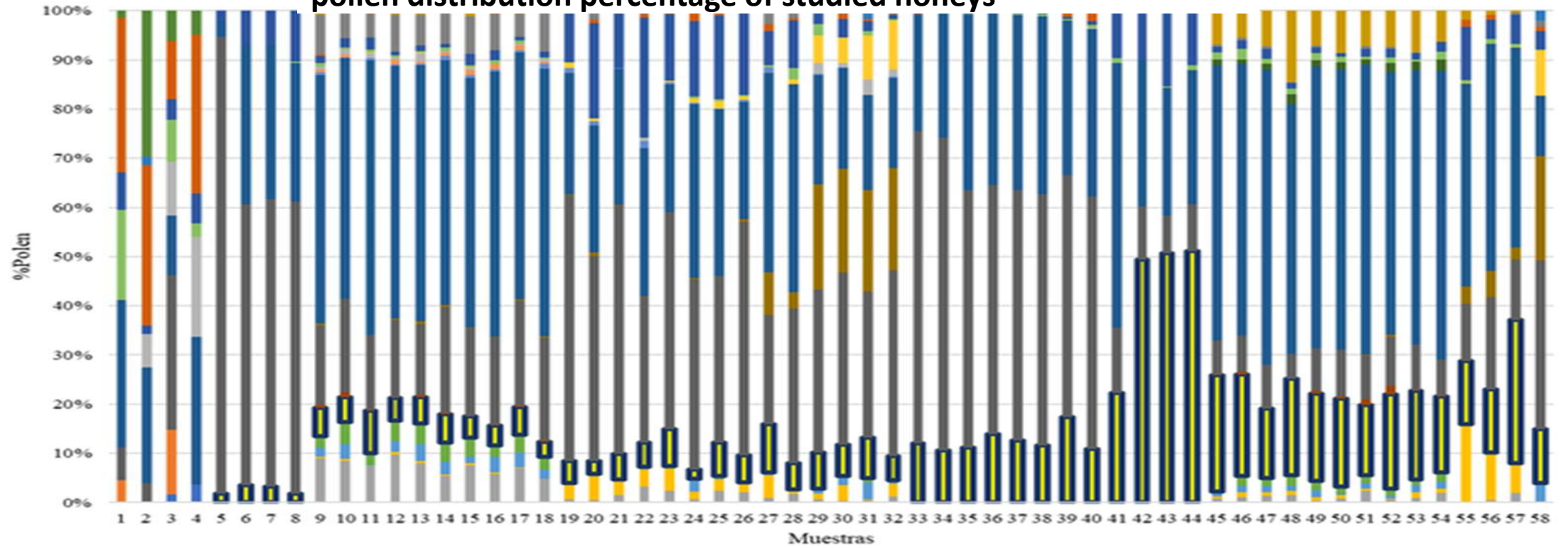


POLLEN ANALYSIS

*Louveaux, J.; Maurizio, A. and Vorwohl, G. Methods of
Melissopalynology. Bee World, 1970 51(3): 125-138*

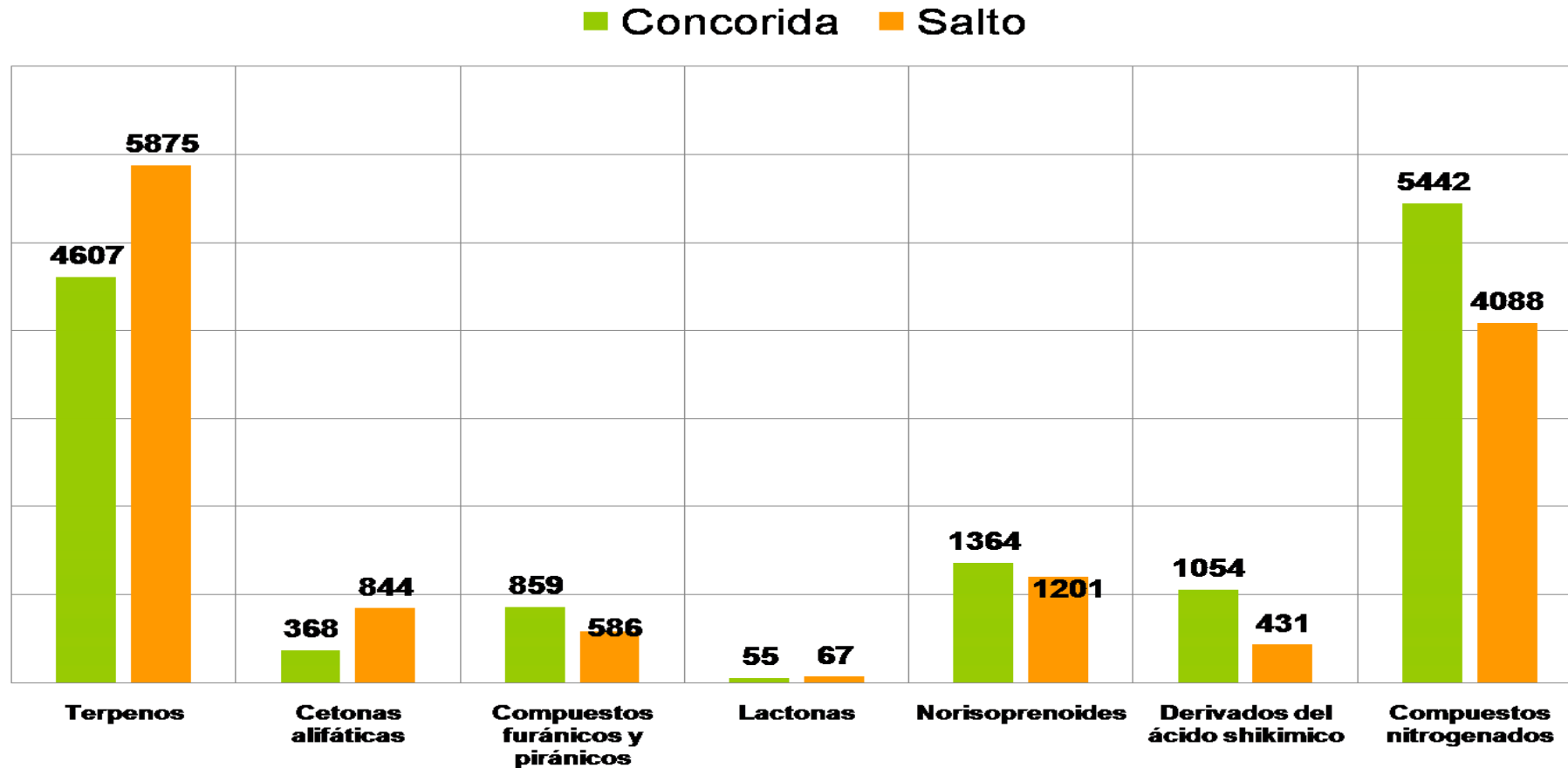


pollen distribution percentage of studied honeys



- Ammi visnaga
- Citrus sp.
- Gleditsia sp.
- Schinus sp.
- Blepharocalyx salicifolius
- Cyperus sp.
- Glycine max
- Scutia buxifolia
- Acicarpa tribuloides
- Echium plantagineum
- Lotus corniculatus
- T. Baccharis spp.
- Brassicaceae
- Eryngium sp.
- Melilotus albus
- T. Palmae
- Casuarina cunninghamiana
- Eucalyptus sp.
- Rosaceae
- Trifolium pratense
- Celtis sp.
- Fraxinus sp.
- Salix sp.
- Trifolium repens

VOLATIL COMPAUNNS ANALYSIS ($\mu\text{g}/\text{Kg}$)



NMR is used to **obtain structural information** useful to understand the structure of components in complex systems such as food.

The **^1H NMR spectroscopy** can also be considered a **fingerprinting technique**.

The **advantages** of the NMR technique with respect to other analytical methods are:

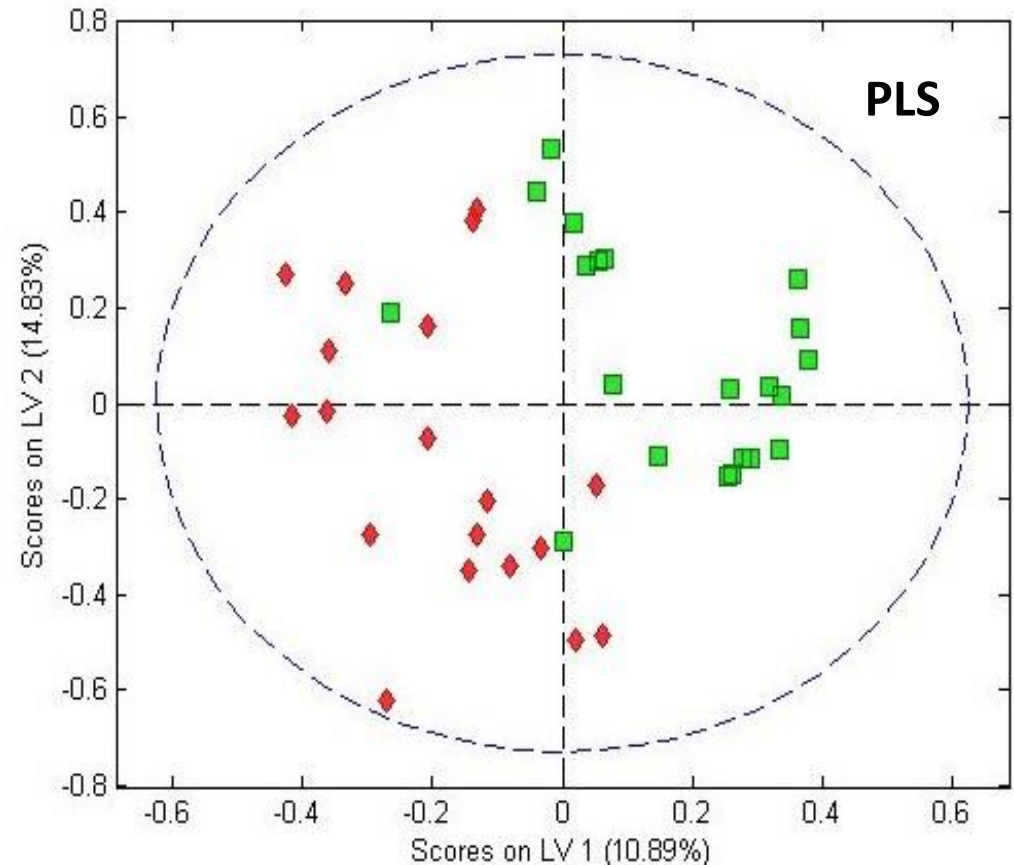
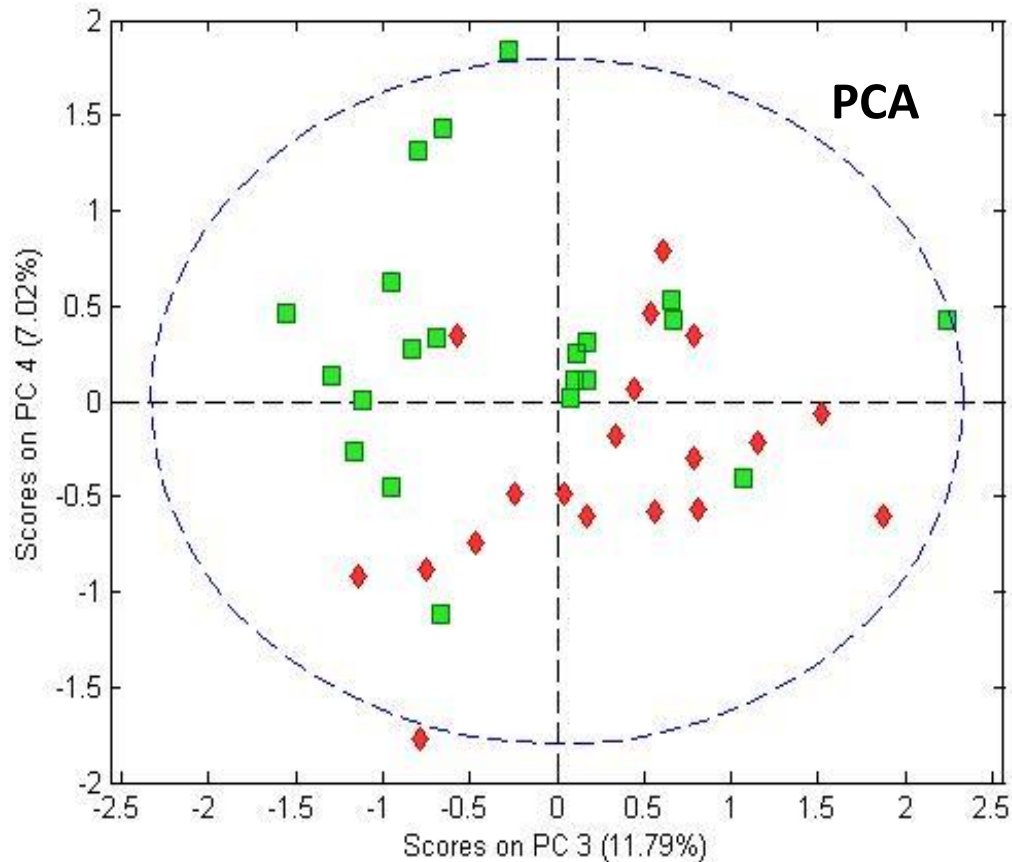
- non-invasive approach
- relatively easy and quick data acquisition
- possibility to provide information on a wide range of metabolites in a single experiment
- sample preparation is almost negligible
- combination of NMR data and chemometric analysis can also be useful for authentication purposes related to food in general

Methodology

- Honey samples were dissolved in CDCl_3 with 0.03% TMS as internal standard
- The ^1H NMR experiments were recorded at room temperature using a a Bruker AVANCE III 500
- Phase correction was performed manually for each spectrum and the baseline correction was applied over the entire spectral ranges
- A database consisting of spectra from authentic samples describing the regular range of product variation was built
- The spectra were normalized to the total area excluding the solvent zone and the data were exported to perform the multivariate statistical analyzes (PCA, PLS) in order to determinate the classification model that shows better prediction ability.

Results

- The results allowed obtaining a good discrimination of citrus monofloral honeys from those of other botanical origins for the same sampling region (Concordia-Argentina, Salto-Uruguay) .
- On the other hand, the citrus honeys from both regions could be discriminated against each other because of their geographical origin.



Conclusions

- The application of chemometric methods to ^1H NMR spectra allowed to discriminate the citrus honeys produced in the Provinces of Concordia (Argentina) and Salto (Uruguay).
- This work allowed us to initiate a database of mnr spectrum of citrus honeys of the region in study
- Moreover, the chemometric methods for pattern recognition showed that it is possible to classify the commercial honey samples according to their volatile aroma profile identifying the type of compounds involved.