OP-235 [Beekeeping Technology and Quality]
Mannose in Eucalyptus honey from Uruguay: should it be considered as an indicator of possible adulteration?

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Eucalyptus honey is a type of monofloral honey characterized by its nutraceutical and organoleptic properties. In Uruguay, this type of honey represents about 30% of the national production, 90% of which is exported.
In recent years, the quality and authenticity of honey has become more relevant, leading to the development of new methodologies for the detection of possible adulterations. One of the parameters in current controversy is the presence of mannose which is related to the addition of exogenous sugars or treatment with ion exchange resins. However, there are reports of the presence of mannose in honey from different countries and it can even be a marker of geographical or botanical origin.
The possible presence of mannose of natural origin can lead to unadulterated honeys to be considered suspicious of adulteration, making their exportation difficult.

The aim of this work is to study and quantify the presence of mannose and other minority sugars that appear naturally in Eucalyptus honeys from Uruguay.

A study was carried out on 45 samples of Eucalyptus honey harvested in 3 consecutive years in Uruguay. Samples were supplied directly from the apiaries, minimizing the chances of adulteration and were physico-chemically characterized. Also, their botanical origin was confirmed by melissopalynological analysis. Samples with a percentage of Eucalyptus pollen higher than 70% were considered monofloral honeys and the mannose content of these samples was quantified. To achieve this, sample preparation methodology was developed, and all samples were analyzed using different Nuclear Magnetic Resonance techniques on a Bruker Avance Neo 400 MHz spectrometer equipped with a 5mm PABBO probe.

This study presents the first advances in the development of a methodology for the identification and quantification of mannose in honeys from Uruguay. Mannose was found in the samples in concentrations between 0.01 and 0.05g mannose/100g of honey, with 0.02g/100g being the limit at which honey is suspicious of adulteration.

These results could be of relevance for beekeepers as this could lead to a reconsideration of whether this sugar can be used as a marker of adulteration in honeys of this botanical origin as it can to be found naturally.

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Mannose in *Eucalyptus* honey from Uruguay: should it be considered as an indicator of possible adulteration?

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Eucalyptus spp. Honey from Uruguay

90% of the honey produced in Uruguay is exported and 30% of this is *Eucalyptus* honey

1 million hectares of plantations dominated by the genus *Eucalyptus*, mainly *Eucalyptus grandis.*

The beekeeping sector has focused part of its production on honey originating from *Eucalyptus spp.* forests.

In Uruguay it is not sold as a differential product but blended with other honeys, losing its value.

the initial aim of this work was to characterize this type of honey in order to increase its added value.

Honey Harvest in Uruguay in 2024

Estimated production is 12,000 tons of honey

2,600 registered beekeepers

600.000 beehives → US$ 35.000.000

Exportations

From Uruguay's annual honey production ➔ 15-30% *EUCALYPTUS SPP. HONEY*

Representative samples

01 25 samples from de 7 departments ➔ 2020

02 19 samples from 7 departments ➔ 2021

03 07 samples from 4 departments ➔ 2022

Paysandú
Rio Negro
Rivera
Tacuarembó
Durazno
Florida
Treinta y Tres

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INITIAL WORKING SCHEME

samples of *Eucalyptus* honey from 7 departments of the country

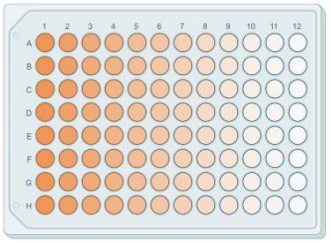
Pollen analysis
Method proposed by Loveaux et al [1].

Physico-chemical characterisation

Conductivity	Sugars
Moistur	Acidty
Colour	pH
Diastase	
Ash	

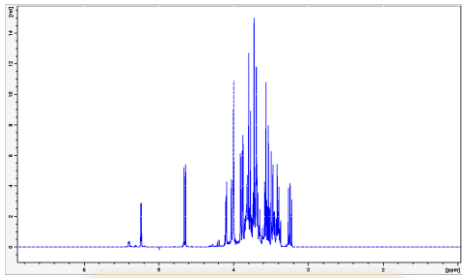
Hydroxymethylfurfural (HMF) [2]

Antioxidant capacity and total polyphenol content
Folin & Ciocalteu, ABTS and ORAC methods.



Antimicrobial activity
Determination of Minimal Inhibitory Concentration (MIC) [6].

Amount of H_2O_2
Peroxide test M Quant®



NMR spectra Determination of Mannose

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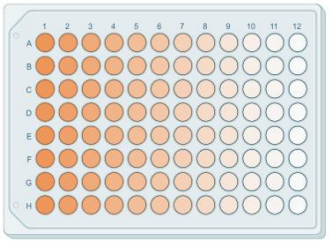

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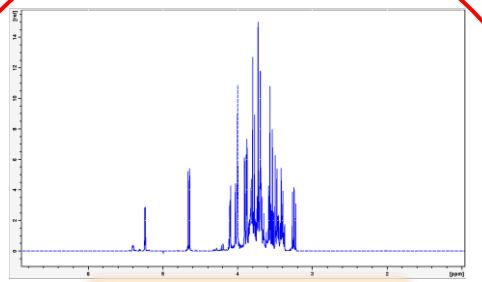

NMR spectra Determination of Mannose

Current issue

Study by the EU Food Fraud Network

- 46% of the samples were found to be suspected of non-compliance with the provisions of EU Directive 2001/110/EC on honey, with 56% of Uruguayan samples possibly suspected of adulteration.

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European Commission

JRC TECHNICAL REPORT

EU Coordinated action to deter certain fraudulent practices in the honey sector

Analytical testing results of imported honey

Ždiniaková, T., Lörchner, C., De Rudder, O., Dimitrova, T., Kaklamanos, G., Breidbach, A., Respaldiza, A., Vaz Silva, I.M., Paiano, V., Ulberth, F., Maquet, A.

2023

Currently, tests to detect possible adulteration involve the presence of **mannose.**

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Currently, tests to detect possible adulteration involve the presence of **mannose.**

It could be found naturally in monoflower honey from *Eucalyptus* trees in Uruguay.

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AIM OF THIS WORK: **is to study and quantify the presence of mannose and other minority sugars that appear naturally in Eucalyptus honeys from Uruguay.**

OPTIMIZATION OF SAMPLE PREPARATION

sampling

250 mg of honey are weighed (known density and moisture content)

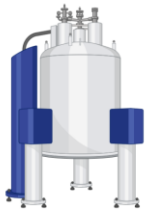
D_2O phosphate buffer D2O (pH 3-4) + potassium biphthalate as internal standard and TSP 0.01%.

mix for 1 min

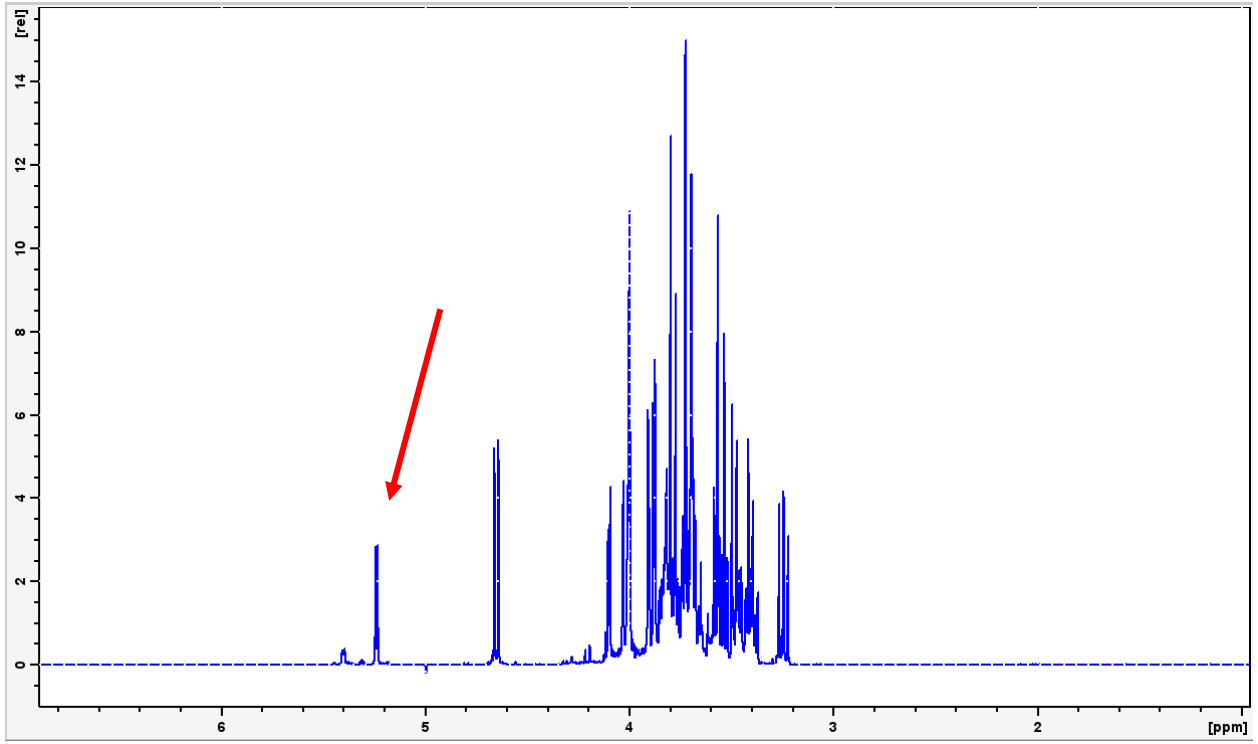
centrifuge for 10 min at 10000 rpm

transfer to NMR tube

Analysis



Why is it so complicated to quantify MANNOSE?



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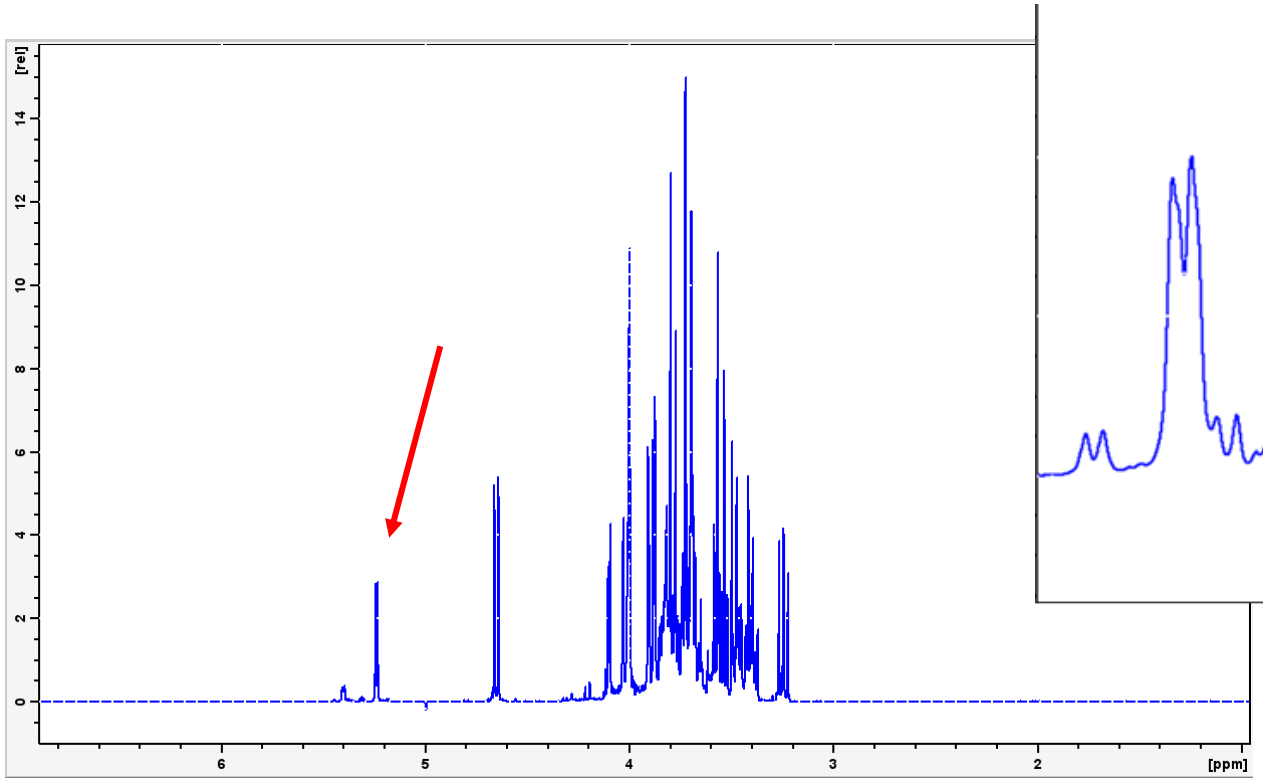
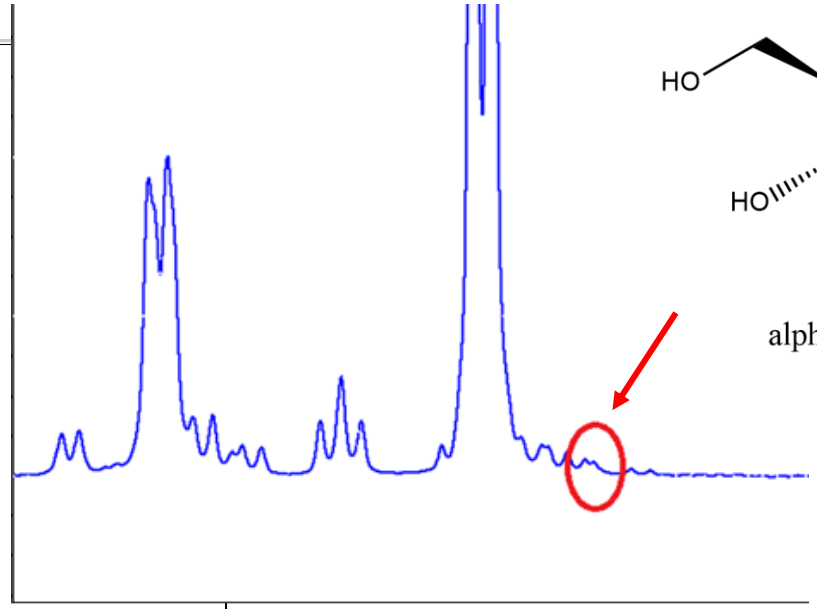
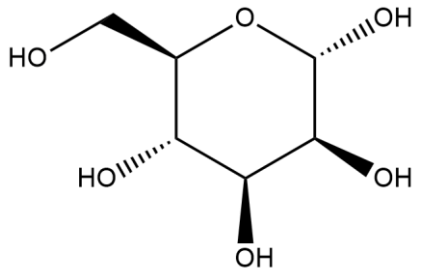






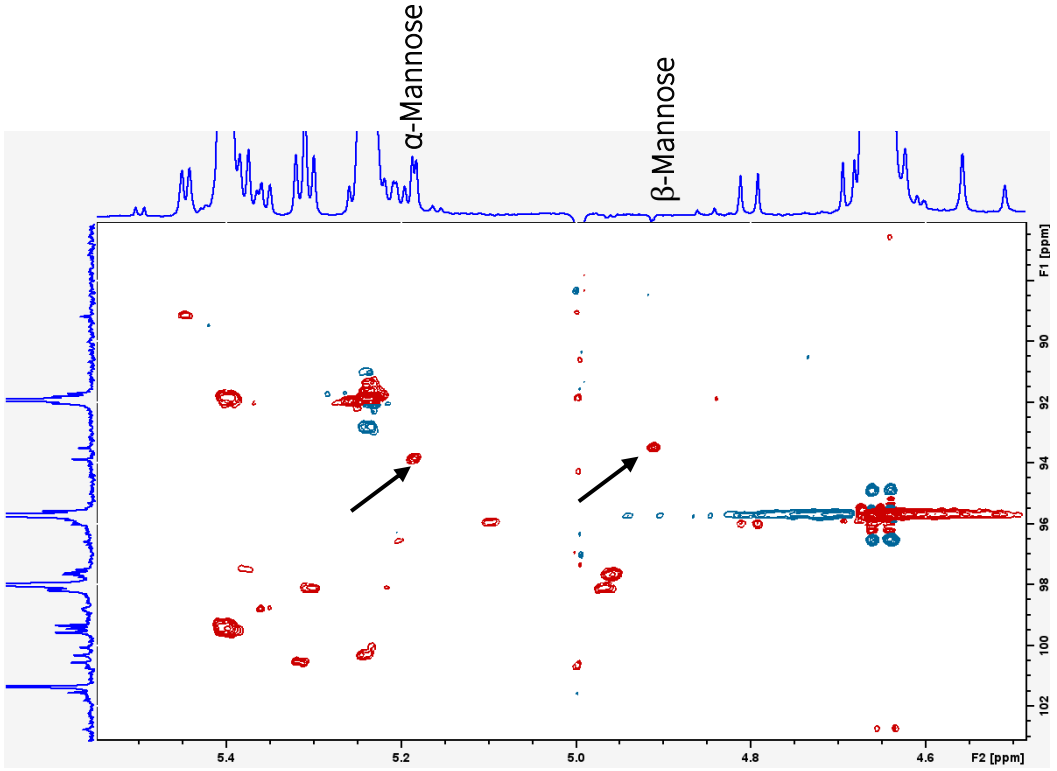
alpha mannose

RESULTS

- Developed a method for preparing samples for H-NMR analysis was successfully optimized.
- Developed a method for quantifying mannose in honey samples was optimized with an LOQ = 0.02 g mannose /100 g of honey.
- Developed a method for the identification of mannose under 0,02% in honey samples using HSQC

RESULTS

Developed a method for the identification of mannose under 0,02% in honey samples using HSQC



SAMPLE RESULTS (1° harvest)

SAMPLE	DEPARTMENT	mg Mannose/100g honey	sd	PASS/FAIL
F1	Florida	0,045820492	0,0008	
F2	Florida	0,07780859	0,0003	
F3	Florida	0,046530997	0,0012	
F4	Florida	ND		
T1	Tacuarembó	0,057557953	0,0016	
T3	Tacuarembó	0,055953254	0,0008	
P1	Paysandú	0,055953254	0,0008	
P2	Paysandú	ND		
P3	Paysandú	0,077081145	0,0031	
P4	Paysandú	0,02		
D1	Durazno	0,045831018	0,0017	
D2	Durazno	0,02		
D3	Durazno	0,057078108	0,0008	
D4	Durazno	ND		
TT1	Treinta y Tres	0,054023505	0,0031	
TT2	Treinta y Tres	0,045395251	0,0030	
RN1	Río Negro	0,042972028	0,0020	
RN2	Río Negro	0,045765876	0,0015	
RN3	Río Negro	0,02		
R1	Rivera	0,055609819	0,0043	
R2	Rivera	0,054017818	0,0011	
R3	Rivera	0,067731254	0,0024	
R4	Rivera	0,062220466	0,0023	

- In only 3 samples mannose was not detected
- In 3 samples mannose was detected but not quantified (0,02%)
- 21% of the samples presented mannose between 0,02-0,05%
- The majority of the samples exceeded 0,05% of mannose

CONCLUSIONS

- This is a preliminary study of mannose in *Eucalyptus* honey from Uruguay, which seems to be naturally found in this kind of honey
- The rest of the harvests showed similar results
- We are working on tracking down the source of this sugar

Could it come from the flowers?

Is *Eucalyptus* honey from Uruguay an exception?

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Acknowledgements

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