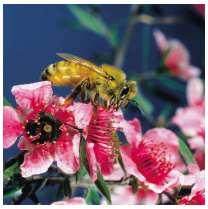

Potential of NMR to detect honey adulteration

adrian.charlton@fera.co.uk

Honey authenticity: determination of exogenous sugars by NMR, 13 November 2019

Fera Science Ltd.

- A leading supplier of scientific solutions, evidence and advice across the Agri-Food supply chain in Europe – “from farm to fork”.
- Joint venture between UK government and Capita

www.fera.co.uk

Honey authenticity @ Fera

J. Agric. Food Chem. 2008, 56, 5451

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Application of Cryoprobe ^{1}H Nuclear Magnetic Resonance Spectroscopy and Multivariate Analysis for the Verification of Corsican Honey

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Department for Environment, Food and Rural Affairs, Central Science Laboratory, Sand Hutton, York YO41 1LZ, United Kingdom

Food Chemistry 118 (2010) 987–994

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Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

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Identification of botanical biomarkers found in Corsican honey

James A. Donarski, Stephen A. Jones, Mark Harrison, Malcolm Driffield, Adrian J. Charlton *

Central Science Laboratory, Department for Environment, Food and Rural Affairs, Sand Hutton, York YO41 1LZ, UK

PAPER www.rsc.org/methods | Analytical Methods

Quantitative NMR spectroscopy for the rapid measurement of methylglyoxal in manuka honey

James A. Donarski, Dominic P. T. Roberts and Adrian J. Charlton*

ELSEVIER

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

FOOD CHEMISTRY

Analytical Methods

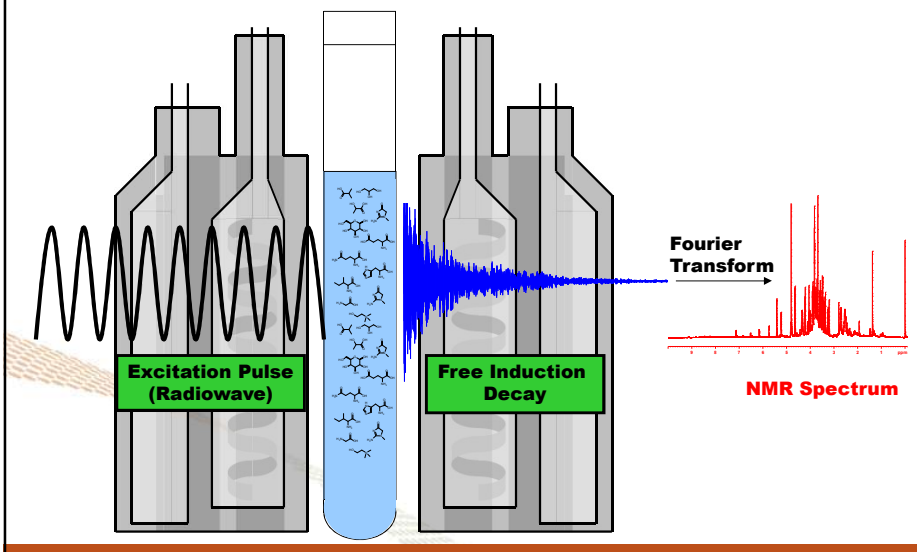
Development of primer and probe sets for the detection of plant species in honey

I. Laube [a,*], H. Hird [b], P. Brodmann [c], S. Ullmann [d], M. Schöne-Michling [c], J. Chisholm [b], H. Broll [a]

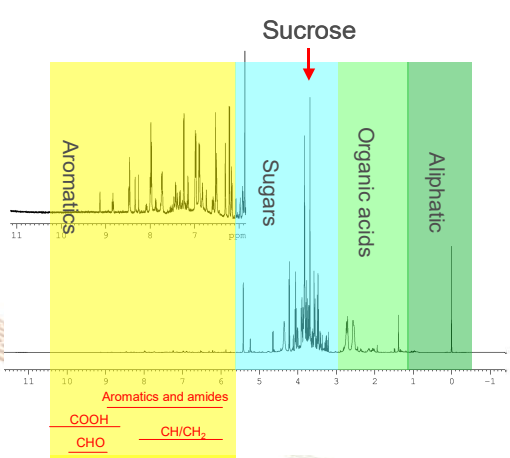
Detecting sugar addition?

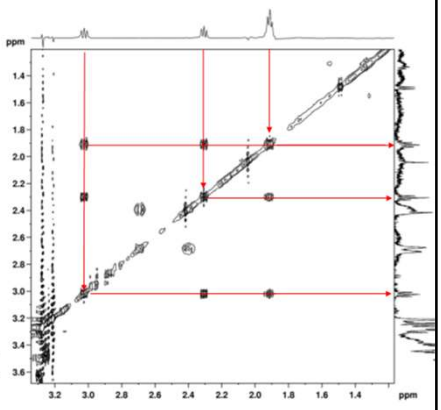
- C3 plants predominate and include sugar beet
- C4 approx 3% of all vascular plants e.g. cane
- Most honey is made from the nectar of C3 plants
- C4 sugar addition is detected using EA-IRMS
- C3 sugar addition is difficult to detect and current isotopic methods are limited due to nature!
- NMR has been proposed as a complimentary approach to detect sugar adulteration
- LC-HRMS profile of oligosaccharides also useful and under development, in collaboration with the the honey industry and international researchers

NMR spectroscopy



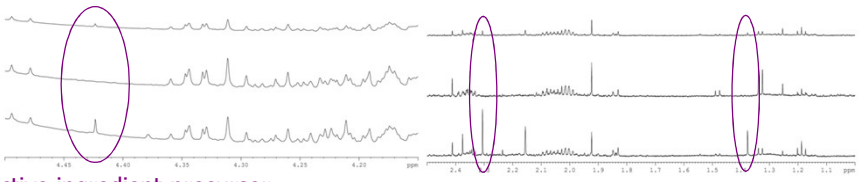
NMR data





- 1D spectra: High sensitivity, accurate chemical shifts.
- 2D spectra: High information content and resolution.

Profiling of Manuka honey (2008)

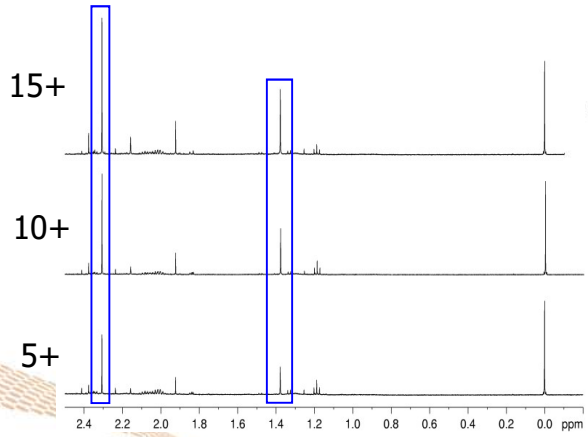


Active ingredient precursor (Dihydroxyacetone)

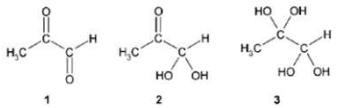
Active ingredient (methylglyoxal)

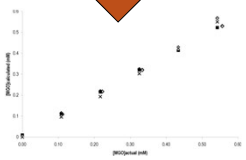
- Upper trace: Kanuka honey
- Centre trace: EU honey
- Lower trace: Manuka honey (5+)

qNMR of Manuka honey (2010)



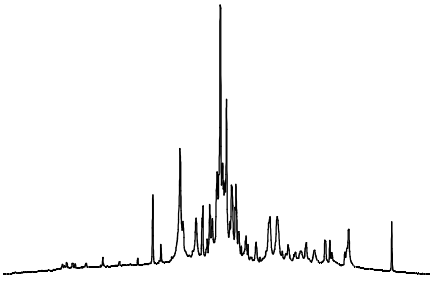
Methylglyoxal



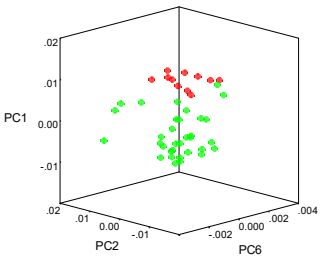


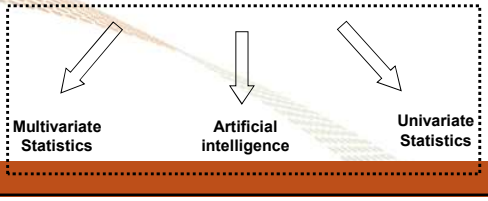
- Alkyl proton unresolved from water signal so methyl signal used for quantification
- MGO has a mono and a dihydrate in equilibrium so need to use both signals

Spectral fingerprinting



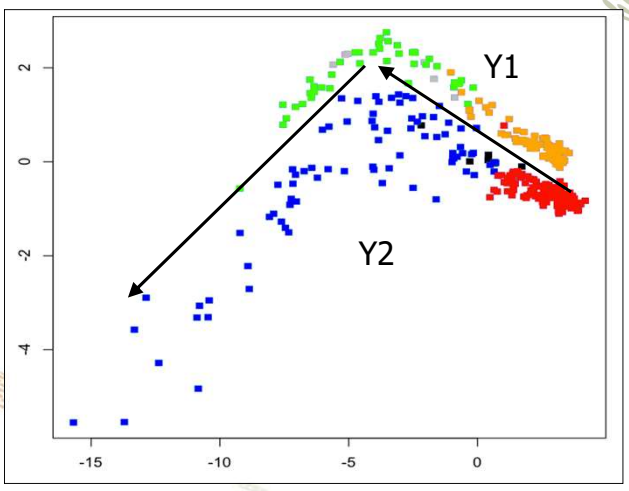
Spectral fingerprint





Computationally intensive
Data handling and bioinformatics tools required

Vintage



PCA plots shows the difference between years for Manuka honey

NMR and sugar adulteration

- Recent work at Fera has focussed on determining the robustness of NMR spectroscopy for the detection of sugar syrups in honey
- Required following the use of NMR by commercial laboratories resulting in claims of sugar adulteration being made against some UK (and international) honey producers
- A thorough investigation into the marketing and application of the technology has highlighted several findings

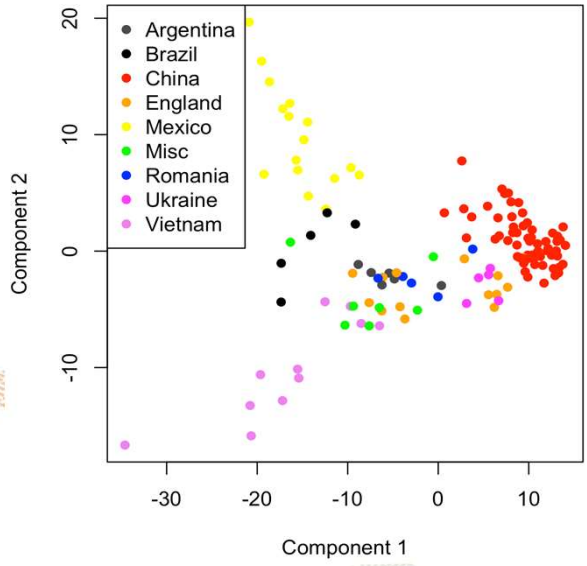
Key findings

1. Current databases of honey NMR spectra may not be representative of international market sources
2. Databases should take into account variation due to seasonality and permitted practises such as blending
3. Potential for unexpected overlapping resonances at lower field strengths and impact on quantification not fully explored
4. Some key markers used to imply adulteration have not been identified/ disclosed so cannot be validated
5. NMR analysis results for immature honeys and blends seem to be most problematic due to confusion about permitted practises

Honey Protect (2018)

- Fera have agreed a project with BHIPA to construct a database of honey NMR spectra that is most relevant to the UK market
- BHIPA and Fera have agreed a sampling plan and procedure. BHIPA have taken representative samples for analysis
- Conventional honey parameters such as C4 sugars have been recorded
- NMR spectra have been recorded on samples that have all tested to be authentic using other means and for which the sample provenance is well understood

Partial least squares



Good separation between countries suggests that there are unique parts of the NMR spectrum which relate to country of origin

PLS-LDA

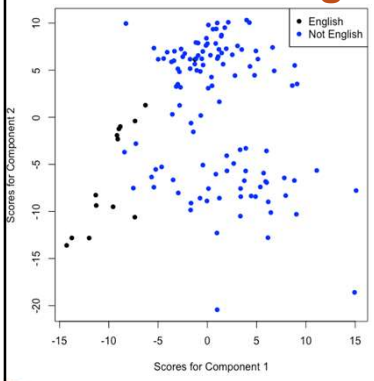
Performing PLS-LDA on the data, trying to classify we are able to get 75% correct classification.

Ukraine and the Misc. country are not very well classified due to the low number of samples

Training Set		Predicted								
		Argentina	Brazil	China	England	Mexico	Misc.	Romania	Ukraine	Vietnam
Real	Argentina	4	0	0	1	0	1	0	0	0
	Brazil	2	4	0	0	0	0	0	0	0
	China	0	0	61	0	0	0	0	0	0
	England	3	0	1	9	0	0	0	1	0
	Mexico	0	0	0	0	16	0	0	0	0
	Misc.	1	0	0	2	0	4	0	0	0
	Romania	0	0	0	0	0	1	3	1	0
	Ukraine	0	0	0	0	0	0	0	5	0
	Vietnam	0	0	0	0	0	4	0	0	7

Test Set		Predicted								
		Argentina	Brazil	China	England	Mexico	Misc.	Romania	Ukraine	Vietnam
Real	Argentina	3	1	0	0	0	0	0	0	0
	Brazil	0	0	0	0	0	0	0	0	0
	China	0	0	30	0	0	0	0	0	0
	England	1	0	0	5	0	0	0	0	0
	Mexico	0	0	0	0	4	0	0	1	0
	Misc.	0	0	0	0	0	0	0	0	0
	Romania	0	1	0	0	0	1	0	0	0
	Ukraine	0	0	0	0	0	0	1	0	0
	Vietnam	0	0	0	0	0	4	0	0	4

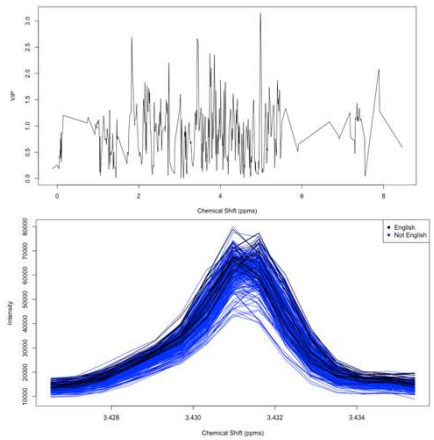
PLS-LDA English Honey



Training Set		Predicted	
		English	Non English
Real	English	13	1
	Non English	2	115

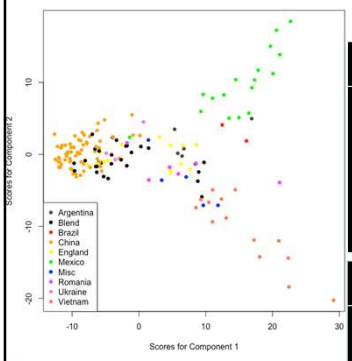
Test Set		Predicted	
		English	Non English
Real	English	6	0
	Non English	2	48

English honey can be distinguished from other world honey



Chemical shifts with the highest VIP scores are not related to specific biomarker, but subtle differences in honey comositioon due to e.g. climate

PLS-LDA with blends



Training Set		Predicted									
		Argentina	Blend	Brazil	China	England	Mexico	Misc	Romania	Ukraine	Vietnam
Real	Argentina	1	0	0	0	4	0	0	1	0	0
	Blend	0	19	0	5	2	0	1	1	0	0
	Brazil	0	0	2	0	0	0	0	0	0	0
	China	0	0	0	65	0	0	0	0	0	0
	England	0	1	0	0	13	0	0	0	0	0
	Mexico	0	1	0	0	0	16	0	0	0	0
	Misc	0	0	0	0	3	0	1	0	0	1
	Romania	0	0	0	0	0	0	0	5	1	0
	Ukraine	0	0	0	0	0	0	0	0	3	0
	Vietnam	0	0	0	0	0	0	1	0	0	13

Test Set		Predicted									
		Argentina	Blend	Brazil	China	England	Mexico	Misc	Romania	Ukraine	Vietnam
Real	Argentina	2	0	0	0	2	0	0	0	0	0
	Blend	0	5	0	8	0	0	0	1	0	0
	Brazil	2	0	0	0	0	1	1	0	0	0
	China	0	1	0	25	0	0	0	0	0	0
	England	1	0	0	0	5	0	0	0	0	0
	Mexico	0	0	0	0	0	4	0	0	0	0
	Misc	0	0	0	0	0	0	0	1	0	1
	Romania	0	1	0	0	0	0	0	0	0	0
	Ukraine	0	0	0	1	1	0	0	0	1	0
	Vietnam	0	0	0	0	0	0	0	0	0	5

If we include the blended samples in our analysis the classification rate decreases.

Next steps

- Identify compounds that differentiate the honey by country of origin using chemical shifts and 2D NMR
- Prepare honey samples adulterated with sugar syrup and investigate how this changes the NMR spectrum
- Further investigate the influence of floral type on the composition of honey
- Continue populating the database

General recommendations

- Validate methods of analysis for honey, particularly NMR, LC-IRMS and DNA based pollen tests
- Database QA is critical for implementing a successful honey monitoring programme
- Criteria for stating that a honey is adulterated need to be more transparent
- Improve understanding of honey production within and particularly outside of the EU
- Unify approach internationally as similar work is being undertaken particularly in US but also China and NZ

Acknowledgements

Fera Colleagues
Rowse
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University of York
EU
UKHIPA