NMR, a platform for comprehensive screening of olive oil ingredients and properties

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Olive Bioactives: applications and prospects
July 5, 2016 Le Studium Conferences Orleans

NMR Spectroscopy of complex mixtures

Analysis of crude natural products and other materials

Some examples of applied NMR Screening

- screening of plant or marine species extracts for new drug leads
- identification of impurities in drugs or chemical products
- identification of unknowns in environmental samples like groundwater, rivers, lakes, soil-extracts, or outlet of wastewater treatment plants
- identification of biomarkers or drug metabolites in body fluids
- screening of food & beverages like fruit juices, wine, milk, honey, edible oils, ...

Food Quality Control and Frauds

Rapid detection tool needed

Police in Chongqing's Hechuan district have discovered a production site for **fake honey** and confiscated about 500 kilograms of the fake nectar, the national broadcaster said in a report on Sunday.

"The **artificial honey** contained zero per cent real honey," the report said. 2013

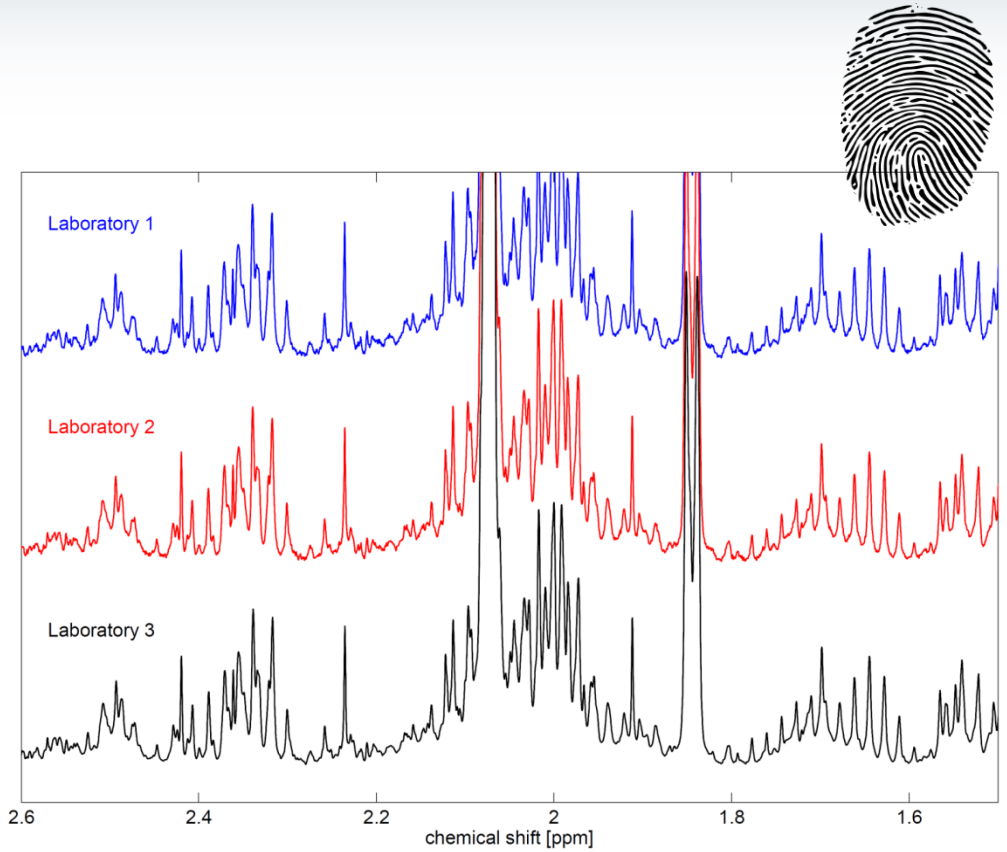
Buckets of fake honey seized in Chongqing in April. Screenshot from CCTV News, June 17, 2013

7 well known Olive Oil brands from Italy were shown to fake EVOO 2015

120 Sickened, 11 Die From Vinegar Likely Tainted With Antifreeze China **2011**

Features of 1H-NMR Profiling

- primary method for quantification
  no need to calibrate each compound
- high reproducibility
  even inter-laboratory
- non-targeted detection of all protons
- 1H-NMR profile can be regarded as unique fingerprint of the sample
- long-term build of reference databases possible
- retrospective analysis possible
  also quantification of further compounds



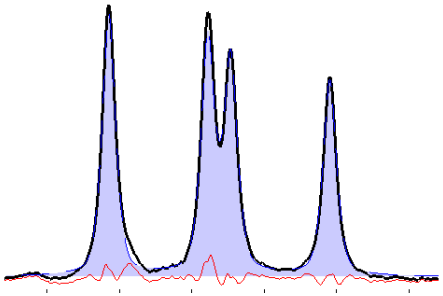
preparation and acquisition in 3 different labs (wine sample)

FoodScreener™
- Full Automated Analyses -

Quantification

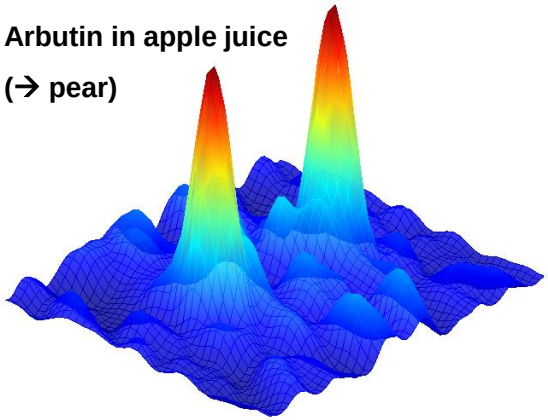
Quinic acid in apple juice

concentration: 798 mg/L



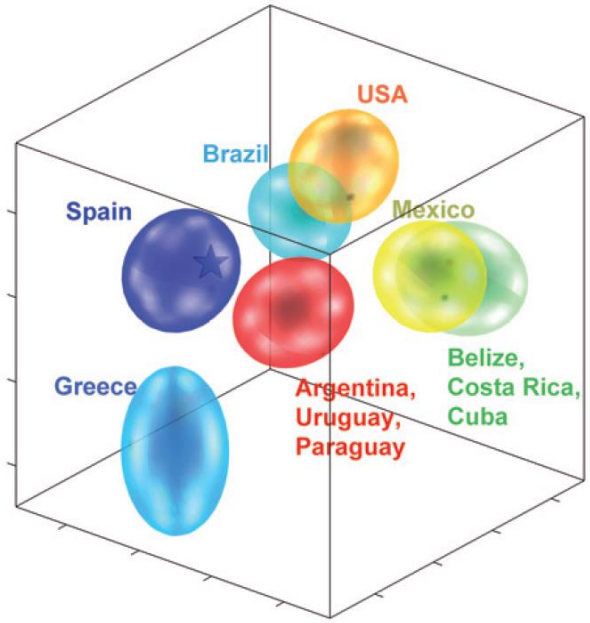
Arbutin in apple juice

(→ pear)



Classifications

- Authenticity Control
- Geographical origin
- Botanical Variety

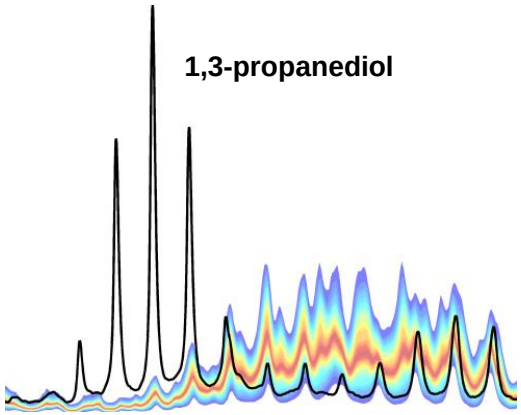


Origin of Orange Juice

Non-Targeted Verifications

- Detection of any deviations
- Check for foreign fruit
- Adulterations (Sugar/Syrup)
- Quality Aspects

1,3-propanediol



Analysis Report Wine-Profiling™

Sample ID: Italy-Sicilia-Syrah

Additional Sample Information

Variety: Syrah
Country: Italy
Region: Sicilia
Type of Wine: red

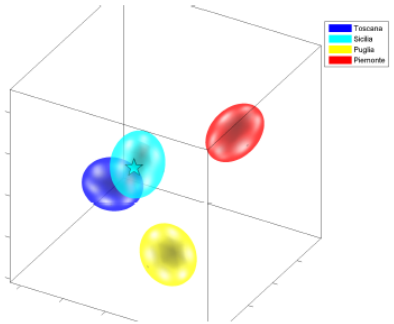
Measuring Date: 13-Nov-2012 07:11:26
Reporting Date: 02-Dec-2014 16:40:31, Version 3.0.0 (alpha), 10 pages

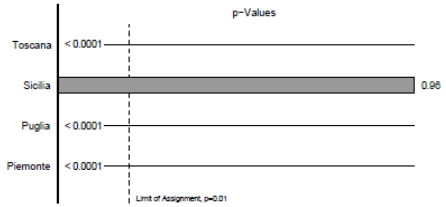
Results Summary

Type of Analysis	Analysis ID	Result	Status
Classification Analysis			
Red Wine Country	WI-1105-01/0681	In-Model	●
Italian Variety	WI-1103-01/0681	In-Model	●
Italian Region	WI-1102-01/0681	In-Model	●
Targeted Analysis			
Quantification	WI-Q/0583	–	○
Comparison with NMR Reference Database	WI-QC/0097	–	●
Untargeted Verification Analysis			
Univariate Verification	WI-2015-01/0105	In-Model	●
Multivariate Verification	WI-2015-01/0105	In-Model	●

Model: Italian Region (Analysis-ID: WI-1102-01/0681)

Result: Declared region *Sicilia* is consistent with classification result.





Standard Parameters:

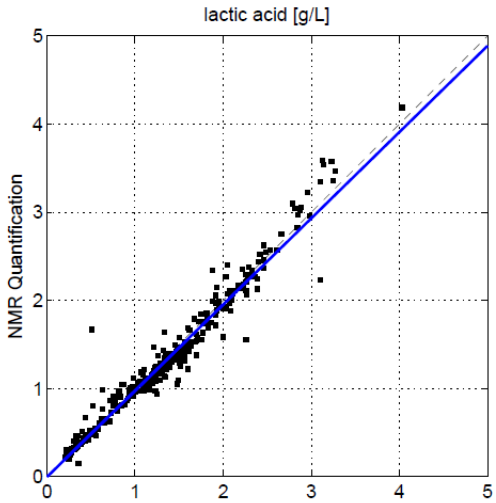
Compound	Value	Unit	LOQ	Flag	Official Ref. min	Official Ref. max	Wine-Profiling™ NMR reference database
total alcohol*	106.6	g/L	-	○	-	-	98.2 … 126.5
total alcohol-v*	13.5	%vol	-	○	-	-	12.4 … 16.0
ethanol	104.8	g/L	5.0	○	-	-	97.4 … 126.3
ethanol-v*	13.3	%vol	-	○	-	-	12.3 … 16.0
glycerol	8.8	g/L	0.5	○	-	-	6.3 … 11.4
glucose	2.0	g/L	0.5	○	-	-	<0.5 … 5.3
fructose	1.8	g/L	0.5	○	-	-	<0.5 … 6.0
glucose/fructose*	1.08	-	-	○	-	-	not available
sucrose	<0.2	g/L	0.2	●	-	-	<200 mg/L in reference set
arabinose	301	mg/L	100	○	-	-	<100 … 646
total sugar (bef. inv.)*	3.8	g/L	1.0	○	-	-	<1.0 … 11.3
total fermentable sugar*	3.8	g/L	1.0	○	-	-	<1.0 … 11.3
tartaric acid	1.9	g/L	0.5	●	-	-	1.2 … 3.3
malic acid	<0.2	g/L	0.2	○	-	-	<0.2 … 0.4
lactic acid	1.4	g/L	0.2	○	-	-	0.7 … 3.4
citric acid	<200	mg/L	200	●	-	1000	<200 … 236
energy value*	3340	kJ/L	-	○	-	-	3060 … 3950
bread units*	0.32	1/L	0.2	○	-	-	not available
carbohydrate units*	0.38	1/L	0.2	○	-	-	<0.2 … 1.1

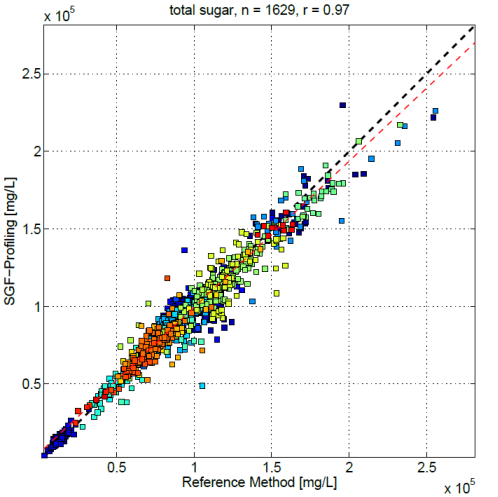
Wine Report Release 3.0

Olive Oil will look similar

Method Validation
- ISO 17025 Accreditation -

- > 20.000 comparisons with reference methods
- Continuous participation in international ring-tests
- ISO 17025: flexible accreditation for 1H-NMR-based analyses on liquid food and food extracts
  - Determination of ingredients (quantification)
  - Measures for authenticity and quality control (statistical analysis, e.g. origin/variety)
- Customers can use our validated methods for the integration in their ISO 17025 accreditation



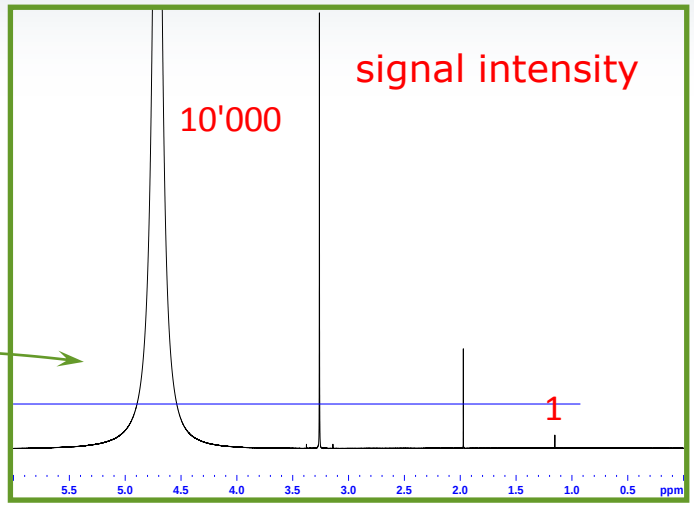


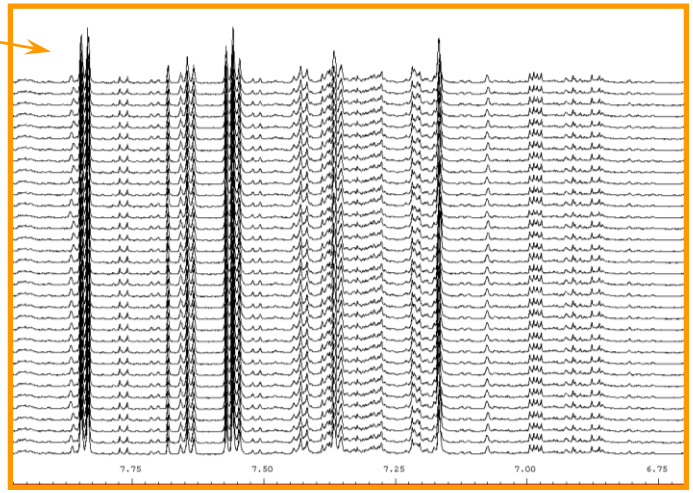
Why using NMR for food quality control?

NMR as an ideal tool for complex matrices

NMR spectroscopy …

- gets along with easy sample preparation
- is quantitative per se
- is targted and untargeted simultaneously within a single experimental run
- is reliable over a high dynamic range
- delivers a multitude of information even in a single experiment
- has excellent reproducibility
- supports either full automation under standardized conditions, or tailored solutions - and almost everything in between
- Allows retrospective analysis, as soon as new models are developed or new quantification is ready
- One quantification reference standard for all compounds

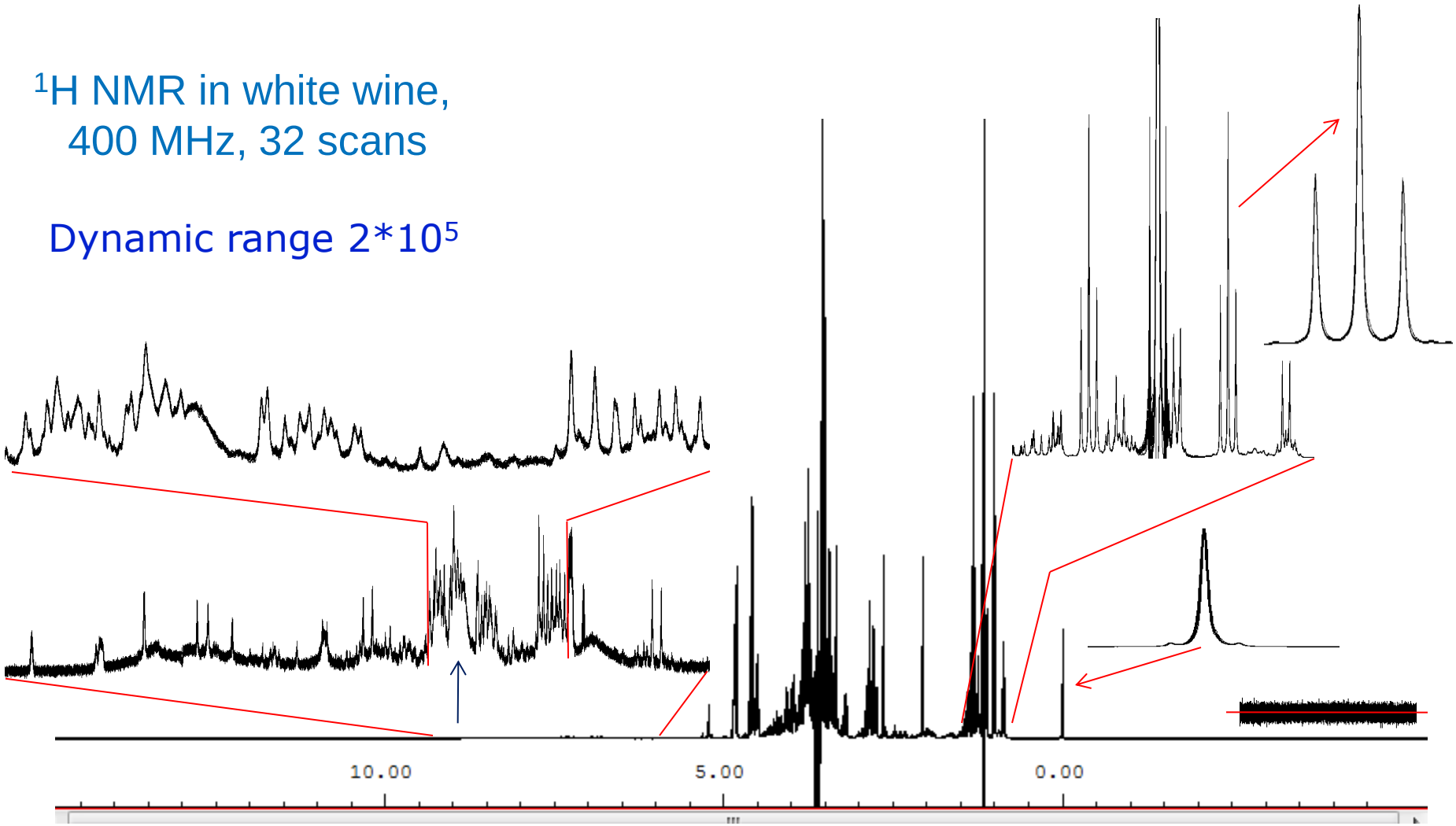




Reproducibility in Wine NMR

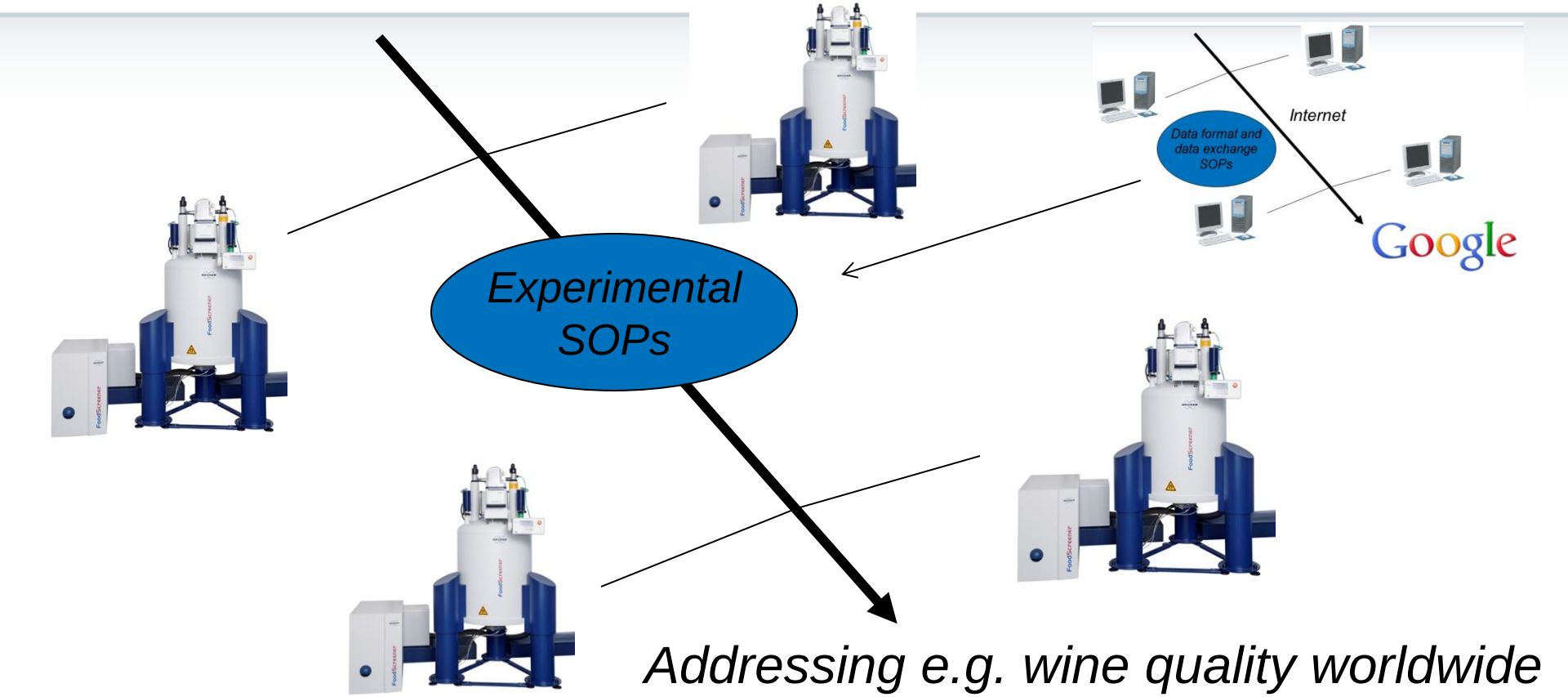
^{1}H NMR in white wine,
400 MHz, 32 scans

Dynamic range $2*10^{5}$



20 replicate preparations

Experimental SOPs act like the Internet to connect spectrometers data output



Addressing e.g. wine quality worldwide

Note: Spectral Databases of food matrices like wine only Make full sense, if complete comparability is guaranteed

FoodScreener 400 MHz-Platform :
New Customers combine solutions, developed by Bruker BioSpin or in future external providers

Example: B1+B3+C1 or B2+B4+C1+C3 .....

External Providers

Solutins on BBIO Basic Platform

Customer 1	Company 2	Company 3
Refreshment Drinks C1	Vodka C2	Tbd. C3

Bruker Standardized Platform Food ISO 17025 ISO 9001

JuiceScreening Module	WineScreening Module	HoneyScreening Module	tbd
B1	B2	B3	B4

Optionally Expandable to **SNIF-NMR**

BBIO
Dedicated NMR Platform
FoodScreener 400 MHz
ISO 9001
ISO 17025

- Iso17025 achieved 05/2015

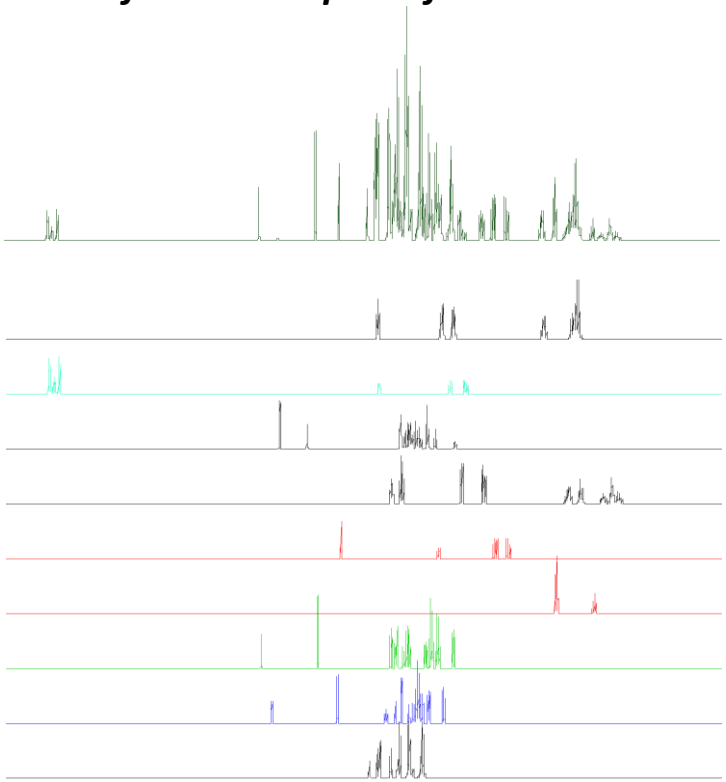
Two NMR screening approaches

Metabolic Profiling and Fingerprinting

Metabolic Profiling

- targeted
- combinable with LC-(SPE)-MS

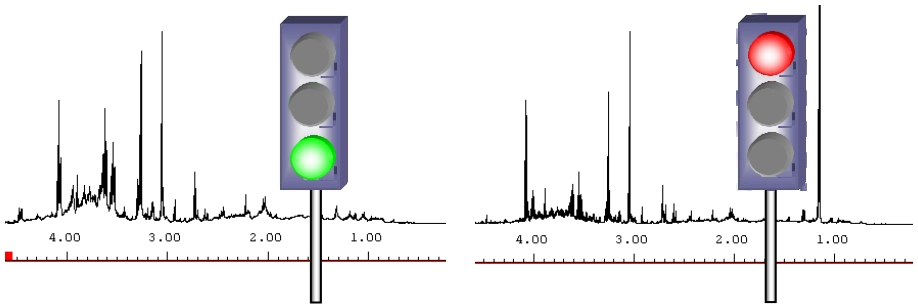
identification & ***quantification***

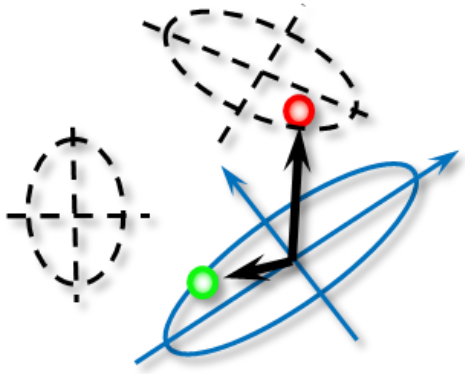


Metabolic Fingerprinting

- non-targeted
- use of statistics

classification & ***discrimination***





Food adulteration
Need for reliable analytics

All the worse - like in other crimes - adulterators always have some lead!

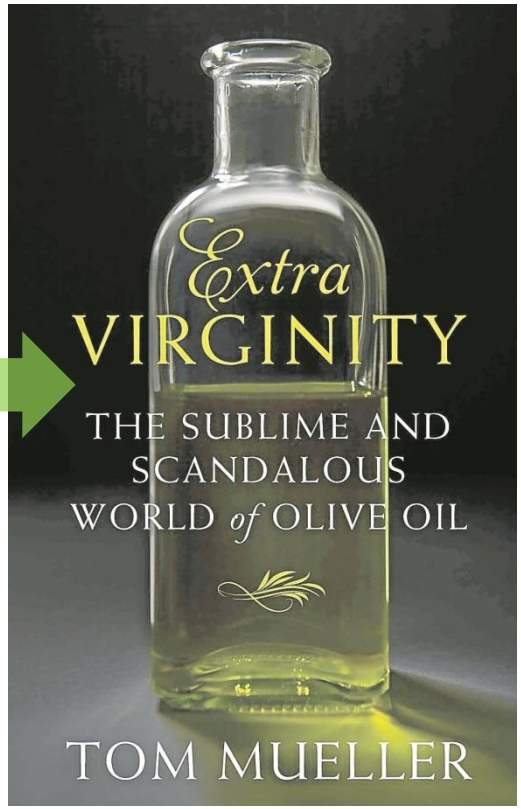
On the other hand, most analytical methods

- may be limited in range, sensitivity or reproducibility
- can be levered out by "elaborated" frauds
- are targeted
- are too expensive to be practized on a grand scale

Thus, adulteration of foodstuff is continuing ...

Food fraud hints can find expression in single compounds within the complex mixture, or can be entangled in subtle matrix effects.

(Cost-)Effective analytical methods have to deal with both tasks simultaneously, and NMR screening can do the job



W W Norton & Co (2011)

Motivation for edible oil screening

The global olive oil market situation

- Global olive oil market is dominated by growing price competition
- Product differentiation based on high quality standards and geographical origin is a highly promising concept to counteract

European Olive Oil Legislation (1992)

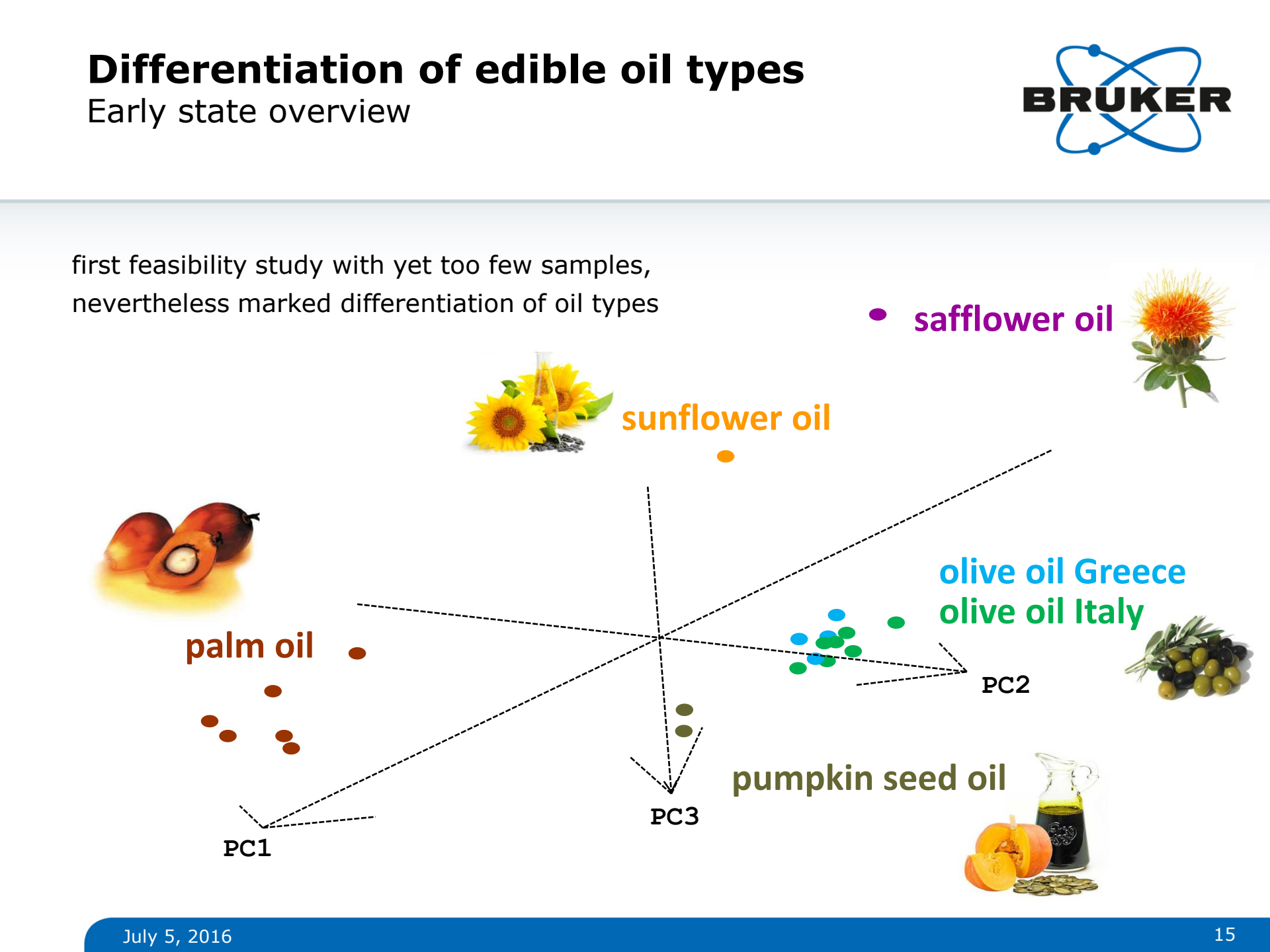
"The EU PDO/PGI regulation (Regulation 510/2006 and its predecessor Regulation 2081/92) provides EU-wide protection to names of agricultural products and foodstuffs that have a close link to their geographic region of production. Such products must be produced in a specified territory and according to a certain production specification."

cited from a report on "Evaluation of the CAP policy on protected designations of origin (PDO) and protected geographical indications (PGI), London Economics, 11-2008

Differentiation of edible oil types

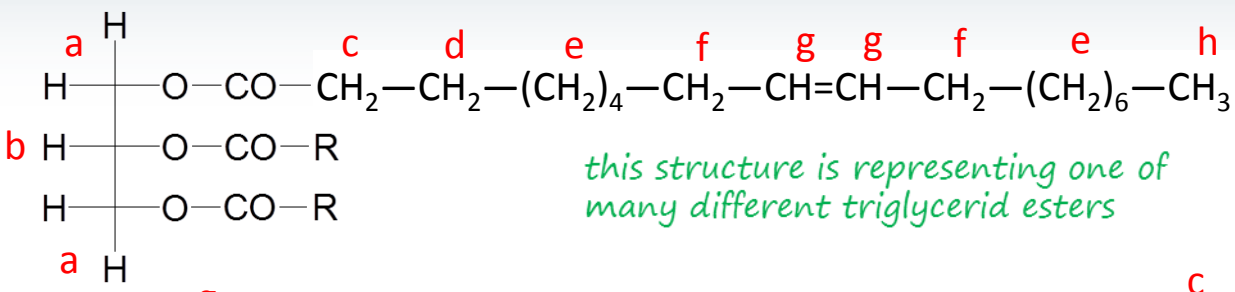
Early state overview

first feasibility study with yet too few samples,
nevertheless marked differentiation of oil types



The Lipid Profile in Olive Oil

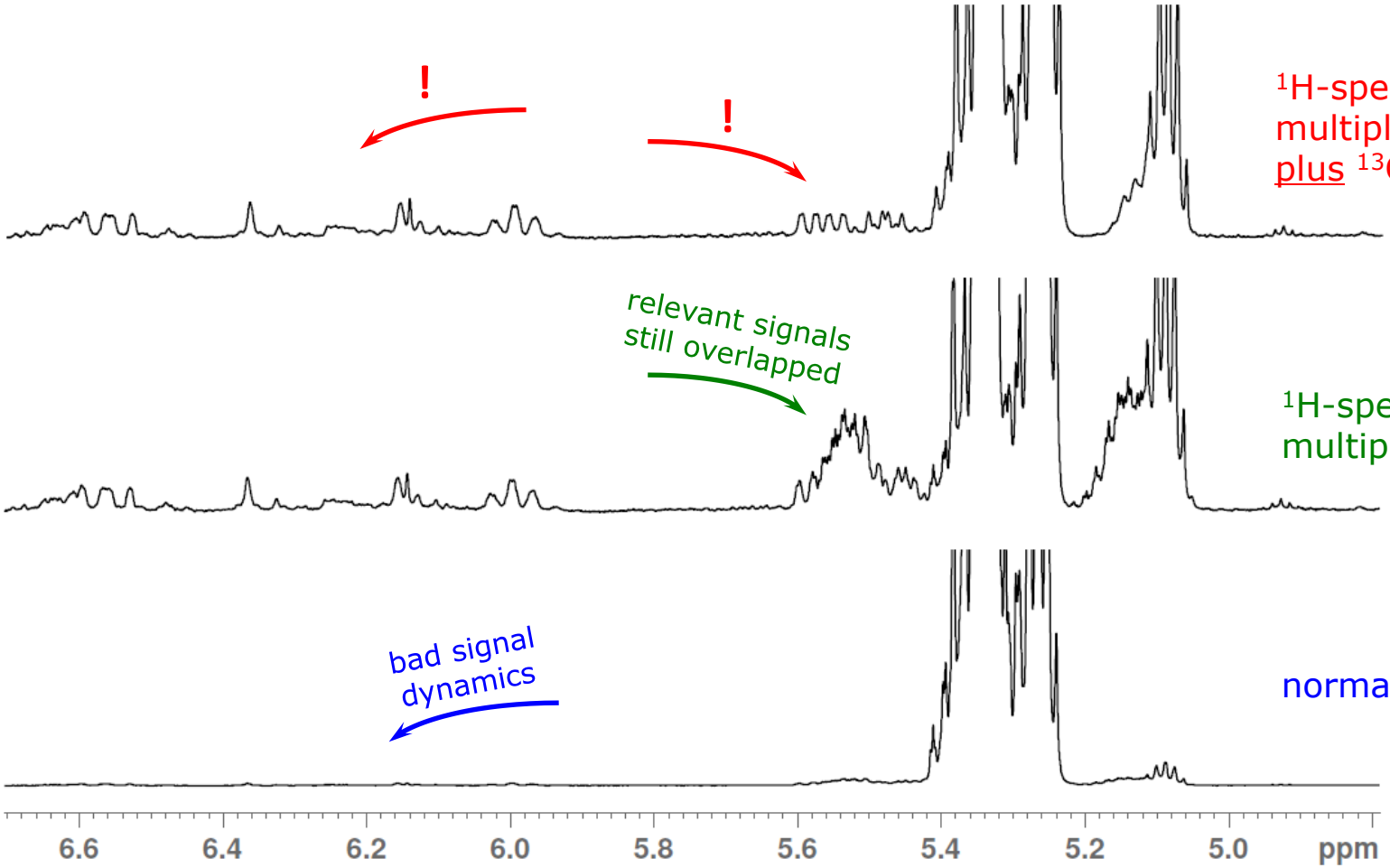
Triglycerid esters



this structure is representing one of many different triglycerid esters

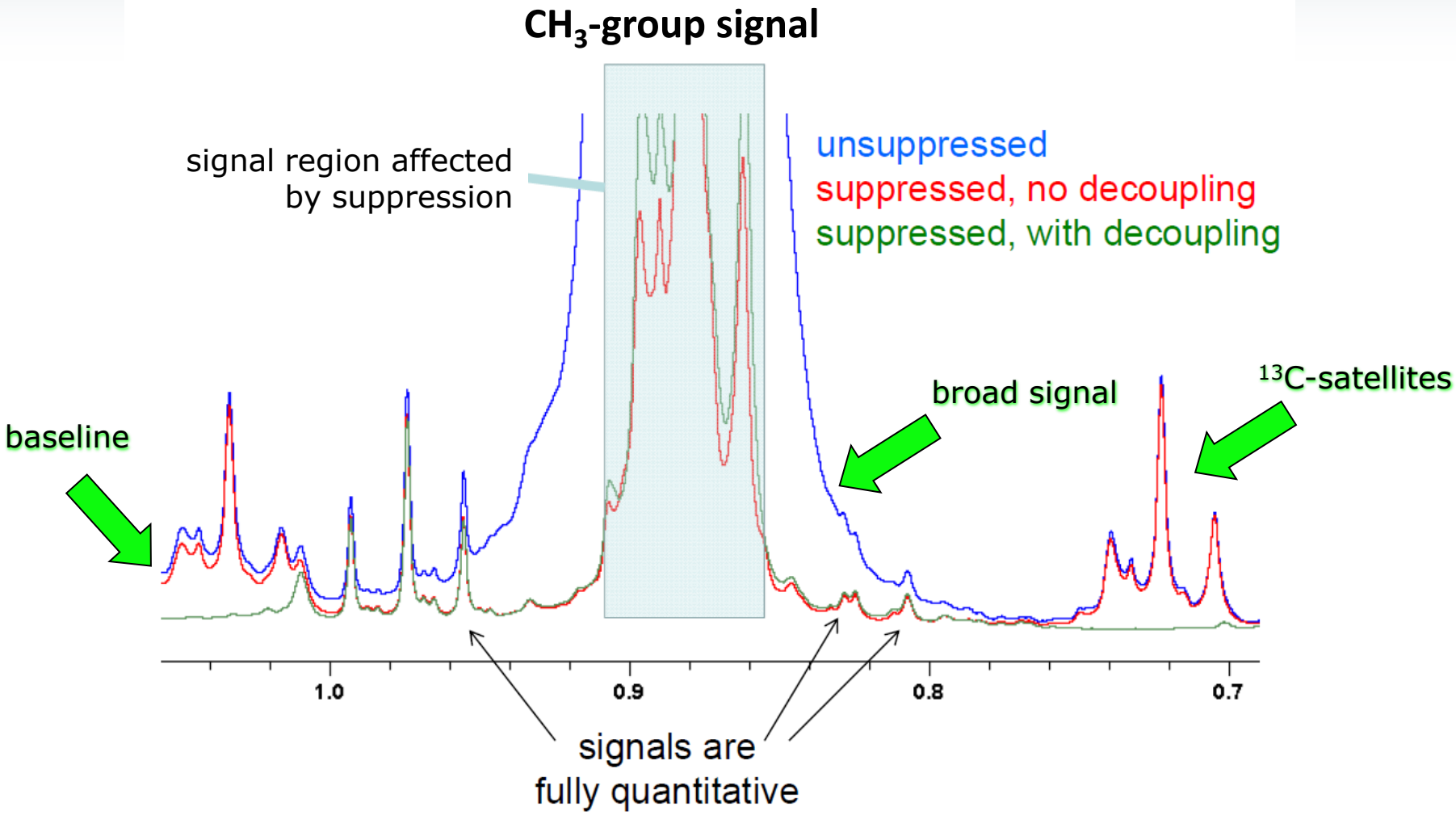
Optimizing access to minor compounds

Avoiding loss of diagnostically relevant signals

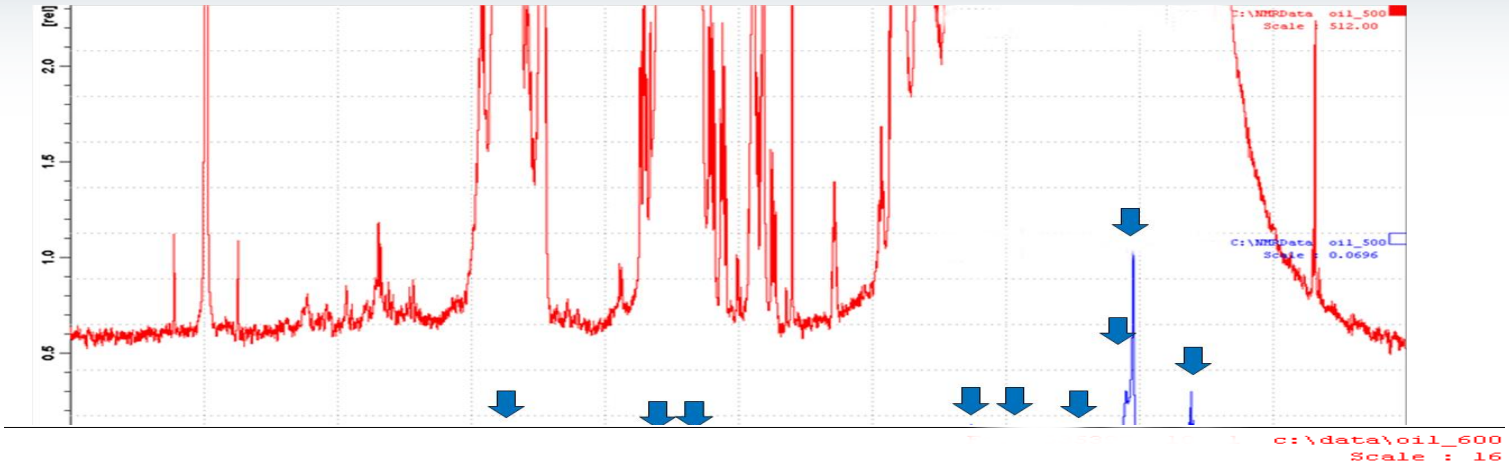


Quantitation of minor compounds

A comparison of the three approaches



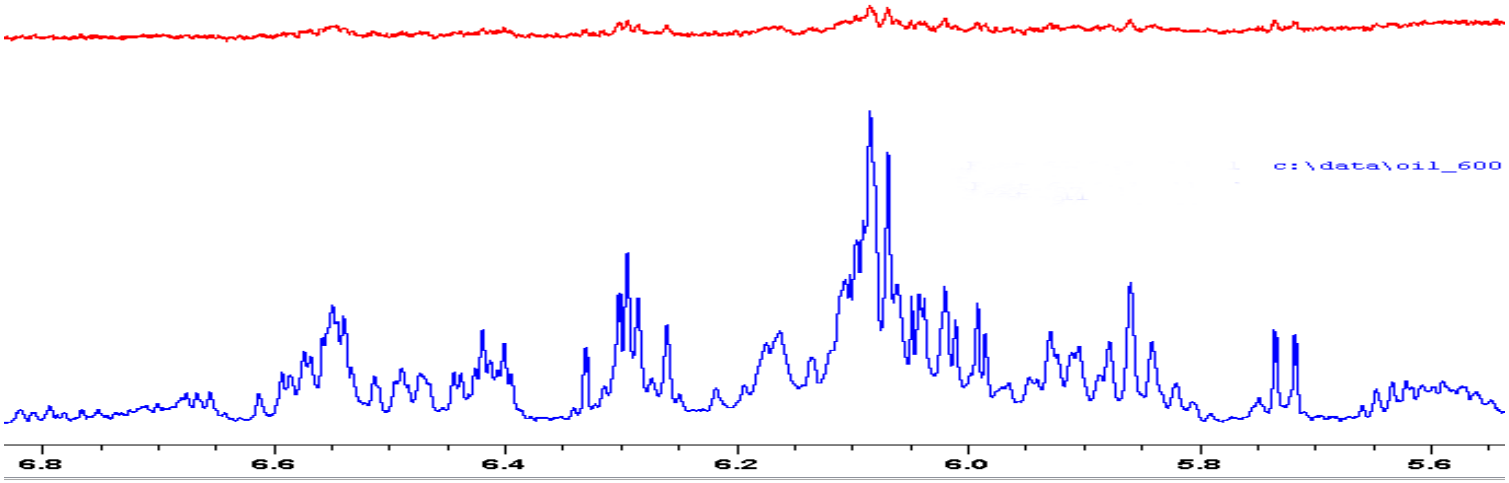
Edible Oil Spectra
Effect of optimizing dynamic range



Multiple Lipid signal Suppression using shaped pulses

Without Lipid Signal Suppression

How to see much more than the tip of the iceberg with NMR



Lipid signals suppressed

Edible oil sample preparation
Standardized Operation Procedure

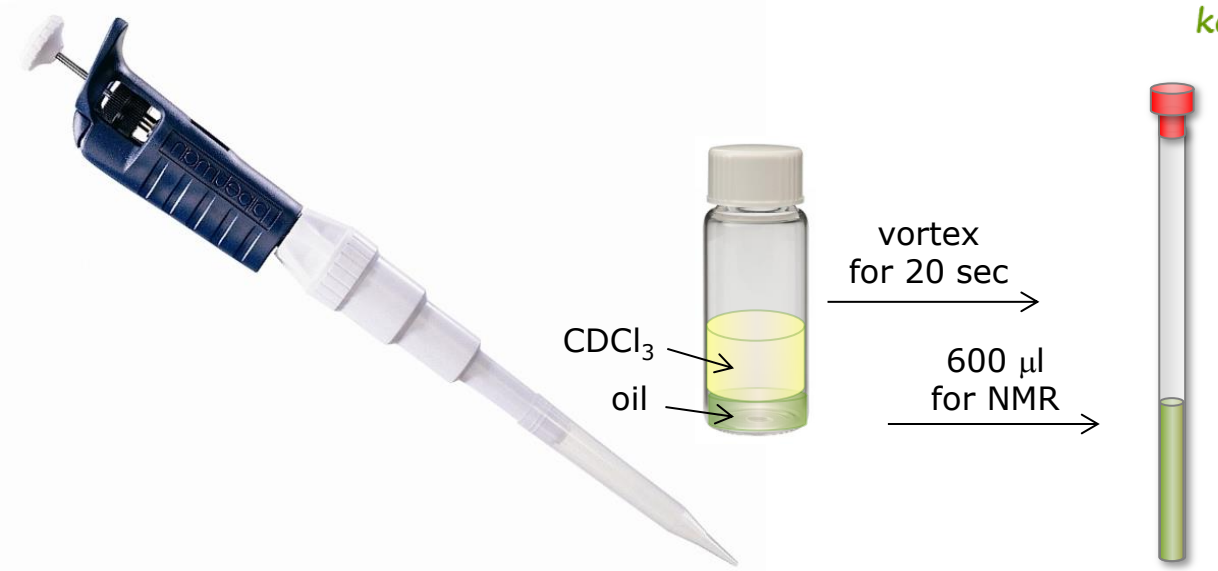
Prerequisite

Use an SOP which allows to generate and compare all types of (edible) oil models

→ same solvent, same oil concentration (and be aware of volatile solvents evaporating from unfused tubes in sample changer queues ...)

SAMPLE ID	OIL WEIGHT (mg)	CDCl3 theo Volume (µl)	FINAL WEIGHT (mg)	CDCL3 Weight (mg)	CDCL3 Volume (µl)	weight OIL / weight CDCl3	CDCl3 Volume difference to ref (µl)
REFERENCE	140,00	700,00	1176,00	1036,00	700,00	13,51351351	0,00
1	139,70	698,50	1176,30	1036,60	700,41	13,47675092	1,91
2	139,80	699,00	1173,80	1034,00	698,65	13,52030948	-0,35

keep as constant as possible

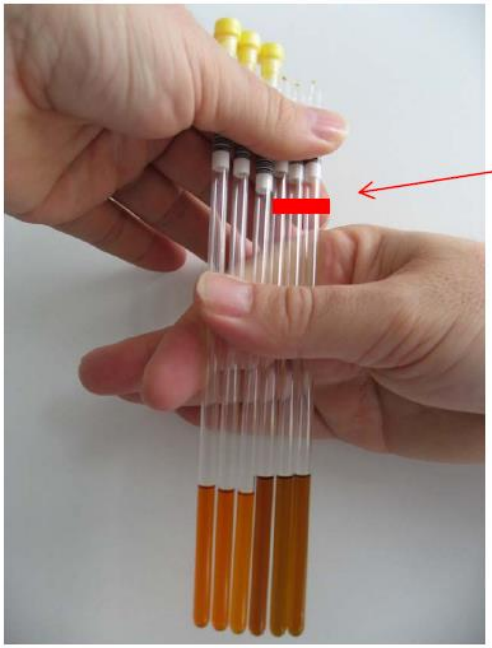


melt-seal tubes or not ?

make sure solvent won't evaporate, but sealing may influence (volatile) oil components

Edible oil sample preparation

Effect of melt sealing sample tubes



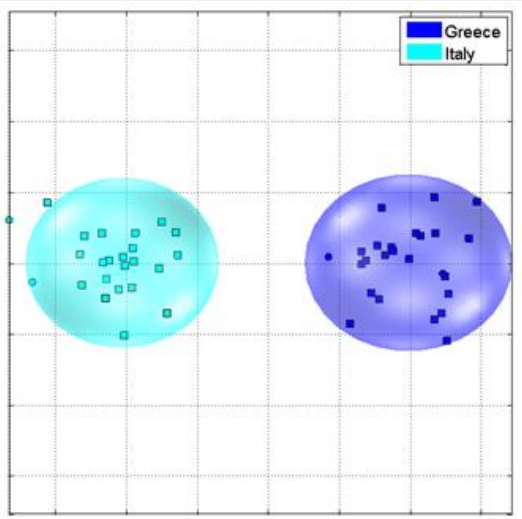
Loss of sample volume after 3 days in samples without melt sealing

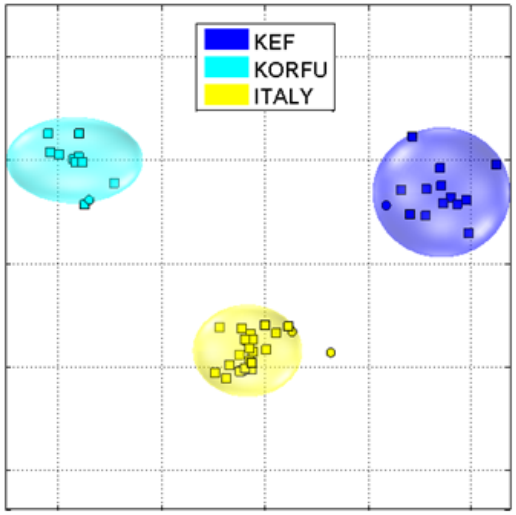
Sample colour changes when applying melt sealing

No melt sealing was applied in order to avoid sample modification. In order to avoid sample volume loss, samples where measured within 12h (no volume loss was observed within that time).

Geographical origin of olive oils

Italy versus Greek Islands





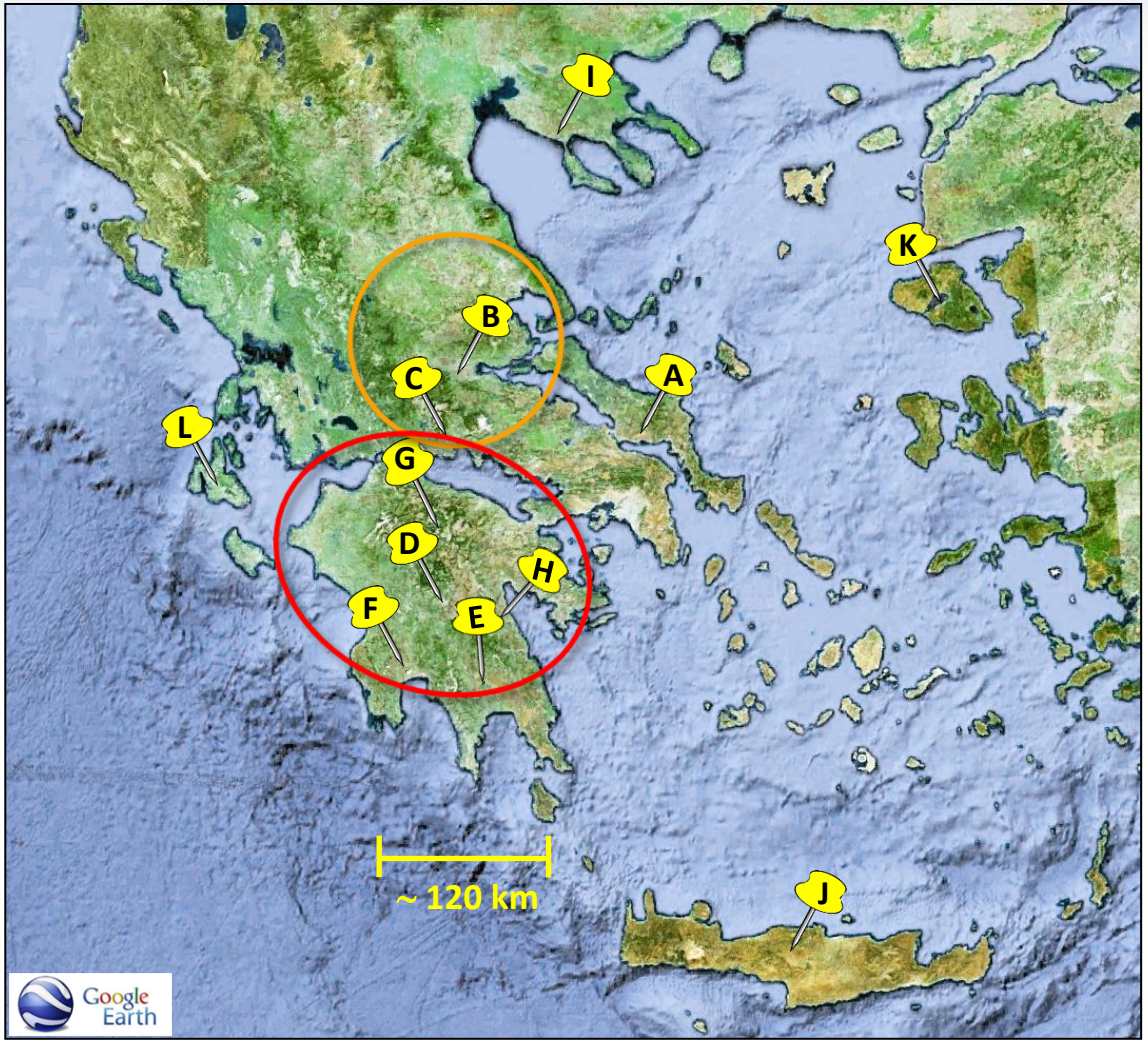
NMR screening of Greek olive oils

Overview of the authentic samples' origins

"Central"

"Peloponnese"

- A Euboea
- B Lamia
- C Amfissa
- D Peloponnese
- E Lakonia
- F Messinia
- G Aigialeia
- H Kynouria
- I Chalkidiki
- J Crete
- K Lesbos
- L Ionian Islands

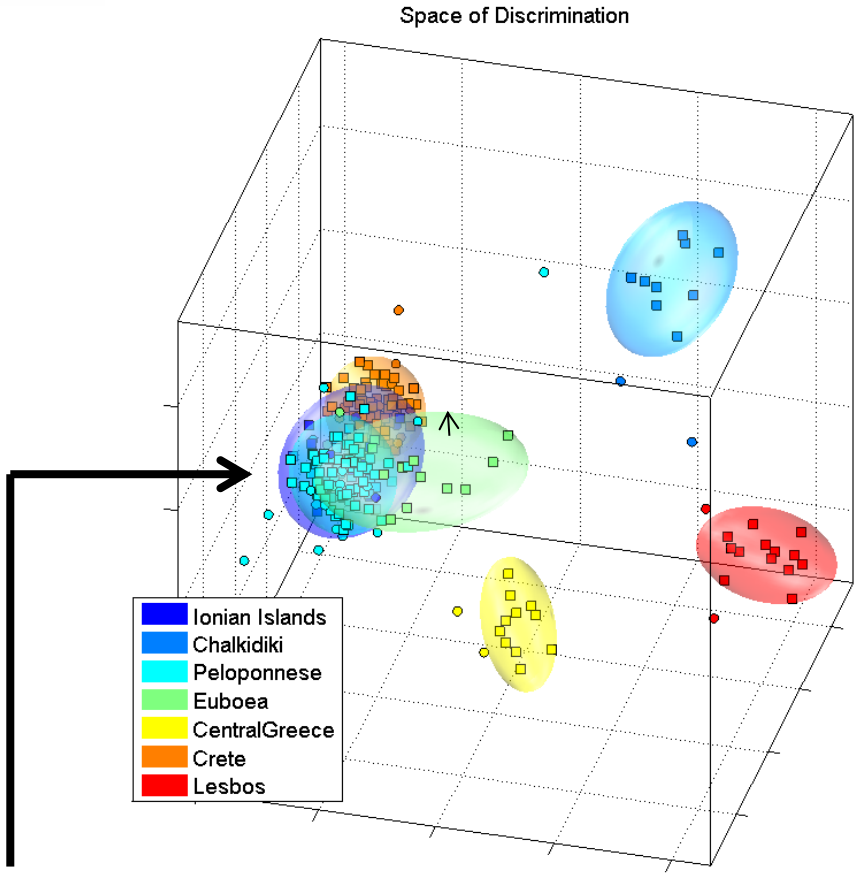


- only groups with >10 samples taken for statistics
- some closely neighbored regions combined to groups

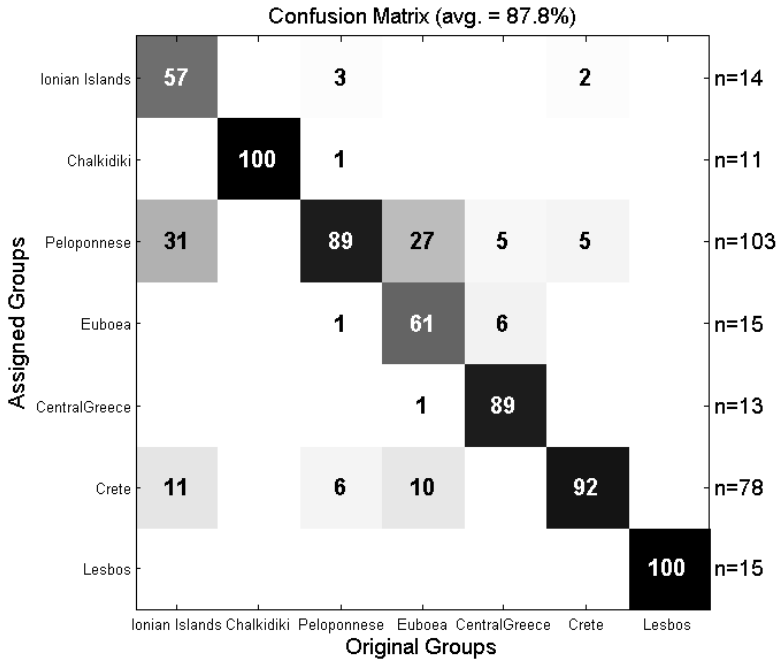
NMR screening of Greek olive oils

Differentiation of geographical origin

based on ^{1}H-NMR spectra of 249 Greek olive oils



despite the small number of samples, group assignment is significantly away from being random



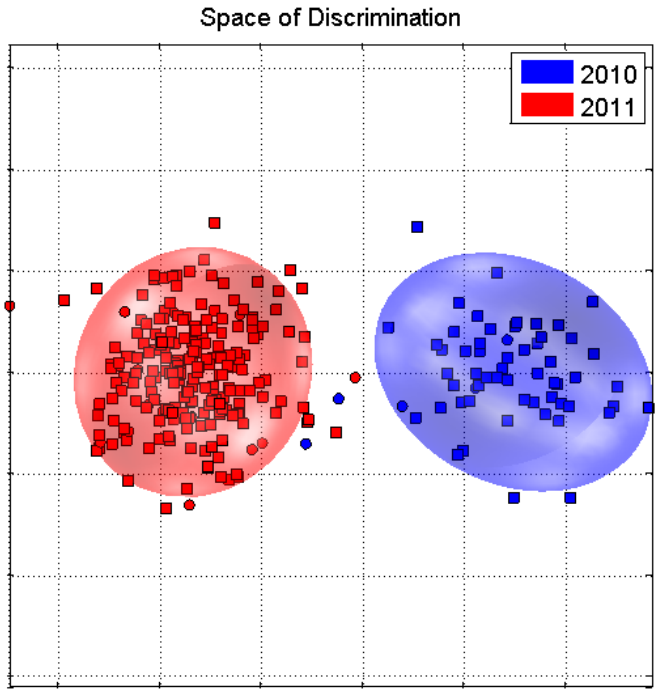
Oil profiles of Euboea and Ionian Islands samples similar to that of Peloponnese samples

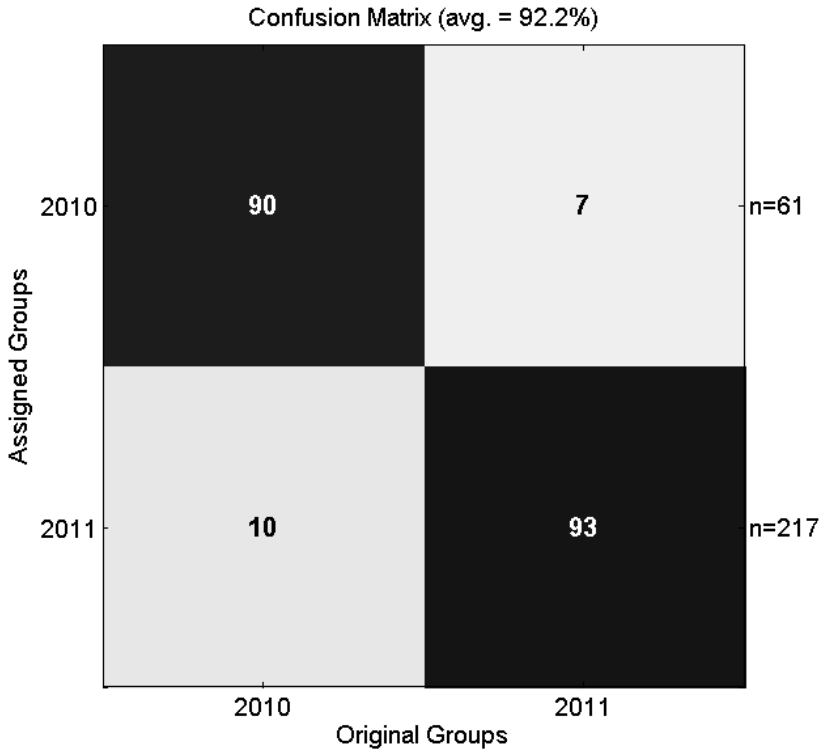
NMR screening of Greek olive oils

Classification by harvesting year

based on ^{1}H-NMR spectra* of 278 Greek olive oils

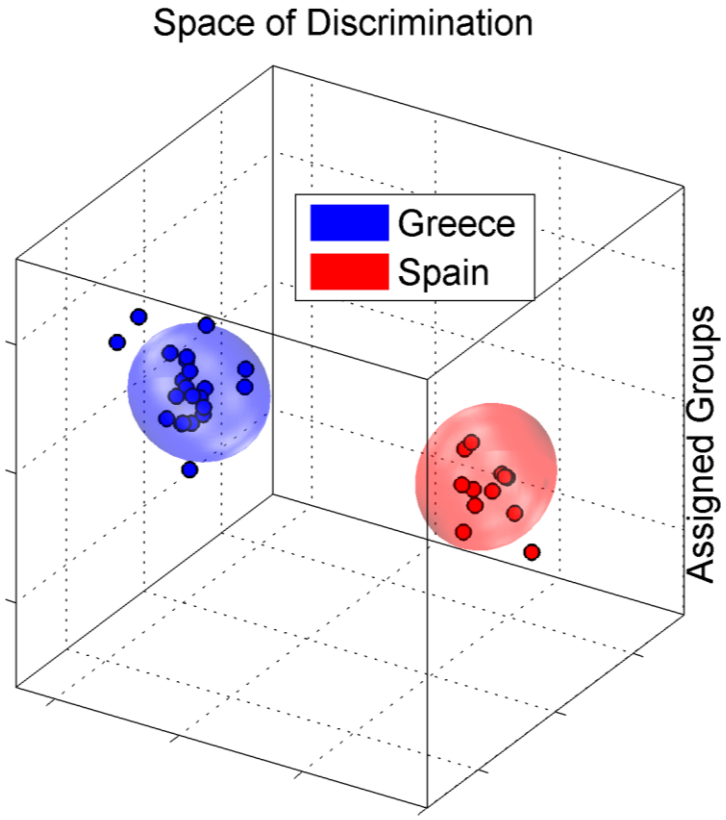
* *all with multiple signal suppression plus ^{13}C-decoupling*

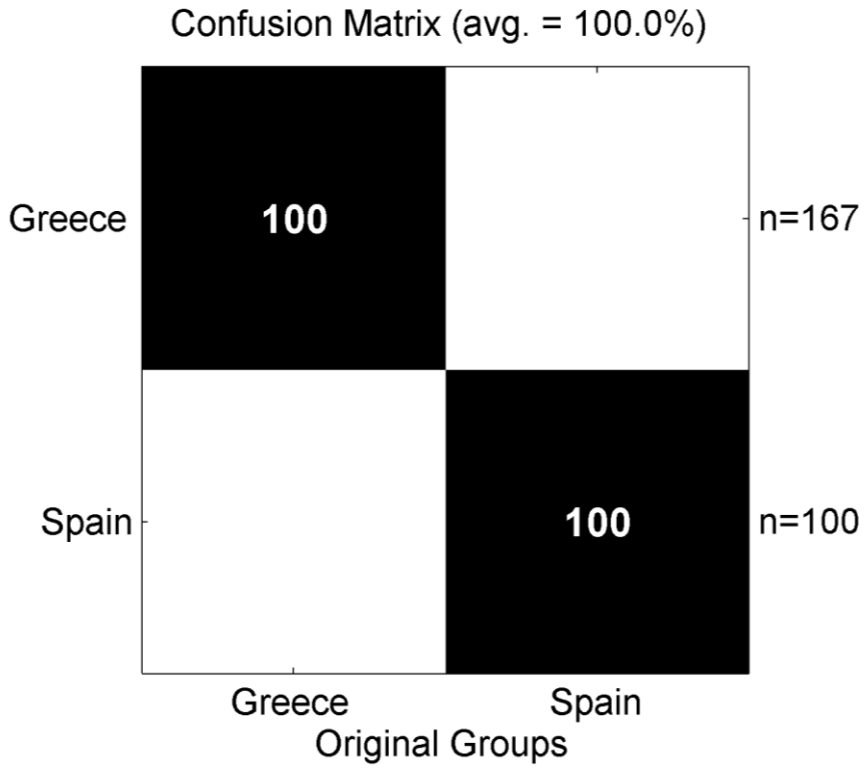




Differentiation of Spanish/Greek Olive Oil Without suppression of lipid signals

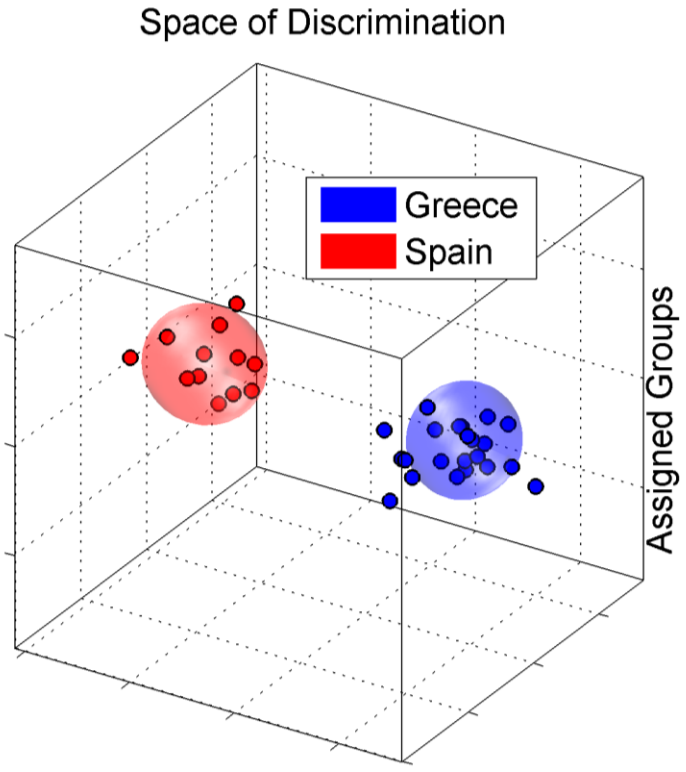
Using lipid region

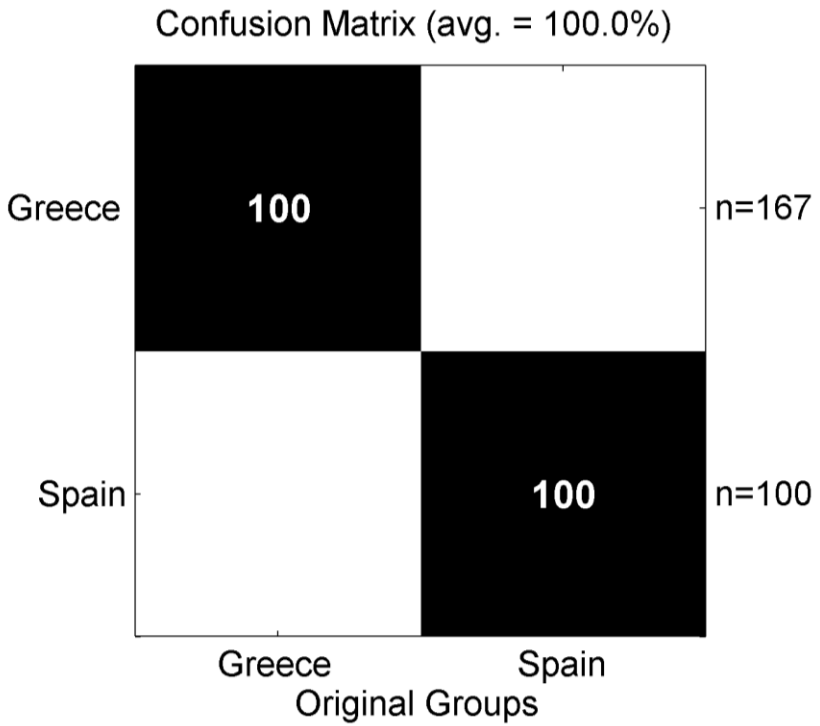




Differentiation Spanish/Greek Olive Oil
With suppression of all large lipid lines

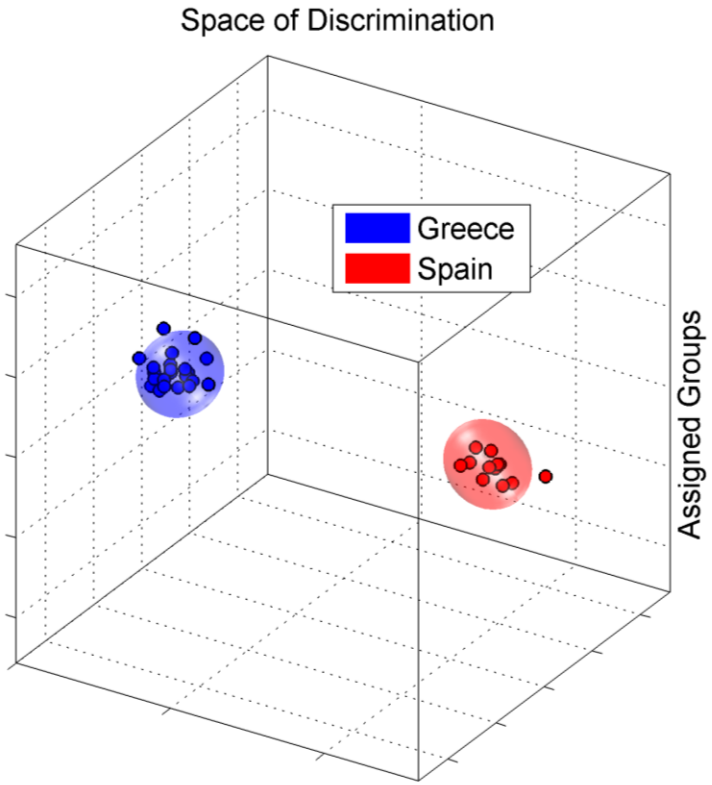
Exclusion of lipid region

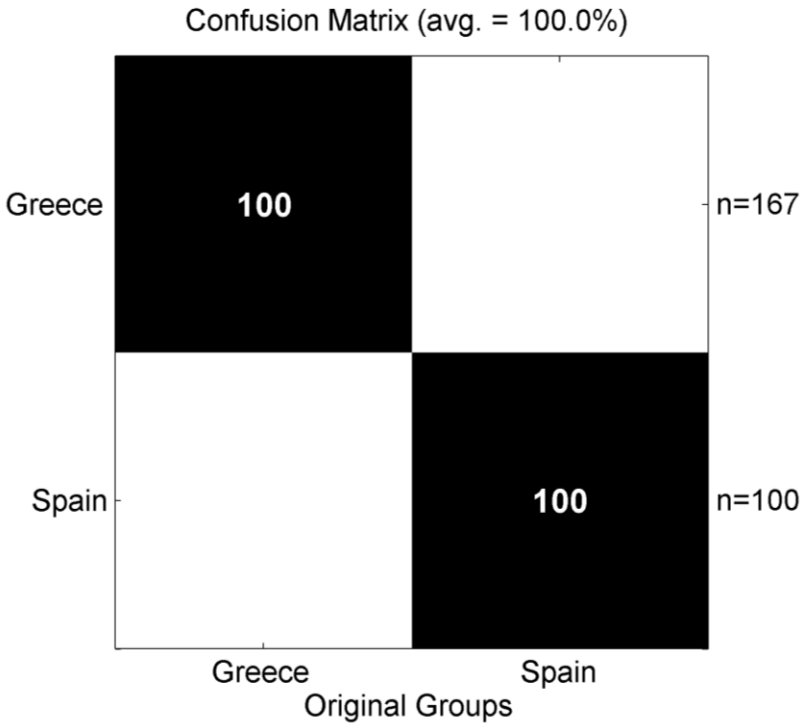




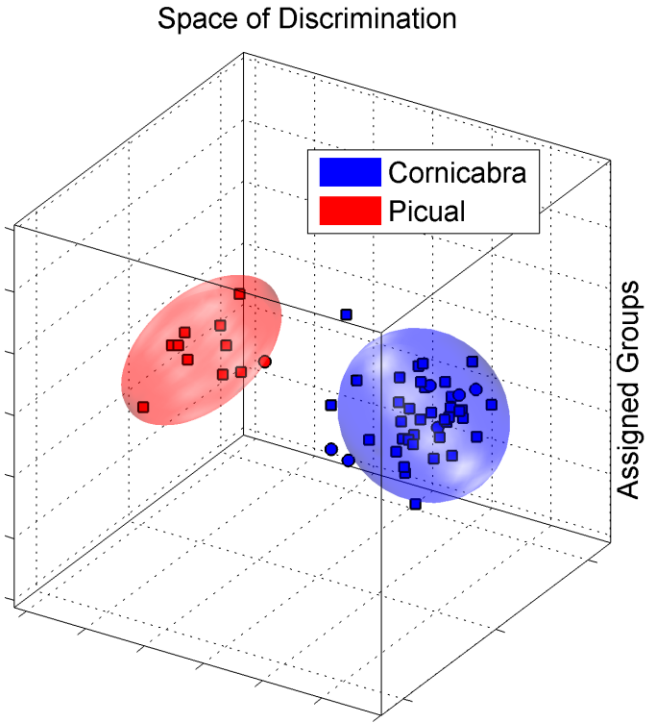
Olive Oil: differentiation Spain/Greece
Combined: without/with suppression

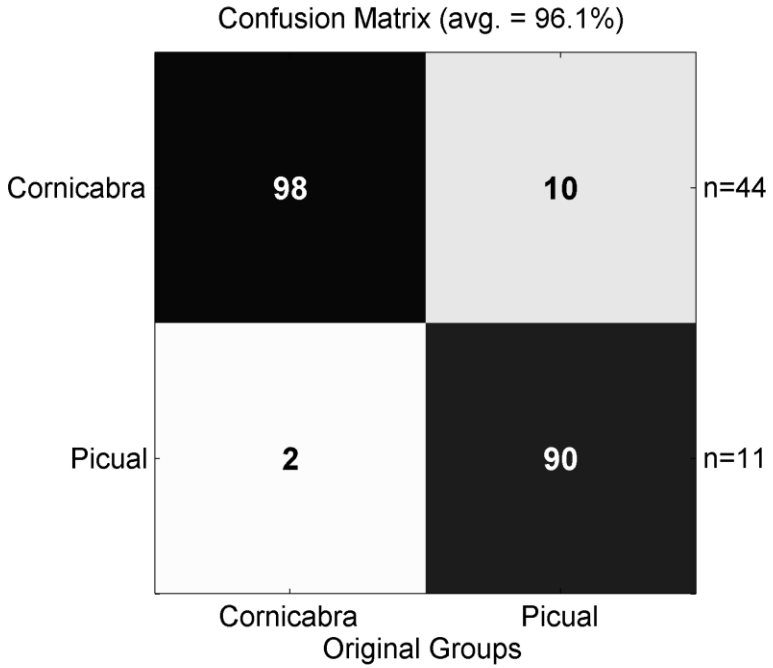
Combined model means using Lipid section from unsuppressed spectra and Other regions from suppressed spectra



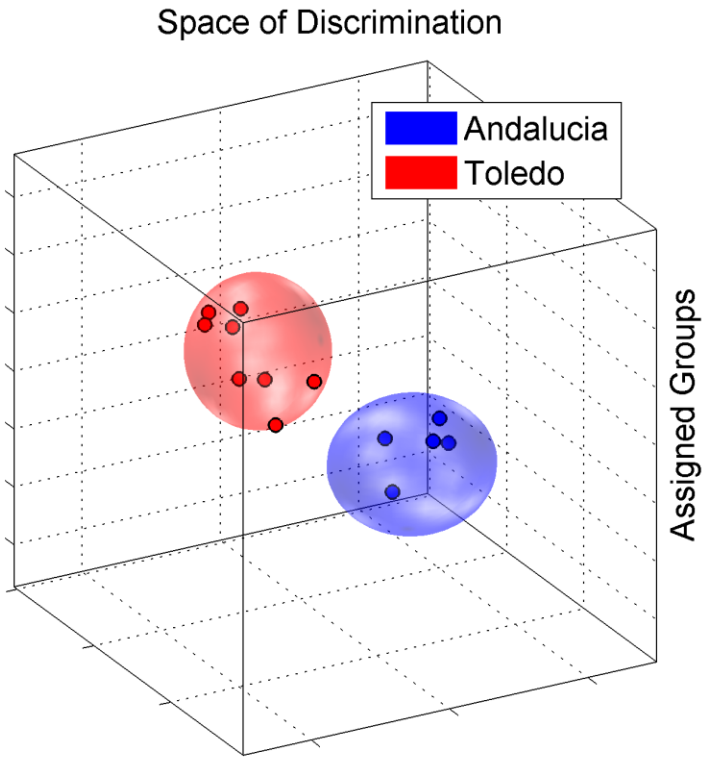


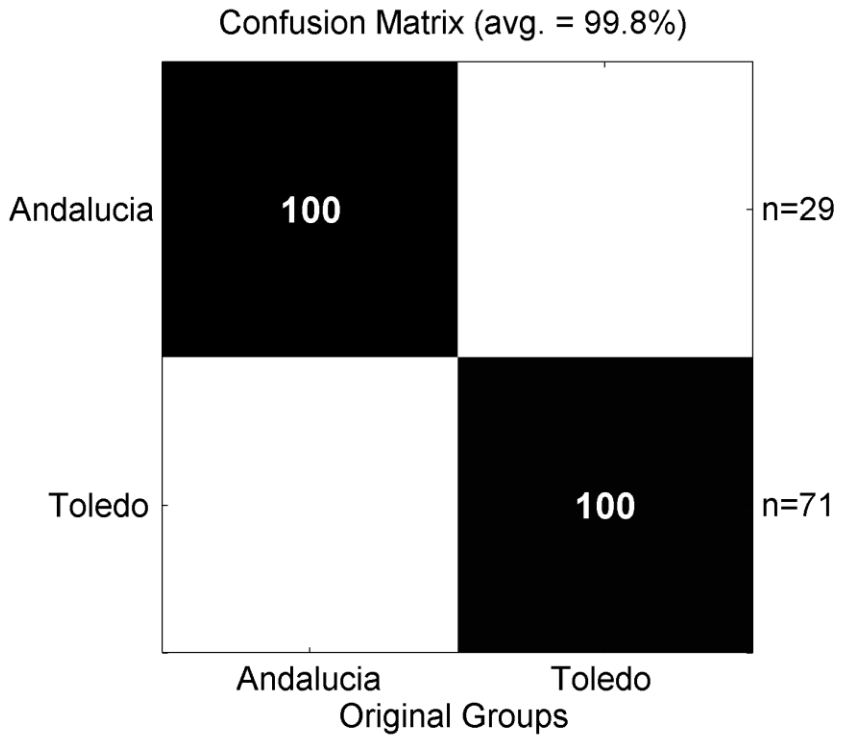
Variety differentiation Spanish Olive Oil



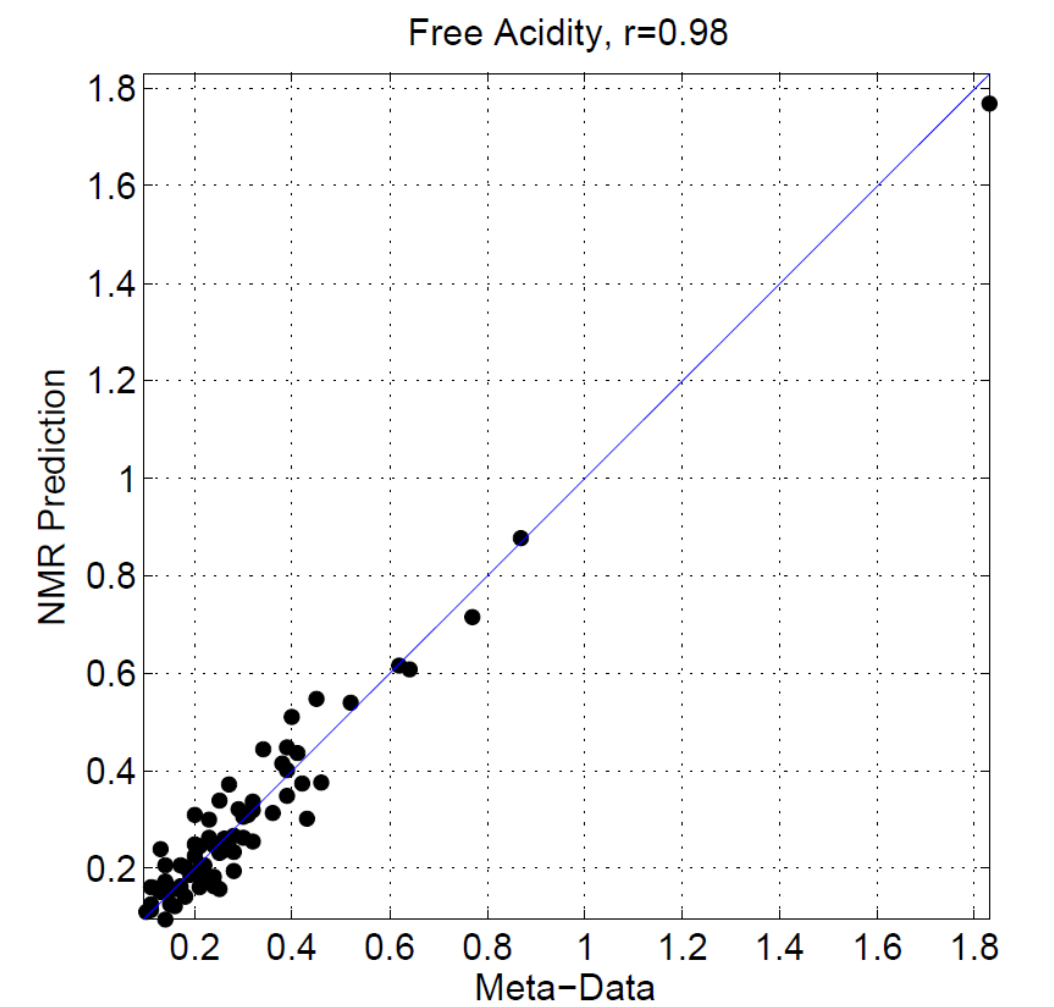


Region differentiation in Spain on Olive Oil Andalucia/Toledo

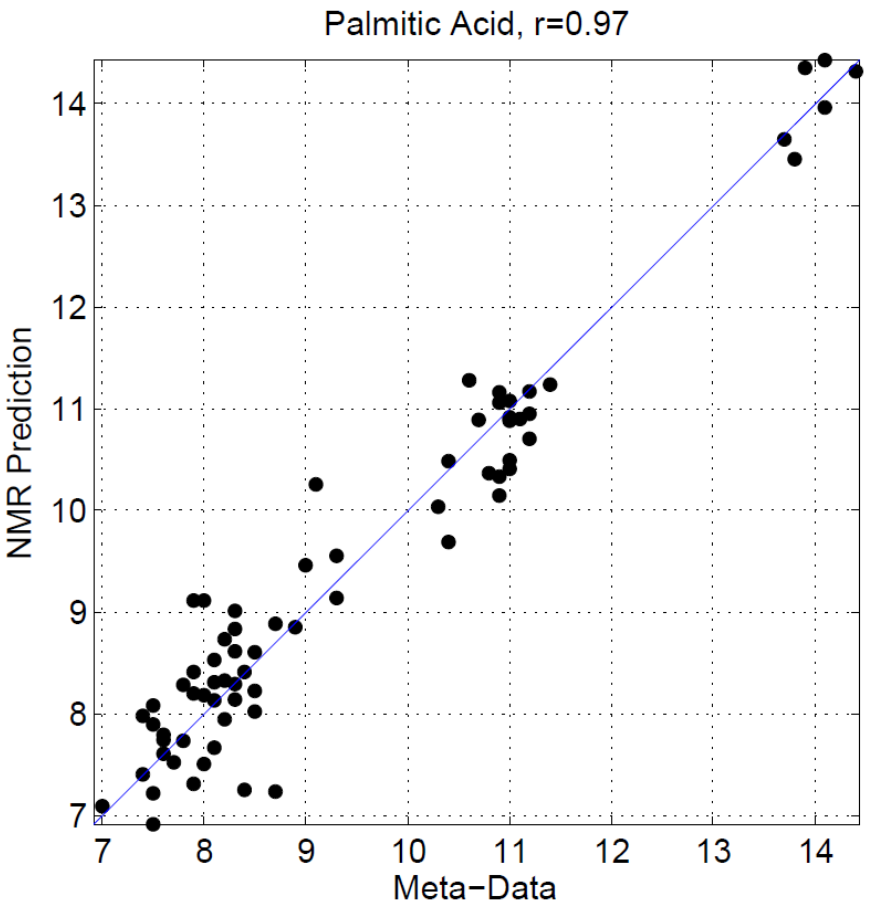


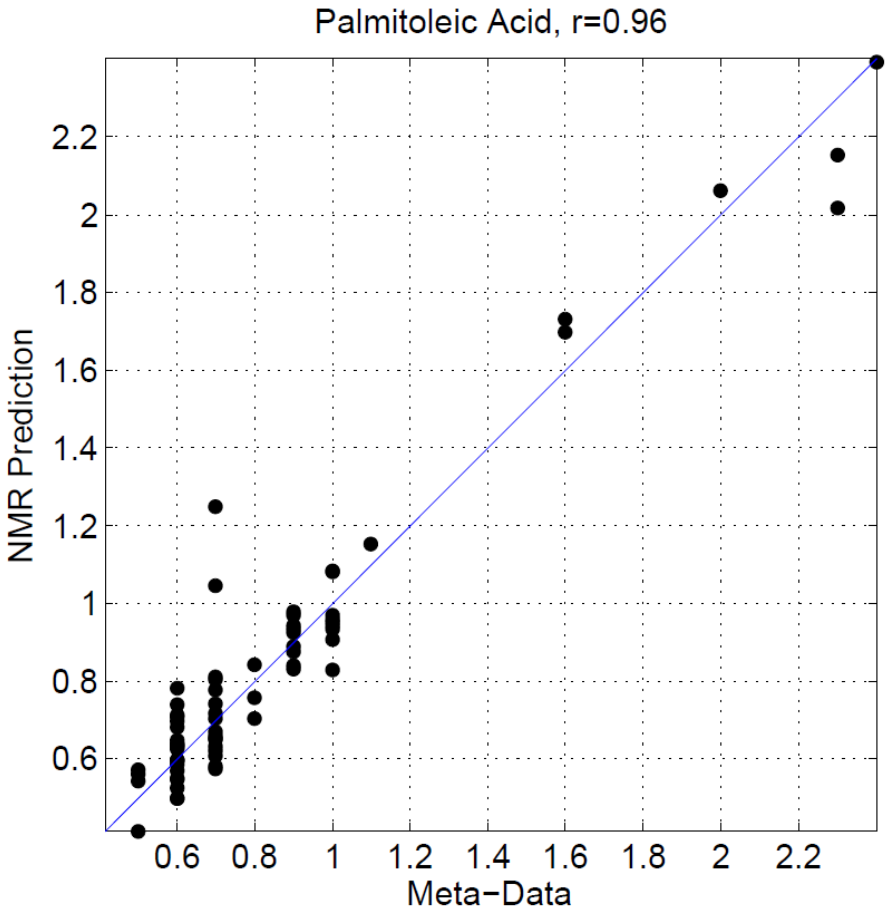


Free Acidity of Olive Oil by Regression Analysis, first results

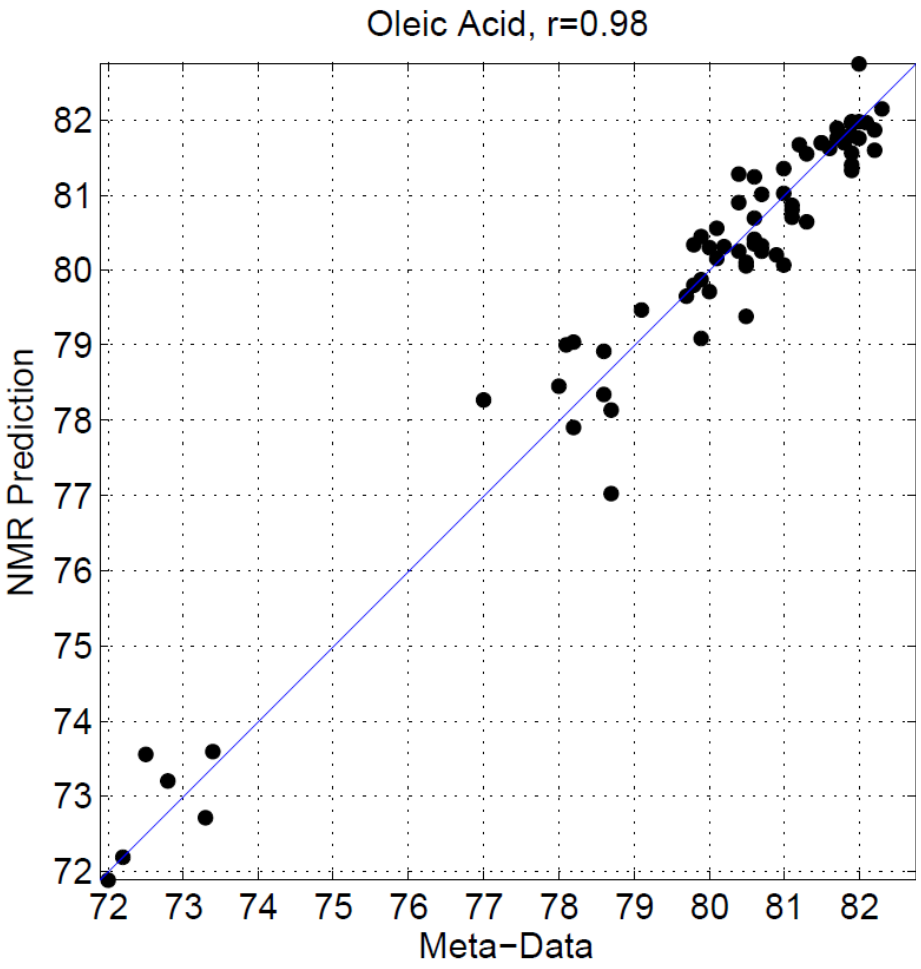


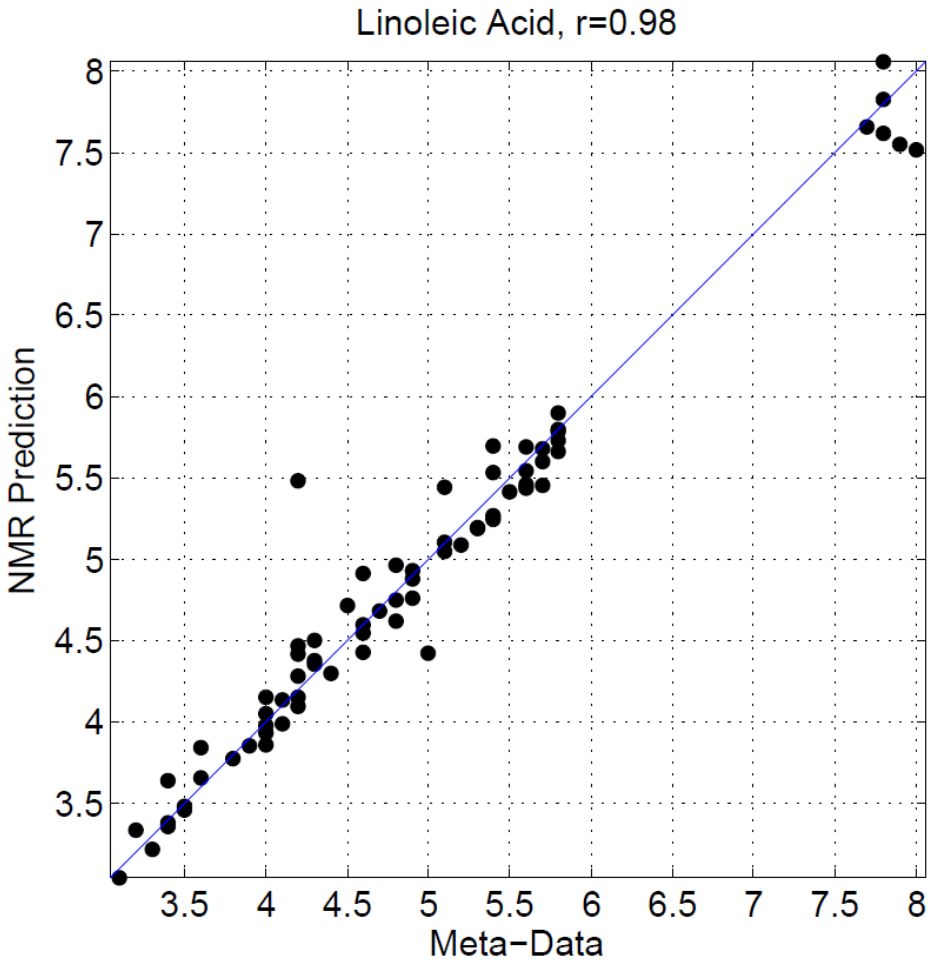
Regression analysis first results Quantification of Palmitic and Palmitoleic Acid





Regression analysis by NMR, first results
Quantification of Oleic Acid





Health Claim Olive Oil

European Food Safety Authority

EFSA Journal 2012;10(8):2848

SCIENTIFIC OPINION

Scientific Opinion on the substantiation of a health claim related to polyphenols in olive and maintenance of normal blood HDL-cholesterol concentrations (ID 1639, further assessment) pursuant to Article 13(1) of Regulation (EC) No 1924/2006[1]

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

This scientific output, published on 7 September 2012, replaces the earlier version published on 7 August 2012[4]

High-density lipoproteins (HDL) act as cholesterol scavengers and are involved in the reverse transport of cholesterol in the body (from peripheral tissues back to the liver). Conversely, low-density lipoproteins (LDL) carry cholesterol from the liver to peripheral tissues, including the arteries.
The Panel considers that maintenance of normal HDL-cholesterol concentrations (without increasing LDL-cholesterol concentrations) is a beneficial physiological effect.

Definition health claim: can be used if 50 mg/kg sum polyphenols is achieved or overcome.

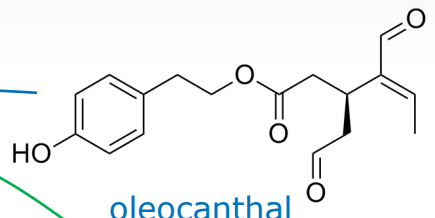
The Polyphenol Pattern

Example - Oleocanthal vs. Oleuropein

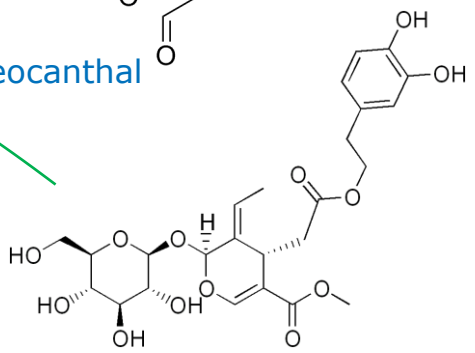
Several quality criteria for EVOO - one is the ratio oleocanthal - oleuropein

Oleocanthal is assumed to have potential as a therapeutic agent

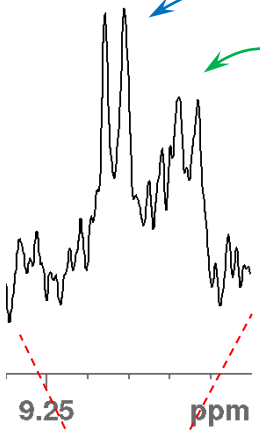
Oleuropein causes the olive oils' bitterness

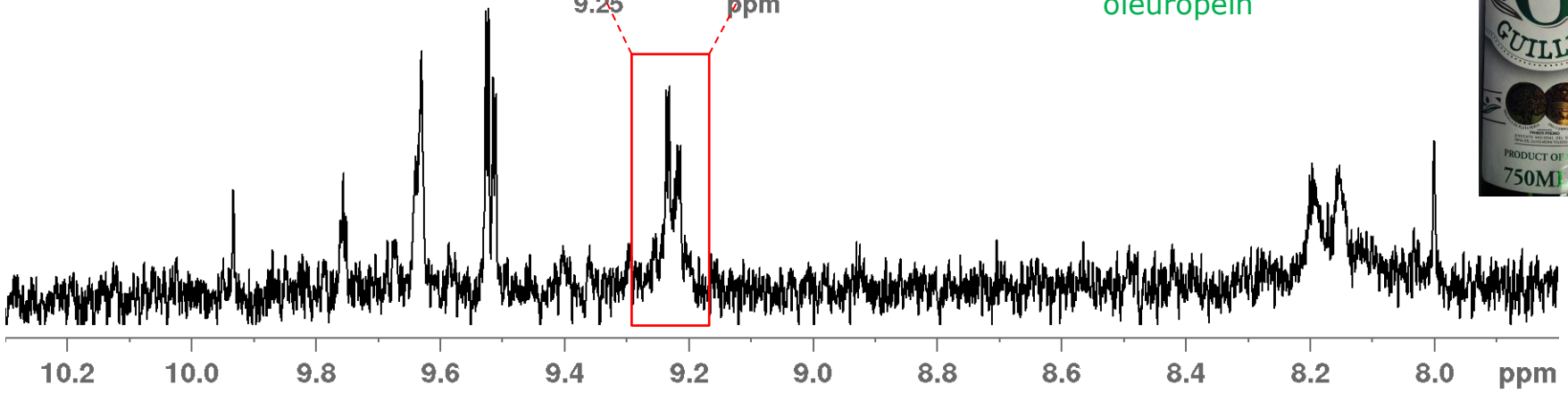


oleocanthal

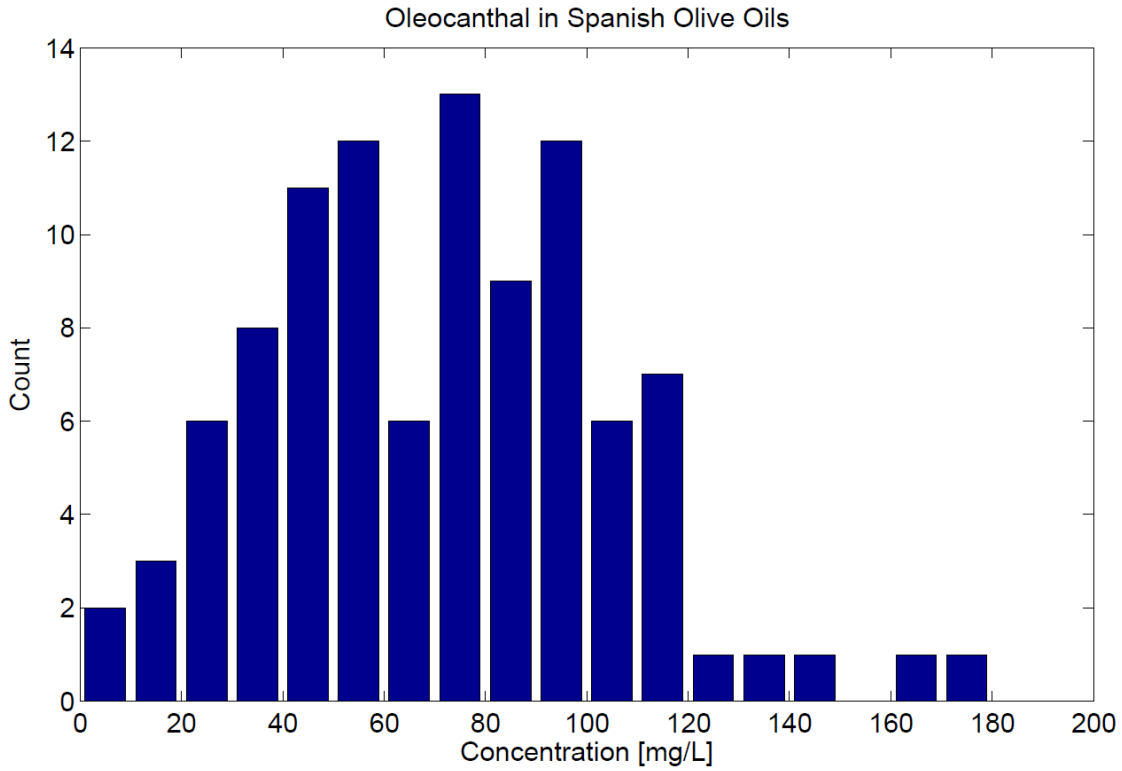


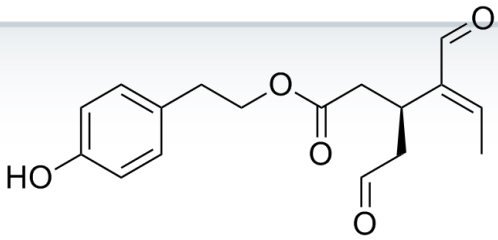
oleuropein



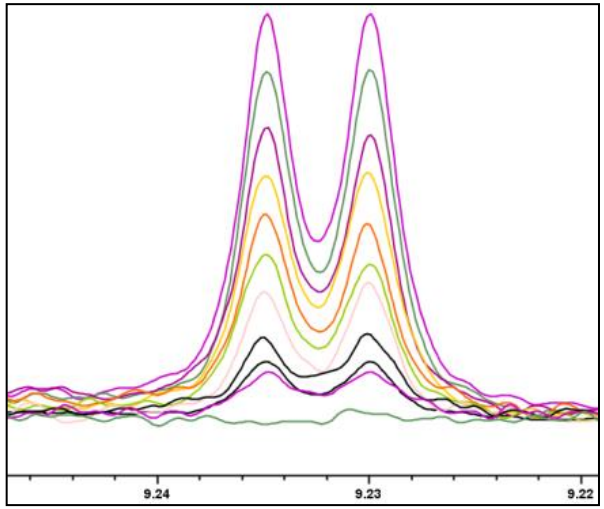


Oleocanthal – direct quantification



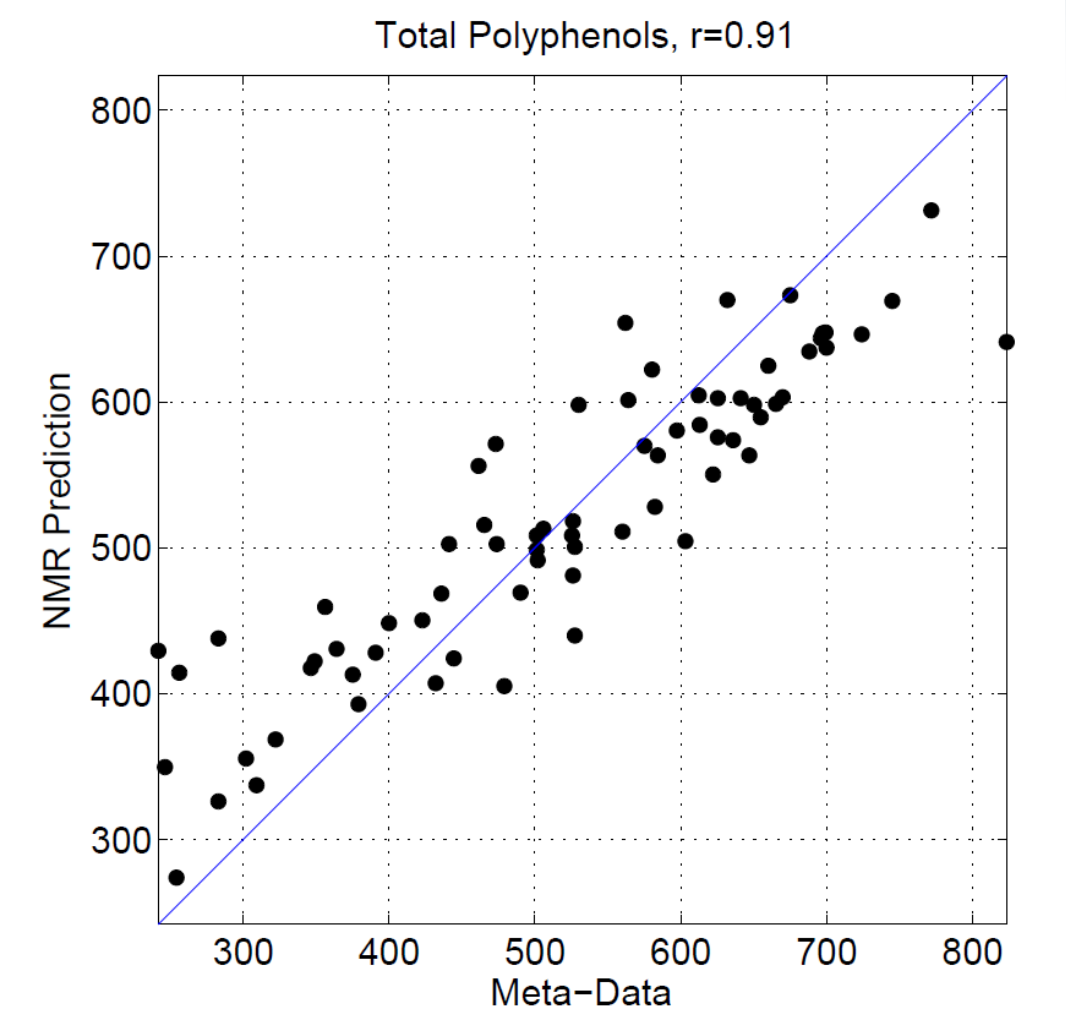


Spiking of oleocanthal



also in progress:
tyrosol
hydroxytyrosol
oleacein

Regression analysis by NMR, first results
Total Polyphenols



IVDr Lipoprotein Subclass Analysis B.I.-LISA™ out of plasma/serum

Extracts from automatic reports

Main Parameters

Key	Parameter	Value	Unit	95% Range of Model	Graphics (*)
TPTG	TG	124	mg/dL	55 - 287	
TPCH	Chol	183	mg/dL	140 - 295	
LDCH	LDL-Chol	86	mg/dL	63 - 202	
HDCH	HDL-Chol	50	mg/dL	36 - 96	
TPA1	Apo-A1	131	mg/dL	113 - 223	
TPA2	Apo-A2	25	mg/dL	27 - 47	
TPAB	Apo-B100	55	mg/dL	35 - 99	

(*) Gray horizontal boxes represent 95% range of model, black vertical lines represent sample value.

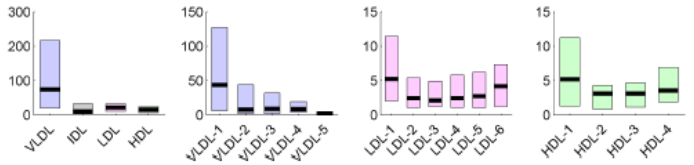
Calculated Figures

Key	Parameter	Value	Unit	95% Range of Model	Graphics (*)
LDHD	LDL-Chol/HDL-Chol	1,70	-/-	0,88 - 3,48	
ABA1	Apo-B100/Apo-A1	0,42	-/-	0,20 - 0,77	

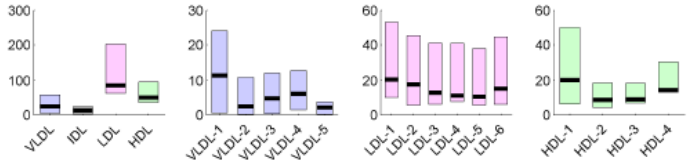
(*) Gray horizontal boxes represent 95% range of model, black vertical lines represent sample value.

Healthy Person compliant to concentration distribution in model in all fractions

Triglycerides distribution (concentrations in mg/dL together with 95% range of model)



Cholesterol distribution (concentrations in mg/dL together with 95% range of model)



Main Parameters

Key	Parameter	Value	Unit	Reference	Graphics
TPTG	Triglycerides	597	mg/dL	50 - 150	
TPCH	Cholesterol	323	mg/dL	110 - 220	
LDCH	LDL Cholesterol	120	mg/dL	70 - 150	
HDCH	HDL Cholesterol	57	mg/dL	35 - 80	
TPA1	Apo-A1	163	mg/dL	90 - 170	
TPA2	Apo-A2	38	mg/dL	25 - 50	
TPAB	Apo-B100	119	mg/dL	40 - 115	

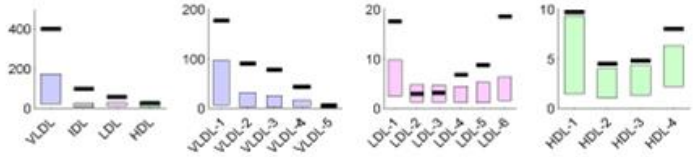
Calculated Figures

Key	Parameter	Value	Unit	Reference	Graphics
LDHD	LDL-Chol/HDL-Chol	2,10	-/-	< 3,00	
ABA1	Apo-B100/Apo-A1	0,73	-/-	0,35 - 1,15	

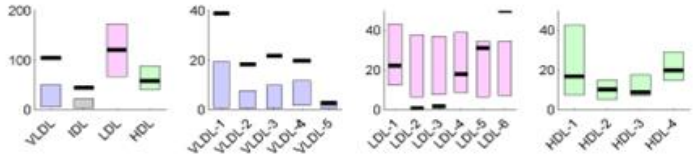
Person having a stroke shortly after blood collection, most fractions out of model ranges

LDL-Cholesterol in model, but subfractions out!

Triglycerides distribution (concentrations in mg/dL)



Cholesterol distribution (concentrations in mg/dL)

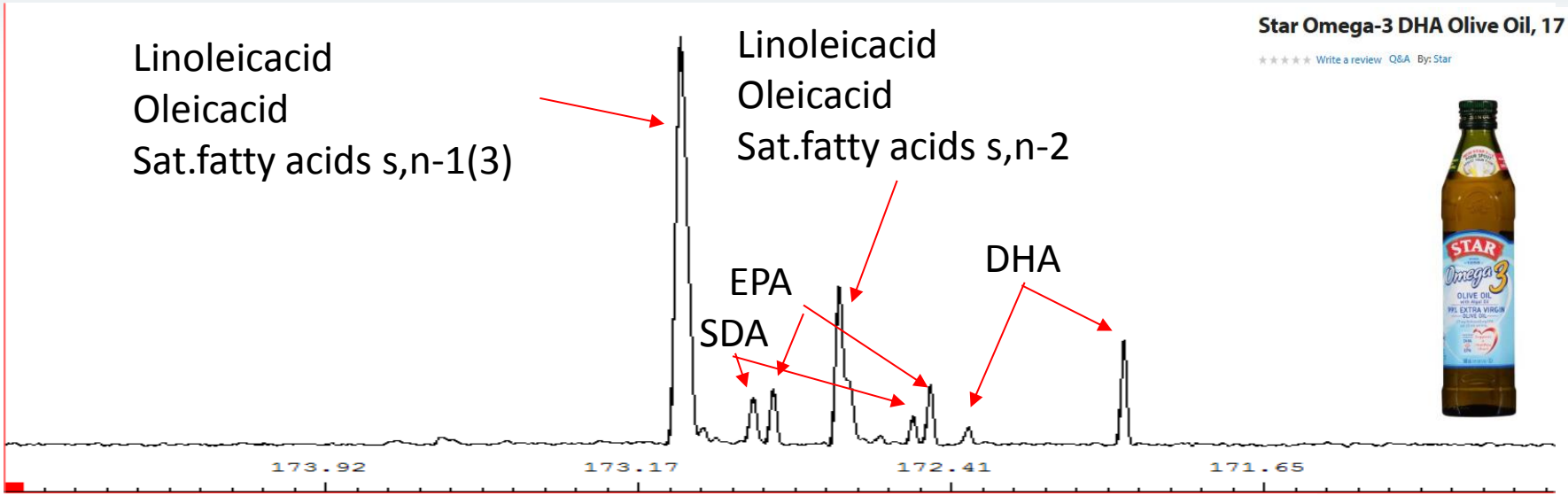


^{1}H NMR analysis of edible oils

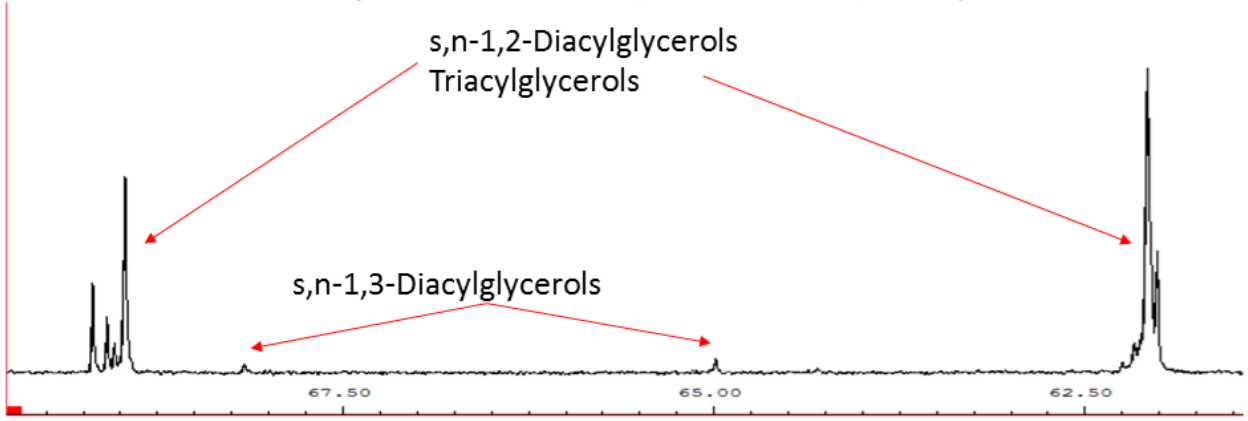
Extra virgin olive oil (EVOO), sunflower oil, and mixes

400/80 MHz comparison of a selected signal

^{13}C-NMR Screening on olive oil



Edible oil inverse gated ^{13}C-NMR (150.92MHz) for quantification



Conclusions

- It could be shown, that 1H-NMR screening of olive oils opens a large number Of parameters, that cannot be obtained conventionally in one measurement
- Targeted Analysis offers 2 possibilities, direct quantification and regression analysis
- Untargeted Analysis shows large promise for
  - Variety differentiation
  - Geographical Origin (country and subregions)
  - Vintage
- With this a tool, checks on quality and authenticity is becoming available
- Next action is to check on differentiation of grades of olive oil VOO, EVOO, …

Acknowledgement

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Li-Hong Tseng

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Claudia Napoli Bruker BioSpin S.I.r. (Milano Italy)

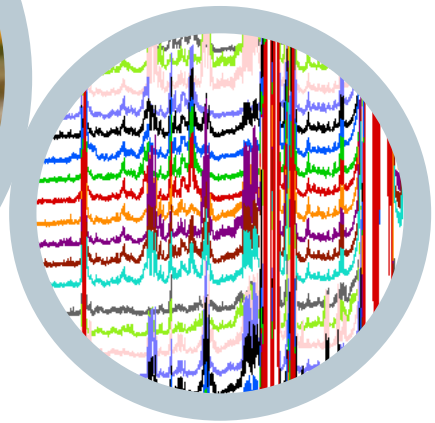
Bruker BioSpin S.I.r. (Milano, Italy)

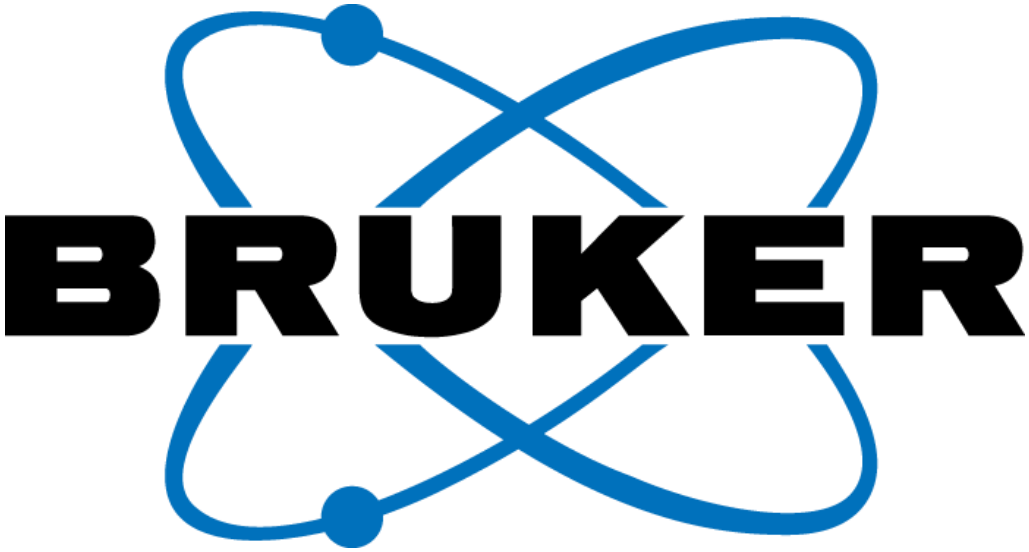
Claudia Napoli

Thank you

for your

attention





Innovation with Integrity