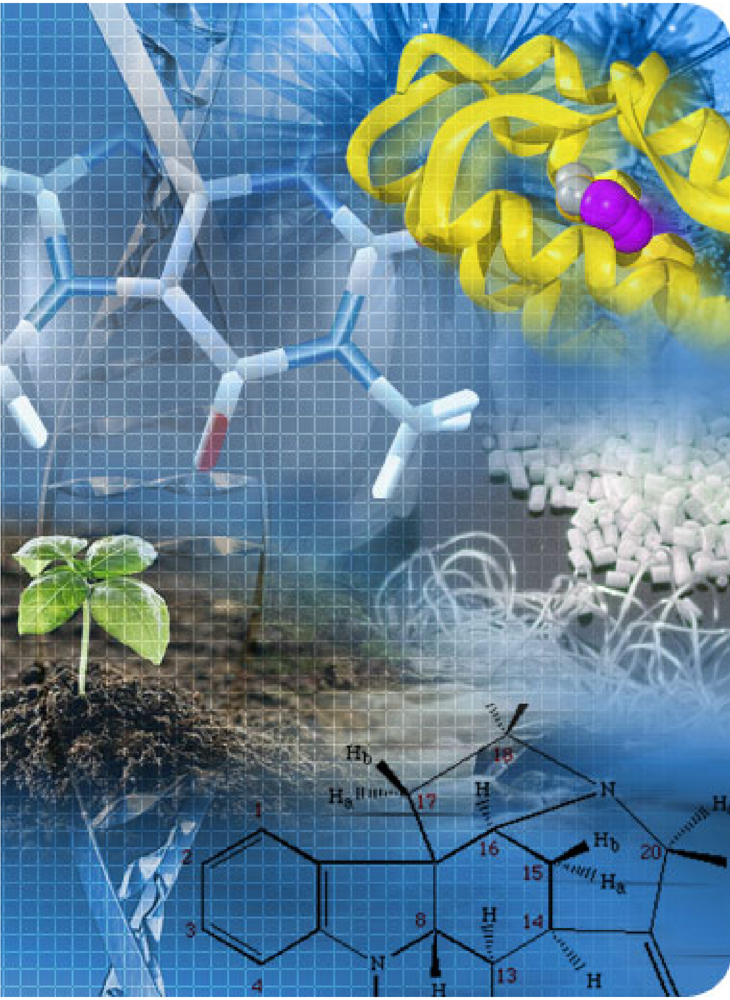
3rd International Conference and Exhibition on Food Processing & Technology
21.07.2014
Las Vegas, USA



FOOD ANALYSIS TO CHECK QUALITY, SAFETY AND AUTHENTICITY BY FULL-AUTOMATED ^{1}H-NMR

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Overview

- What can Nuclear Magnetic Resonance (NMR) accomplish in food analysis

- NMR-based screening features

- The JuiceScreener Concept as template
  - Targeted analysis (quantification)
  - Non-targeted analysis (statistics)

- The WineScreener solution
- NMR in analysis of other food (e.g. edible oil, honey, …)

What can NMR accomplish in Food Analysis?

Non-Targeted Screening / Targeted Screening
in a single measurement

- Conventional food tests are Targeted!

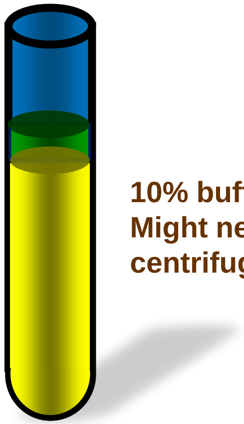
- What is not tested for, will likely be over-looked!

The Non Targeted Screening (NTS) enables the discovery and analysis of unexpected and unknown (!!) deviations, which can not detected with conventional analytical methods!

Over and above that concentration differences of known substances could be detected.

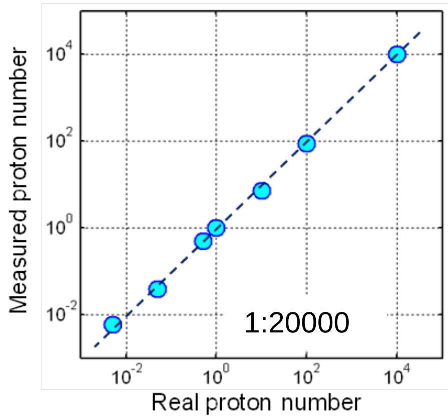
NMR-based Screening Features

Minimal sample preparation

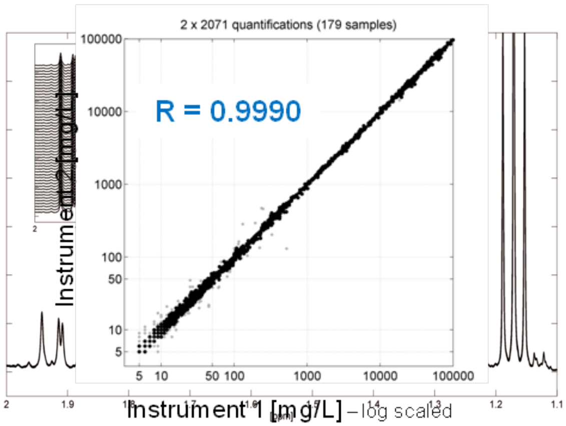


10% buffer addition
Might need centrifugation

Inherently quantitative linearity and huge dynamic range



Highest Reproducibility and Transferability



Intact mixtures

High throughput

Targeted analysis
Quantification of compounds

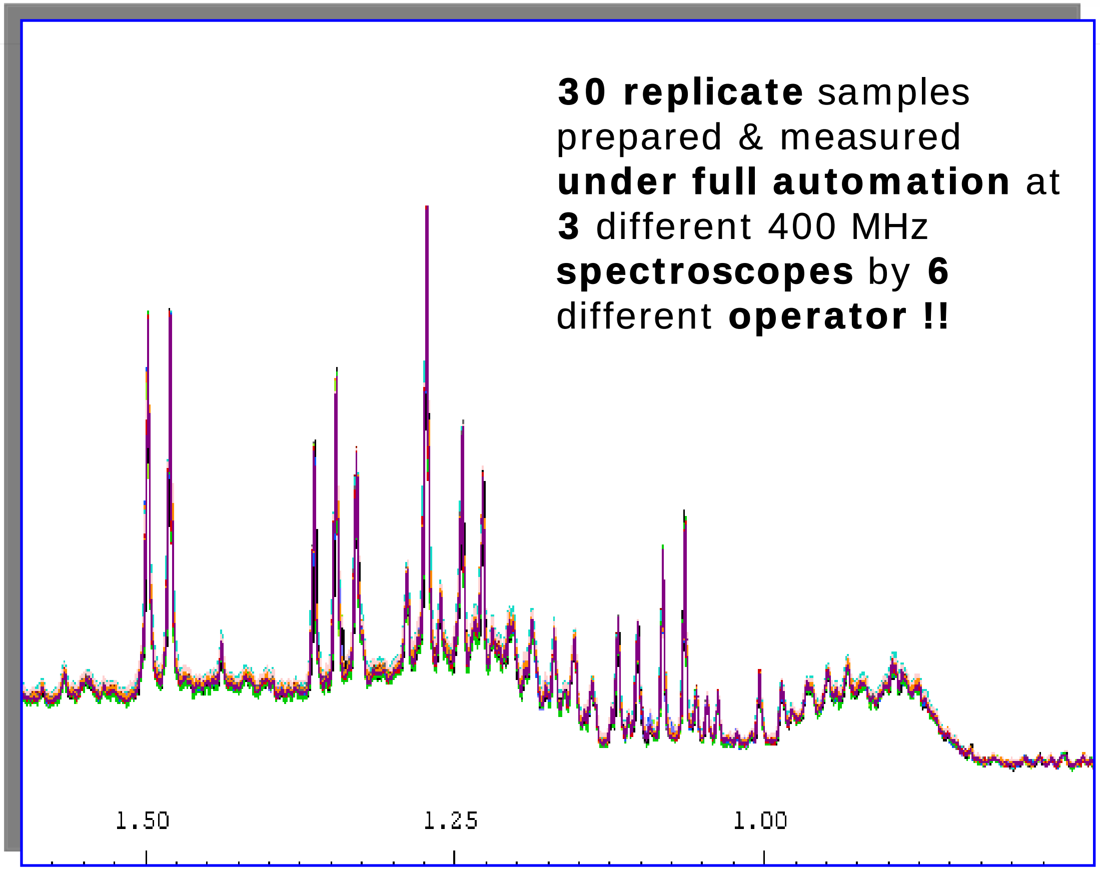
Non-targeted / statistical analysis
Metabolic Fingerprinting
(Classification/ Verification)

Highest reproducibility / transferability

Full automated models need to be applicable to data generated :

- By someone else
- At an other spectroscope
- In another lab
- Anywhere in the world
- At any time

Need of common standard and protocols in order to secure models and their applicability



Juice Quality Control by JuiceScreener / SGF-Profiling™

Fruit Juice Analysis

- **Full automated** push-button system
- Only one measurement (~ **15 minutes**)
- Minimal sample preparation
- Targeted analysis
    - Quantification of more than 30 compounds
- Non-Targeted analysis (up to 10 results)
    - Authenticity
    - Frauds
    - Fruit content
    - Quality
- Database of more than 16.000 juice spectra
- PDF report of all results
- Even retrospective analysis is possible

German Industry Award 2008
Category: Automation

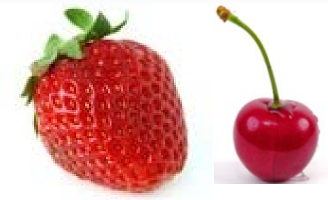
INDUSTRIE PREIS 2008

Greentech Asia Shanghai 2010: Award for most innovative Food Analysis System!

Conclusions made by Quantification

- Sugar Profile (Sucrose, Glucose, Fructose) => Addition of sugar
- Acids Profile => Addition of acid (e.g.: Citric acid in apple juice)
- Ratio Malic Acid/Quinic Acid => Ripeness of the apple
- Ratio Citric acid/Iso-citric Acid in Lemon Juice => Addition of citric acid
- Concentration of Galacturonic Acid => Enzymatic treatment in apple juice
- Concentration of Phlorin in Citrus Fruit => Usage of peels
- Concentration of spoilage parameters => Lactic Acid, Fumaric Acid, Formic Acid, Gluconic Acid
- Detection of Other Fruits => For example, pear in apple juice, citrus fruit in apple juice, grapefruit in orange juice

Example: Fruit content



Fruit content of Red-Fruits Purees

- Red fruits: High value products

  ➡ High number of adulterated products (2008/2009)

- Adulteration :

  Dilution by **addition of sugar**

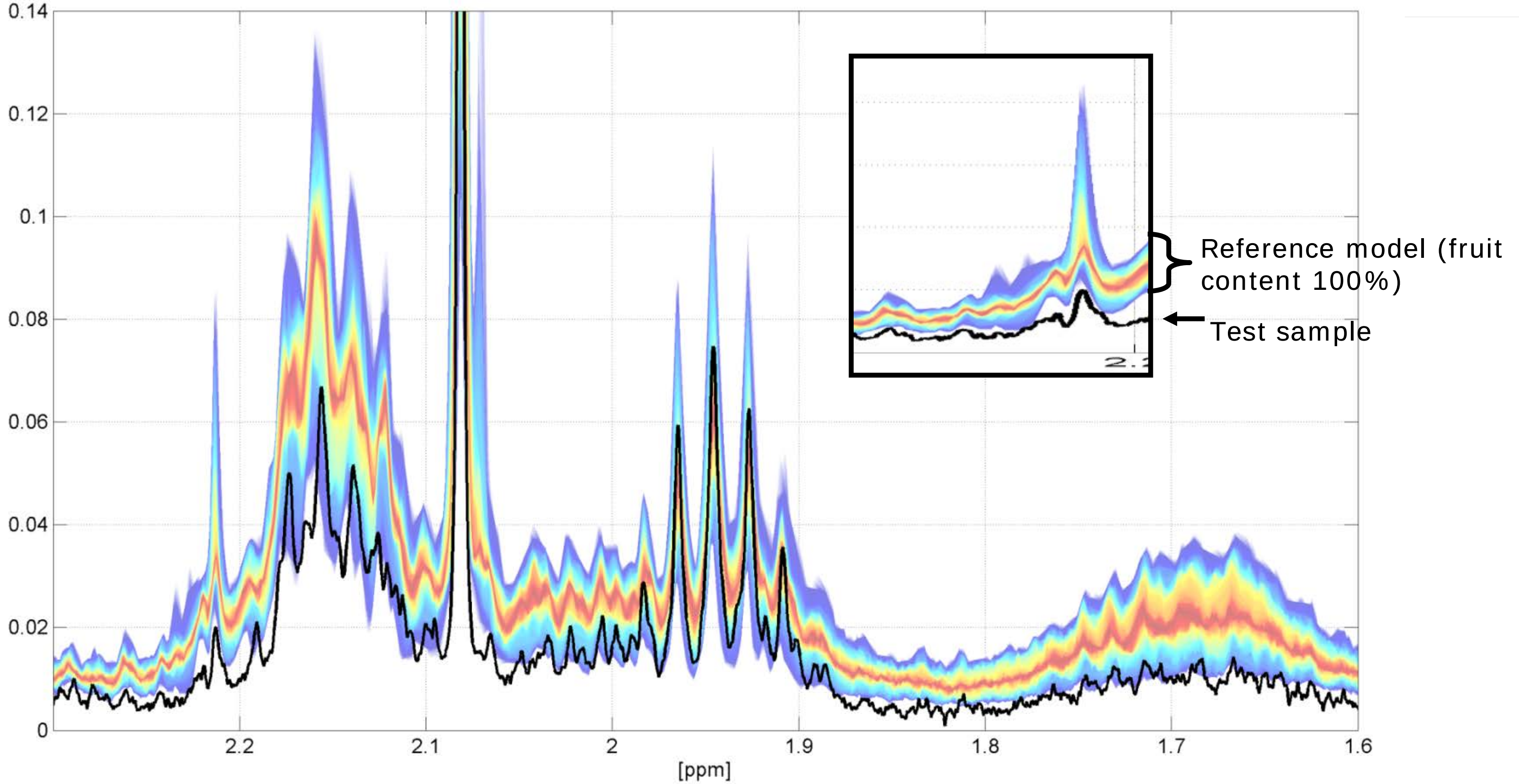
  Compensation by : **Addition of minerals** and

  of 1 or several **amino acids**

  to **adjust** the **sugar / formol ratio**

Fruit Content of Red-Fruits Purees

NMR Results



⇨ **The fruit content is lower in the tested sample**

Fruit Content of Red-Fruits Purees

Results of Conventional Analyses

Conventional parameter (Calculated at Brix 8.5°)

Potassium	2161 ppm	√
Phosphate	636 ppm	√
Magnesium	135 ppm	√
Isocitric acid	106 ppm	√
Formol number	18.9 ml/100ml	√
Citric/isocitric ratio	151	√
Glucose/Fructose ratio	0.82	√

Fruit Content Estimation

105 % √

The results of the conventional analyses have estimated the fruit content of the sample as '***normal***'

but…

Fruit Content of Red-Fruits Purees

Results of Isotopical Analyses

Conventional parameter (Calculated at Brix 8.5°)

Potassium	2161 ppm	√
Phosphate	636 ppm	√
Magnesium	135 ppm	√
Isocitric acid	106 ppm	√
Formol number	18.9 ml/100ml	√
Citric/isocitric ratio	151	√
Glucose/Fructose ratio	0.82	√

Fruit Content Estimation

105 % √

Share of added sugar: 20% at least

Isotopic profile

		Difference	Difference
δ13C- Sugar	-24.7 ‰ V-PDB √	-0.2 ‰ V-PDB √ (Sugar vs Acids)	FAIL !
δ13C- Acids	-24.9 ‰ V-PDB √		
δ13C- Pulp	-23.9 ‰ V-PDB √		-0.85 ‰ V-PDB (Sugar vs Pulp)

The next step in NMR based Food Quality Control has arrived From JuiceScreener to WineScreener

BRUKER

Bruker BioSpin GmbH

Winespin-Analytics

BRUKER

Analysis Report Wine-Profiling™

Sample ID: Bruker_3Oberkircher_2011

Measuring Date: 20-Mar-2012 16:42:27
Reporting Date: 21-Mar-2012 09:32:04, Version: 1.0.0

Additional Information
Variety: Riesling

Results Summary

Type of Analysis	Analysis ID	Result	Status
Targeted Analysis			
Quantification	Q	–	●
Untargeted Verification Analysis			
Univariate Verification	1000/75	In-Model	●
Multivariate Verification	1000/75	In-Model	●

Please note, that Wine-Profiling™ is a screening method with extensive inhouse validation, but it is not an method. Quantitation is regularly validated taking part in official ring tests.

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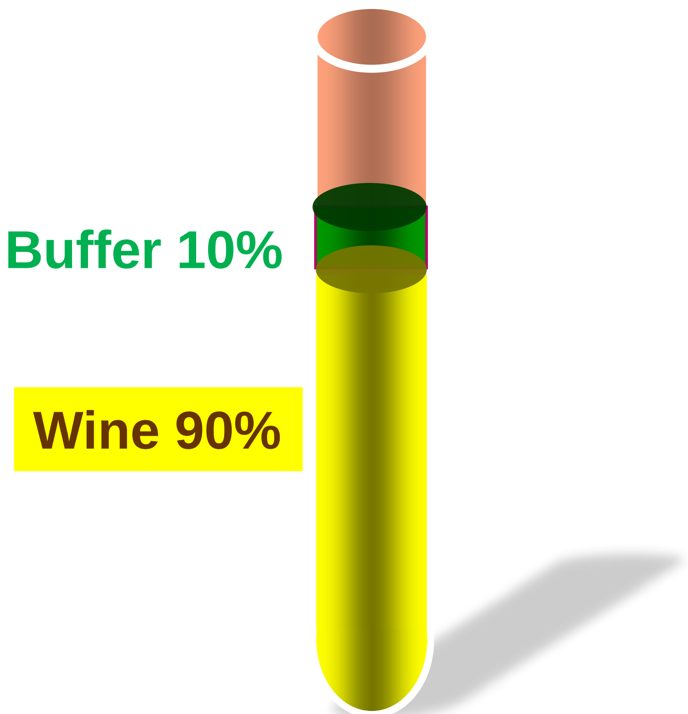
Wine Analysis by WineScreener™

Wine by NMR:

- Identification & quantification of compounds
- Determination of grape variety
- Geographical origin for selected countries
- Company product profile / identity comparison
- Detection of irregularities of any kind
- Vinification / Aging

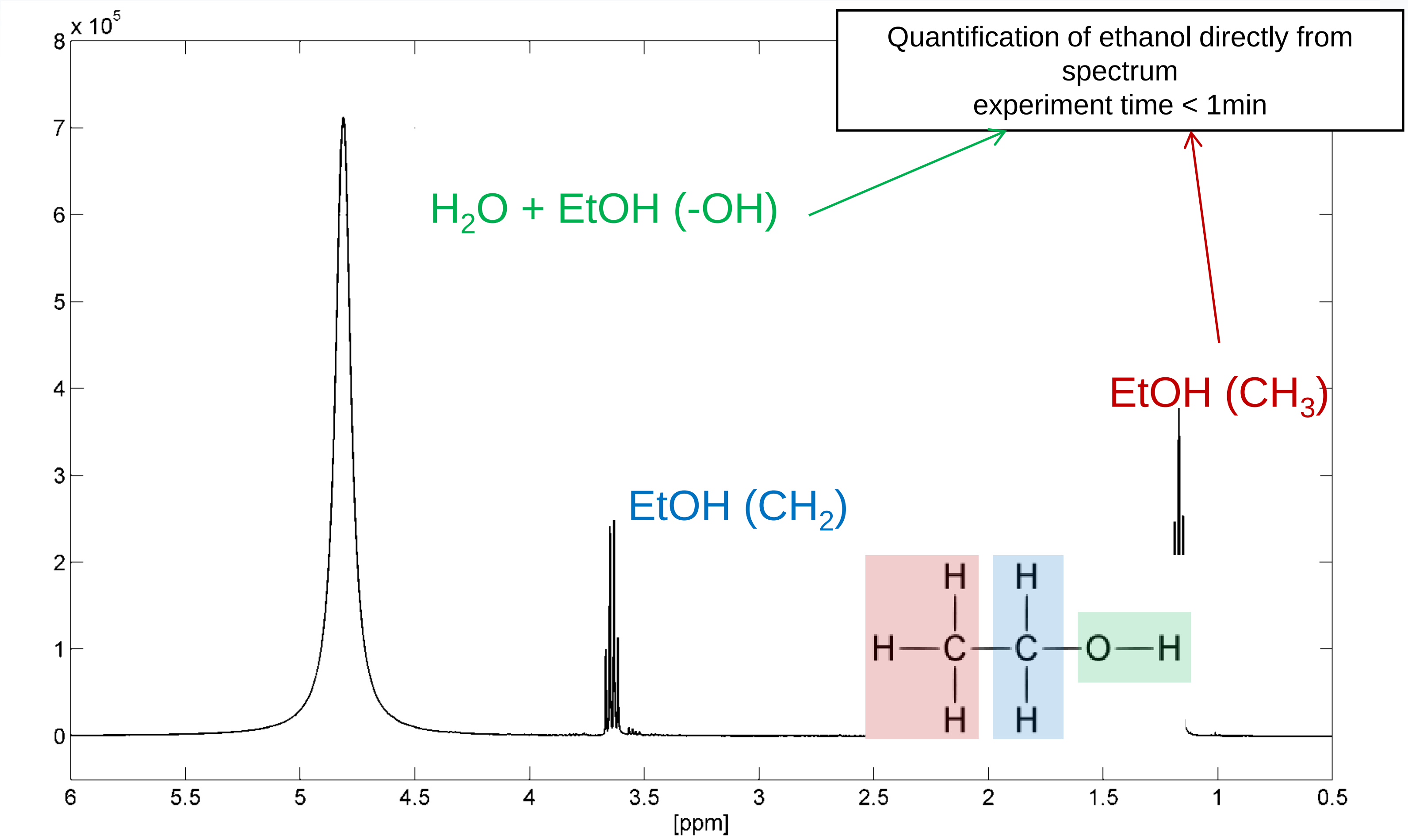
Very simple sample preparation
- can be done manually or by robotic system

pH control by automated pH-titration system

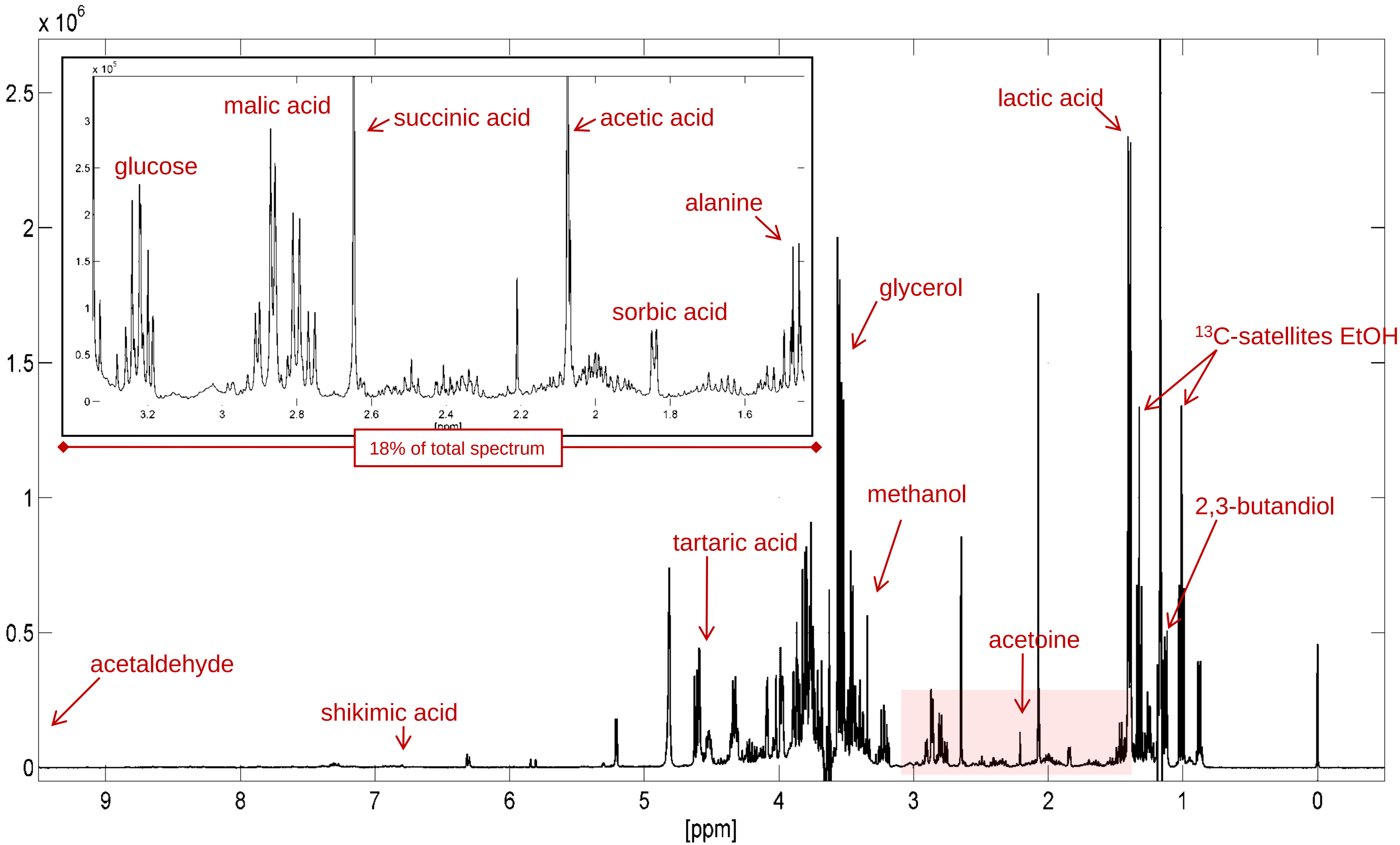


Total sample volume typically 600 µl

^{1}H-NMR spectrum of wine (without suppression)



^{1}H-NMR spectrum of wine (with suppression)



Additional compounds: HMF, trigonelline, sucrose, fructose, citric acid, fumaric acid, proline, …

Targeted Analysis

In the following tables the results of the quantitative analysis are given.

Parameters labelled with * are calculated parameters.

Quantification

Standard Parameters:

Compound
total alcoho
total alcoho
ethanol
ethanol-v*
glycerol
glucose
fructose
glucose/fru
sucrose
arabinose
total sugar
total fermen
tartaric acic
malic acid
lactic acid
citric acid
energy valu
bread units
carbohydrat

Degradation Par

Compound
acetic acid
acetoine
ethylacetate
ethyllactate
formic acid
fumaric acid
gluconic acid
putrescine
cadaverine
HMF
furfural

Higher Alcohols

Compound
methanol
1,3-propanediol
2,3-butanediol
2-methyl-propanol
2-phenylethanol
3-methyl-butanol
acetaldehyde
pyruvic acid
galacturonic acid
succinic acid
glycerol/ethanol*

Amino Acids:

Compound	Value	Unit	LOQ	Official Reference			Wine-Profiling™
				Flag	min	max	NMR Reference Database
4-aminobutanoic acid	<120	mg/L	120	○	-	-	not available
alanine	<35	mg/L	35	○	-	-	not available
arginine	<150	mg/L	150	○	-	-	not available
proline	222	mg/L	150	○	-	-	not available

(Poly-)phenols:

Compound	Value	Unit	LOQ	Official Reference			Wine-Profiling™
				Flag	min	max	NMR Reference Database
caftaric acid	20	mg/L	15	○	-	-	not available
epicatechin	<30	mg/L	30	○	-	-	not available
gallic acid	<25	mg/L	25	○	-	-	not available
shikimic acid	22	mg/L	20	○	-	-	not available
trigonelline	13	mg/L	10	○	-	-	not available

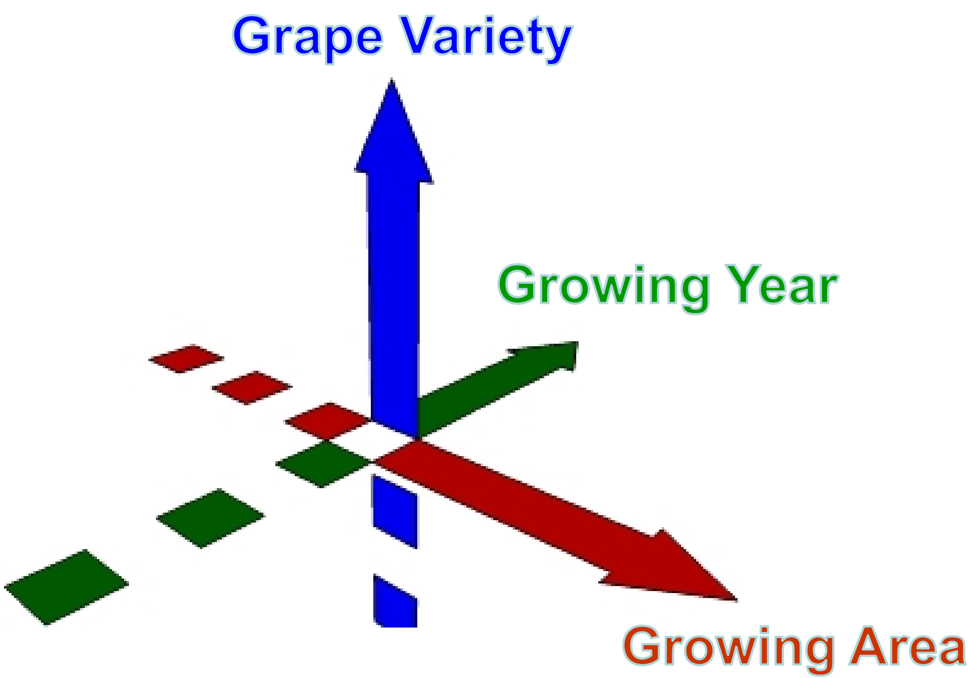
Stabilising Agents:

Compound	Value	Unit	LOQ	Official Reference			Wine-Profiling™
				Flag	min	max	NMR Reference Database
benzoic acid	<10	mg/L	10	●	-	0 c)	not available
sorbic acid	<10	mg/L	10	●	-	200 c)	not available
salicylic acid	<30	mg/L	30	○	-	-	not available

Sources for Reference Values
a) EU-Verordnung
b) Resolution OENO 19/2004
c) Weinverordnung (Germany, 21. April 2009)

Statistical Modelling with authentic wine

- In cooperation with several wine laboratories more than 10.000 wine samples have been collected and measured at 400 MHz
- NMR, once trained, can predict parameters, that are not related to a special molecule
- NMR can deliver statistical results beyond quantification.

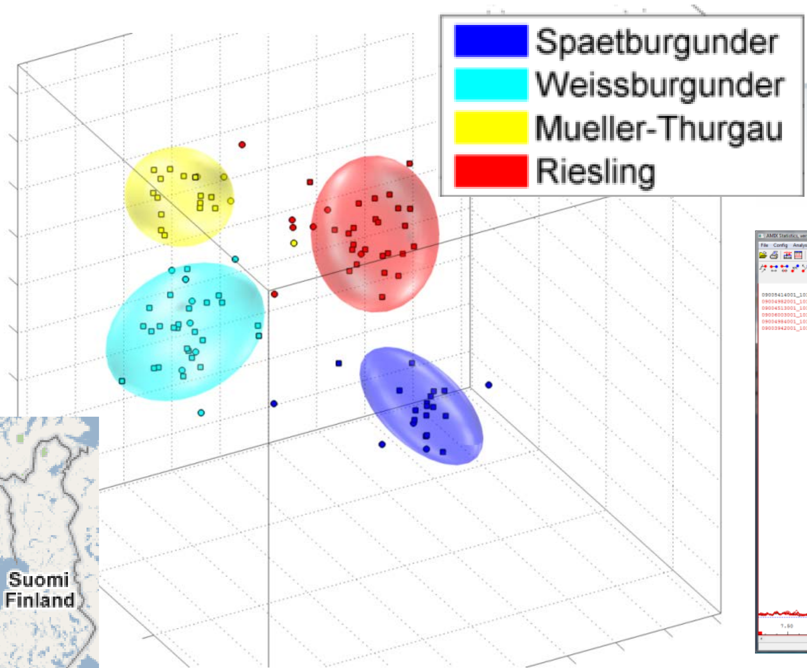


- In statistical modelling, we have to deal with orthogonal parameters that influence the spectra considerably

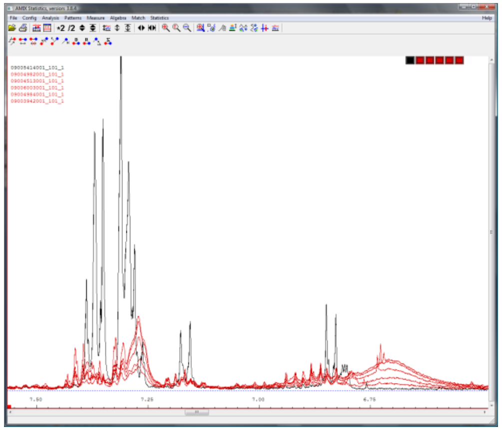
Differentiation of grape varieties
Classification & verification models

Model	Group by	Groups
German/Austria white wine	Varieties	Riesling Weiss, Müller Thurgau, Pinot Blanc/Gris, Welschriesling, Grüner Silvaner, Sauvignon Blanc, Chardonnay Blanc, Grüner Veltliner
German/Austria red wine	Varieties	Dornfelder, Pinot Noir, Blauer Portugieser, Blaue Zweigeltrebe

Wine: Differentiation of grape varieties



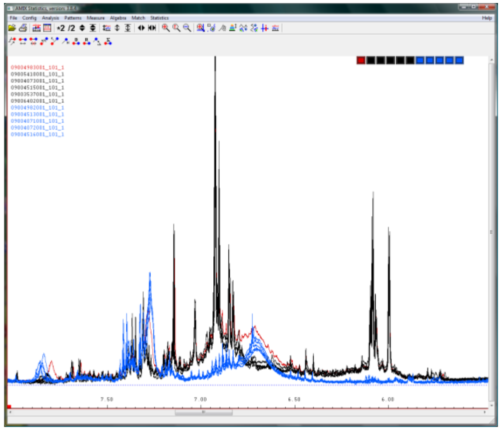
Based on 100
Wines from Baden
(Rhine Valley)



Problems
Within the
Group of
100

Cleaning
Agent left

R.Godelmann
CVUA Karlsruhe



White wine
Added to
Red wine

Differentiation of grape varieties
Classification & verification models

BRUKER

Model	Group by	Groups
World Wide Red Wine	Varieties	Cabernet Sauvignon, Merlot Noir, Syrah, Tempranillo
World Wide White Wine	Varieties	Chardonnay Blanc, Riesling Weiss, Sauvignon Blanc

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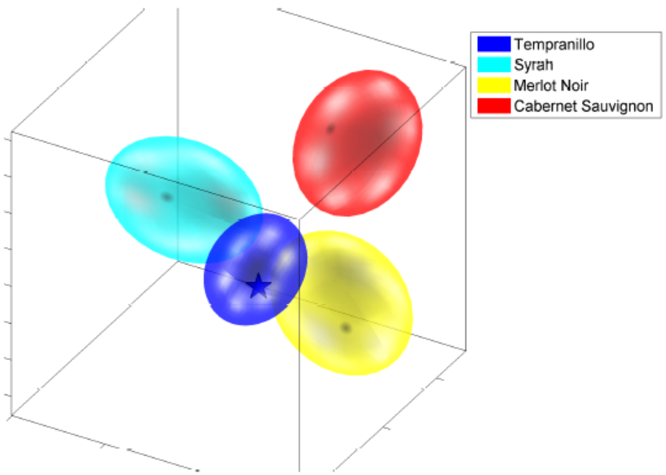
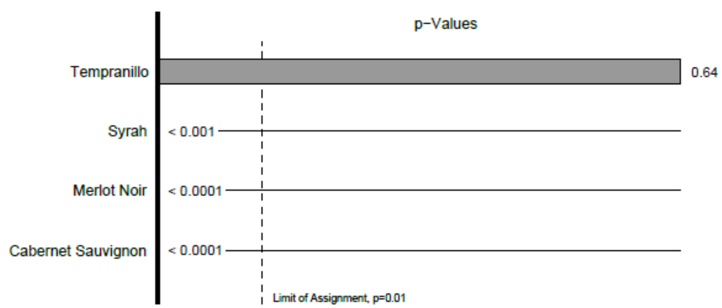
Red wine world wide: Determination of Tempranillo Quantification and concentration profiles

Classification Analysis

Model: Red Wine Variety

Result: Most probable class is *Tempranillo*





Targeted Analysis

In the following tables the results of the quantitative analysis are given.

Parameters labelled with * are calculated parameters.

Standard Parameters:

Compound	Value	Unit	LOQ	Official Reference Flag	Official Reference min	Official Reference max	Wine-Profiling™ NMR Reference Database
total alcohol*	116.5	g/L	-	○	-	-	98.3 – 127.6
total alcohol-v*	14.8	%vol	-	○	-	-	12.5 – 16.2
ethanol	116.1	g/L	5.0	○	-	-	98.0 – 127.0
ethanol-v*	14.7	%vol	-	○	-	-	12.4 – 16.1
glycerol	9.7	g/L	0.5	○	-	-	4.4 – 10.6
glucose	<0.5	g/L	0.5	○	-	-	<0.5 – 4.4
fructose	<0.5	g/L	0.5	○	-	-	<0.5 – 4.8
glucose/fructose*	-	-	-	○	-	-	not available
sucrose	<0.2	g/L	0.2	○	-	-	<200 mg/L in reference set
arabinose	448	mg/L	100	○	-	-	<100 – 510
total sugar (bef. inv.)*	<1.0	g/L	1.0	○	-	-	<1.0 – 9.2
total fermentable sugar*	<1.0	g/L	1.0	○	-	-	<1.0 – 9.2
tartaric acid	2.1	g/L	0.5	○	-	-	1.3 – 2.8
malic acid	<0.2	g/L	0.2	○	-	-	<0.2 – 0.3
lactic acid	1.5	g/L	0.2	○	-	-	0.9 – 3.4
citric acid	<200	mg/L	200	●	-	1000 a)	<200 – 218
energy value*	3650	kJ/L	-	○	-	-	3080 – 3970
bread units*	<0.2	1/L	0.2	○	-	-	<0.2 – 0.8
carbohydrate units*	<0.2	1/L	0.2	○	-	-	<0.2 – 0.9

Differentiation of geographical origin
Classification & verification models

Model	Group by	Groups
German/Austria Area for Riesling	Area	Germany, Austria
German Area for Riesling	Region	Rheinhessen, Württemberg, Pfalz, Mosel, Baden, Rheingau

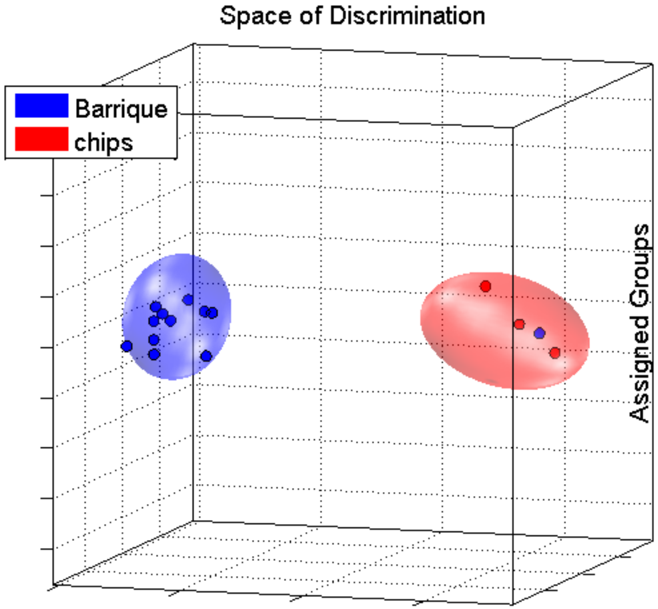
Differentiation of vintage
Classification & verification models

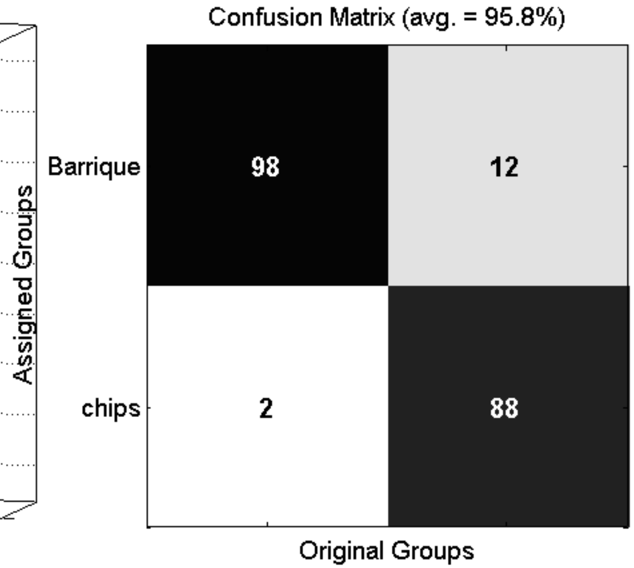
Model	Group by	Groups
German Riesling vintage	Year	2011, 2012

Vinification: Barrique vs. Chips

under development
first results

Or?





- n (Barrique)= 50, n(with chips) = 14
- outlier: Wuerzburg-Wine-118-A13

Cooperation LUA Würzburg

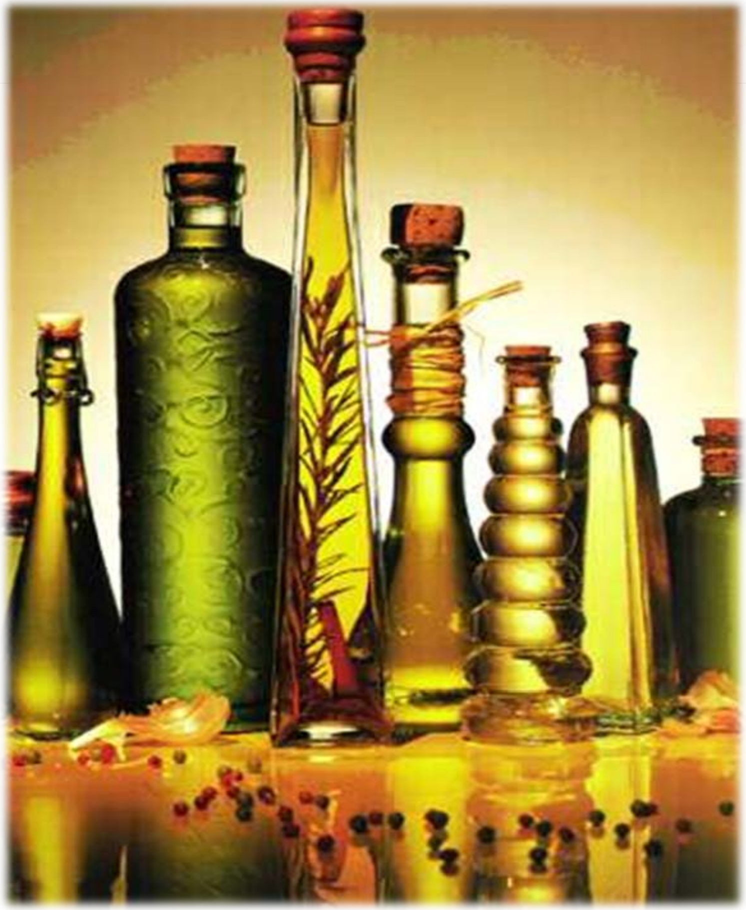
Proof-of-Principle

Same methodology for other areas of mixture analysis

- ***Food***
  - **Edible oil**
  - Honey
  - Coffee
  - Milk powder
  - Soft-Drinks and Energy-Drinks
  - Cheese

Edible Oil (Under development)

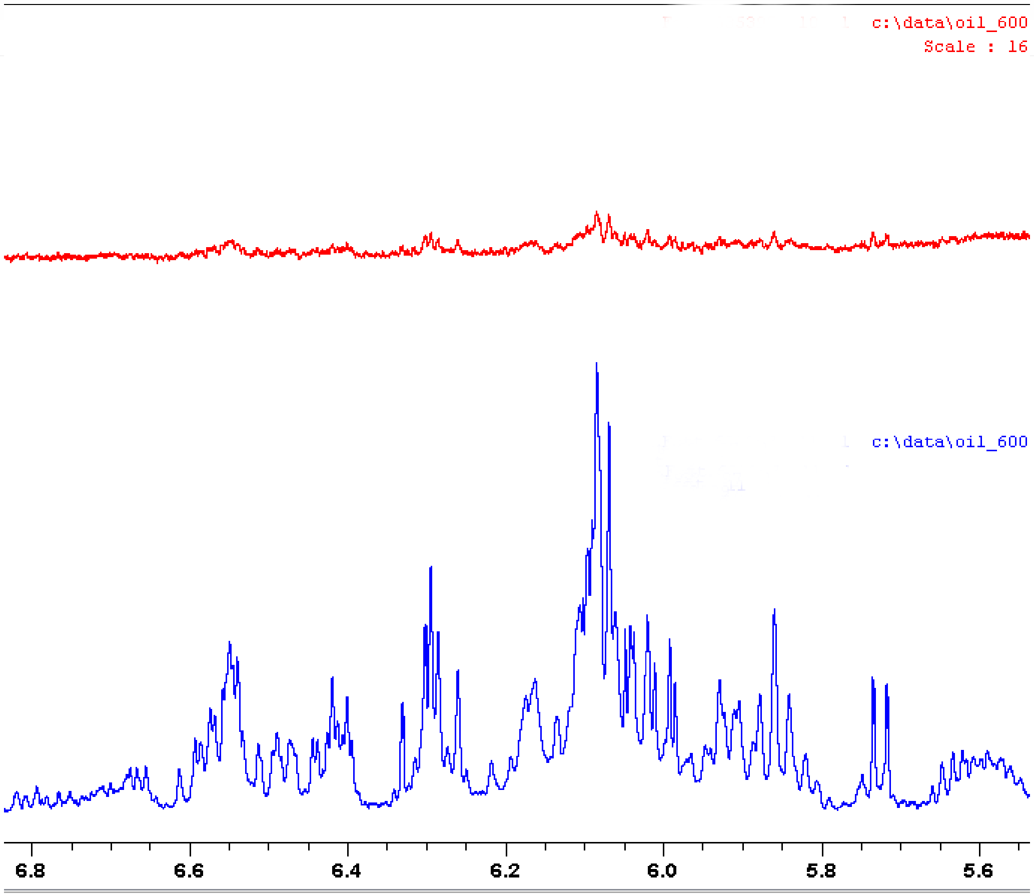
Olive / Palm / Rape seed / Soya bean / ...



- Preparation in $CDCl_3$

Olive Oil: Comparison in Aromatic and Olefinic Region

Non-suppressed against 10-fold suppression



Red = non suppressed

Blue = with 10-fold suppression

Fat dissolved in Chloroform

The NMR lipid profile of edible oils

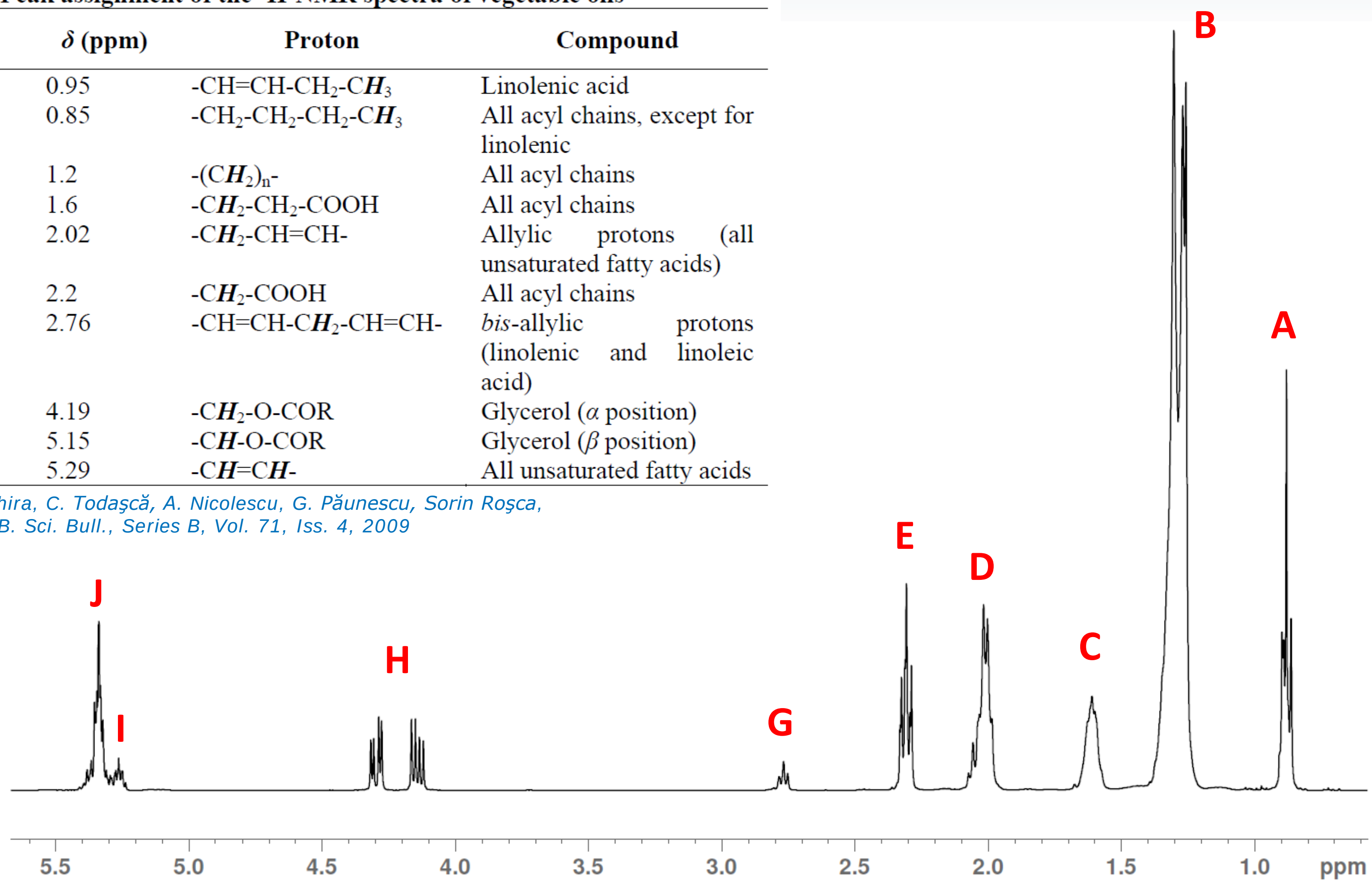
^{1}H signal assignments

under development
first results

Peak assignment of the ^{1}H-NMR spectra of vegetable oils

Peak	δ (ppm)	Proton	Compound
A	0.95	$-CH=CH-CH_2-C\boldsymbol{H}_3$	Linolenic acid
B	0.85	$-CH_2-CH_2-CH_2-C\boldsymbol{H}_3$	All acyl chains, except for linolenic
C	1.2	$-(C\boldsymbol{H}_2)_n-$	All acyl chains
D	1.6	$-C\boldsymbol{H}_2-CH_2-COOH$	All acyl chains
E	2.02	$-C\boldsymbol{H}_2-CH=CH-$	Allylic protons (all unsaturated fatty acids)
F	2.2	$-C\boldsymbol{H}_2-COOH$	All acyl chains
G	2.76	$-CH=CH-C\boldsymbol{H}_2-CH=CH-$	*bis*-allylic protons (linolenic and linoleic acid)
H	4.19	$-C\boldsymbol{H}_2-O-COR$	Glycerol (*α* position)
I	5.15	$-C\boldsymbol{H}-O-COR$	Glycerol (*β* position)
J	5.29	$-C\boldsymbol{H}=C\boldsymbol{H}-$	All unsaturated fatty acids

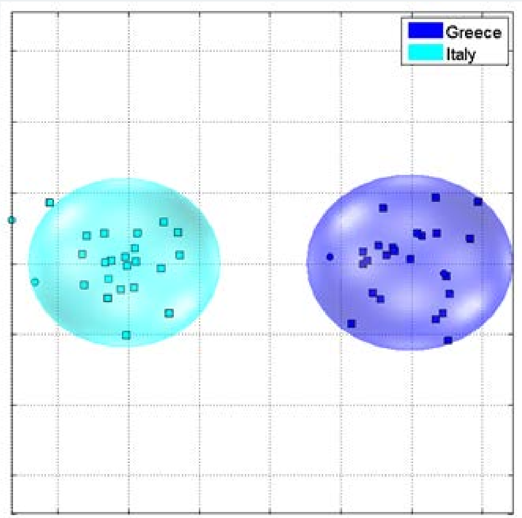
source: N. Chira, C. Todaşcă, A. Nicolescu, G. Păunescu, Sorin Roşca, U.P.B. Sci. Bull., Series B, Vol. 71, Iss. 4, 2009

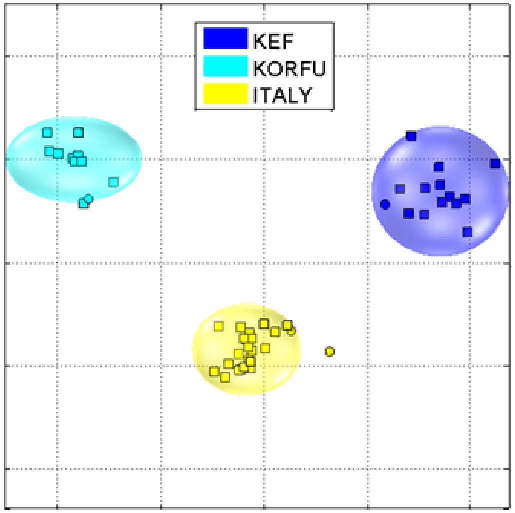


Geographical origin of olive oils

Italy versus Greek Islands

under development first results





Palm oil

Certification of sustainable production

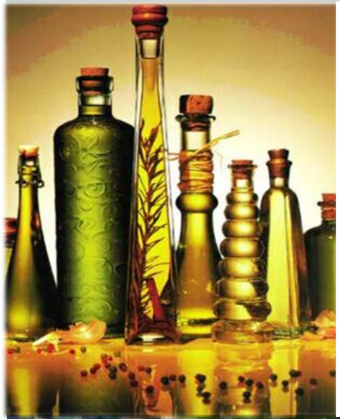
2004 Foundation of the **Roundtable for Sustainable Palm Oil** (RSPO), representing ~50% of global palm oil production, and members of major traders and processing industries

→ RSPO certifications (sustainable production, supply chains)

- palm oil producers/suppliers have fundamental interest in methods confirming oil quality and certification compliance

- **NMR screening methodology** may have the power to narrow palm oil production sites down to single plantations, proving that respective palm oil charges originate only from existing - and not from newly deforested - areas.

Conclusions

FoodScreener systems ➔ a powerful analytical tool

- Minimal sample preparation
- ^{1}H-NMR delivers highest reproducibility and transferibility (lab, user and instrument independent !)
- ^{1}H-NMR combines targeted and non-targeted analysis within one single measurement (Detection of even unknown deviations !)
- Enables a positive identification and quantification of a multitude of compounds

Conclusions

FoodScreener systems ➔
a powerful analytical tool

- Non-disruptive technique
- ^{1}H-NMR is fully quantitative (**one** calibration suffice for **all** compounds)
- Rapid and full automated push-button solution to analyze fruit juice and wine, inclusive automated PDF report of all results (next WineScreener release: Q4, 2014)
- Full automated edible oil and honey screening will be available probably end of 2014

Acknowledgements

University of Athens (Greece)

Emmanuel Mikros

Alexios-Leandros Skaltsounis

University of Ioannina (Greece)

Michael G. Kontominas

University of Bari (Italy)

Francesco Longobardi

Antonio Sacco

Andrea Ventrella

Malaysian Palm Oil Board

Bruker BioSpin GmbH (Rheinstetten, Germany)

Andrea Steck, Claire Cannet, David Krinks, Fang Fang, Léa Heintz, Eberhard Humpfer, Monika Mörtter, Hartmut Schäfer, Birk Schütz, Manfred Spraul

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

Claudia Napoli

DLR Neustadt Rheinland Pfalz:
- Arbeitsgruppe Herr Prof. Dr. Fischer

Universität Koblenz-Landau
-Frau Bansbach

CVUA Karlsruhe:
- Arbeitsgruppe Herr Dr. Godelmann

SGF Niederolm:
- Herr Dr. Rinke, Frau Dr. Koswig

LGL Würzburg:
- Arbeitsgruppe Herr Dr. Wachter, Herr Dr. Christoph

Winespin-Analytics:
- Herr Kost, Herr Langenwalter, Herr Witowski

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markus.link@bruker-biospin.de

You can also get in contact with us using the internet:

- www.bruker.com/sgf

Thank you for your attention