RECENT DEVELOPMENTS IN FOOD SCREENING BY NMR

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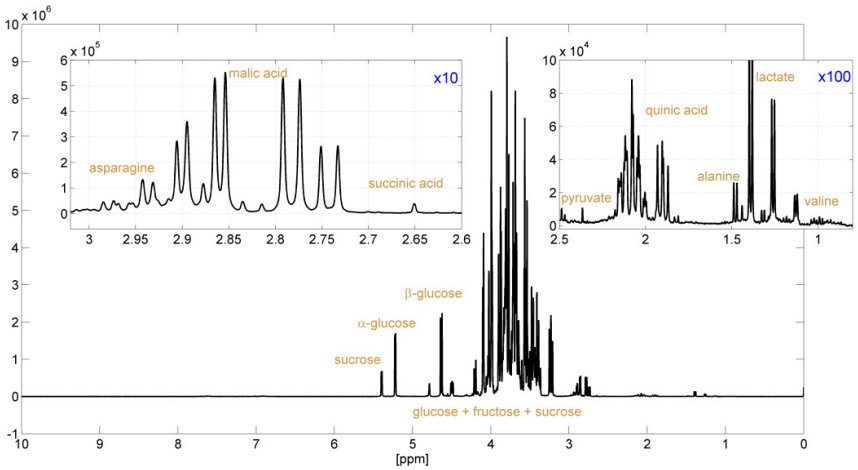
Food Scandals in recent years

http://www.khd-research.net/Food/LM_Skandale_4.html#AKTUELL

Red wine from the test tube	December 2005
Juices in Tetra-Paks polluted by ITX print chemical	January 2006
Benzene in fruit drinks	March 2006
Cheap Vodka poisons thousands in Russia	October 2006
Wine adulteration in Italy	March 2008
Milk scandal in Bavaria	June 2008
Adulteration of wine and champagne by glycerol	June 2008
Poisoned hard liquor in Turkey	March 2009
Artifical (analog) cheese rapidly increasing	April 2009
People died: cheese made from unpasteurized milk	January 2010
10 Mio. Liter falsified Chianti	May 2010
Bavarian mozzarella got blue	June 2010
Melamine in Chinese food	July 2010

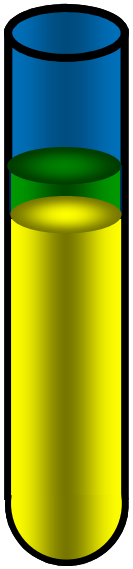
Features of 1H NMR based screening

- All type of samples measurable : liquid, semi-solid, solid
- Quantitative detection of all proton-carrying molecules
- The spectrum corresponds to a **highly specific fingerprint**
- **Targeted** / **Non targeted** analysis in a single experiment



Example of an apple juice spectrum

Advantage of NMR
Minimal sample preparation



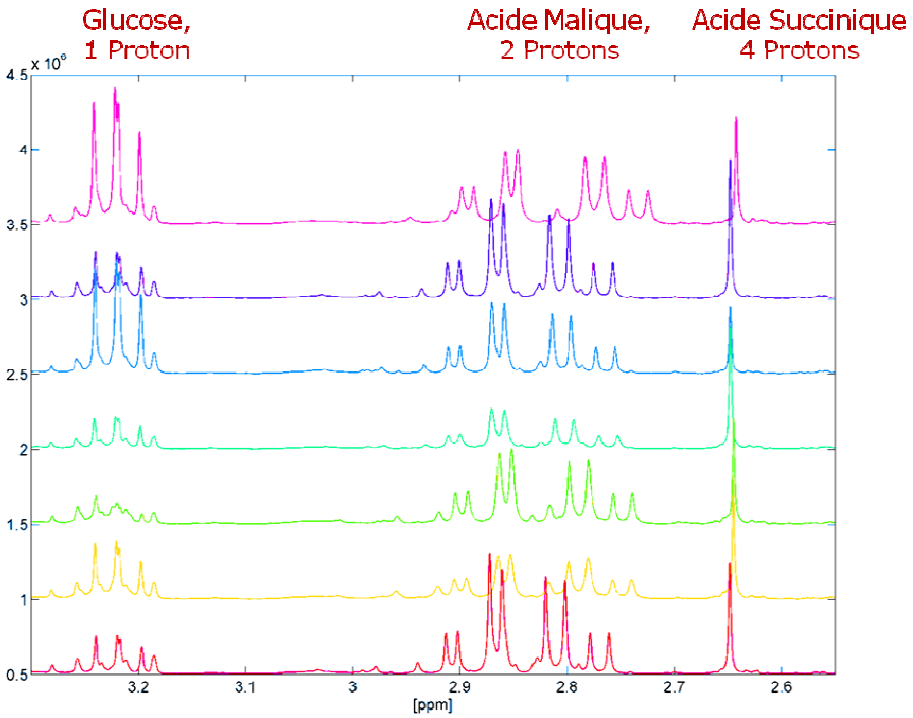
Buffer Solution
10%

Fruit juice / Urine
90%

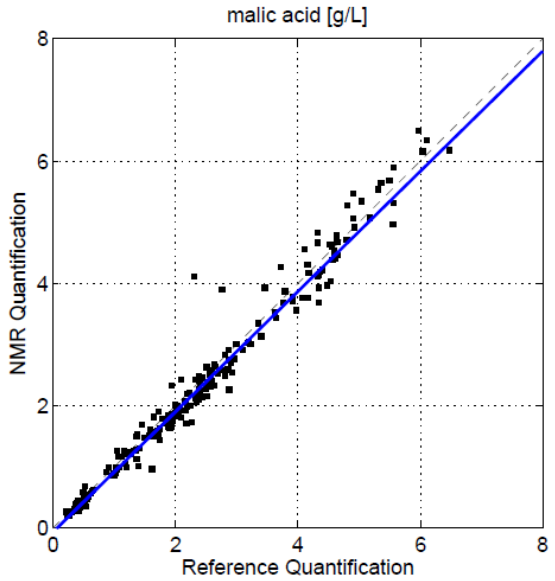
Total sample volume of 600 µl
in NMR tubes of 5mm

Advantage of NMR
NMR is quantitative

- NMR is inherently quantitative

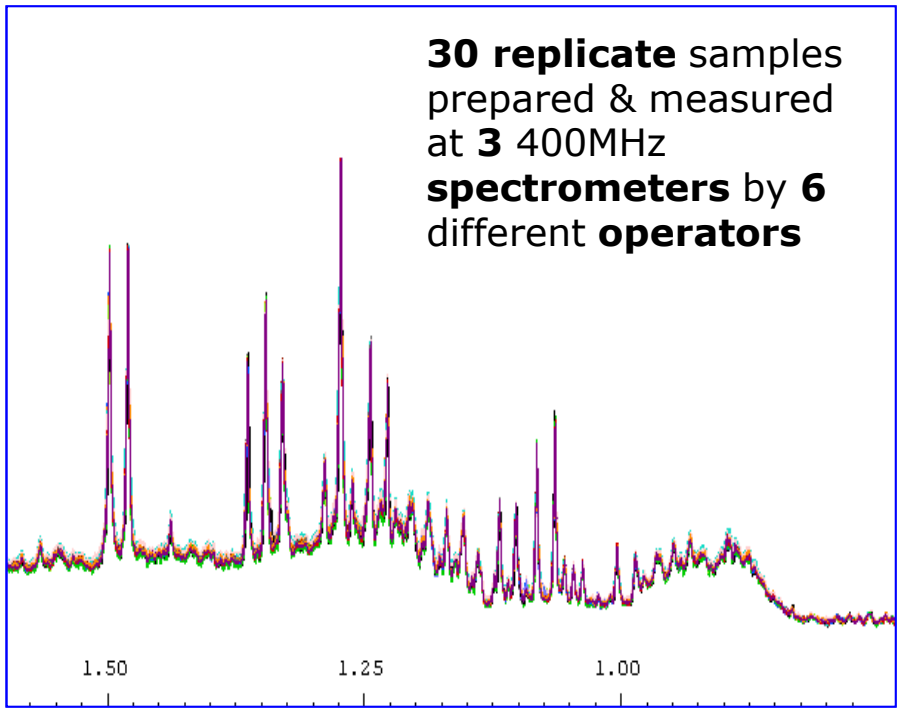


- ✓ Integral of a signal is directly proportional to molar concentration
- ✓ Only one calibration for the quantification of all compounds in a mixture



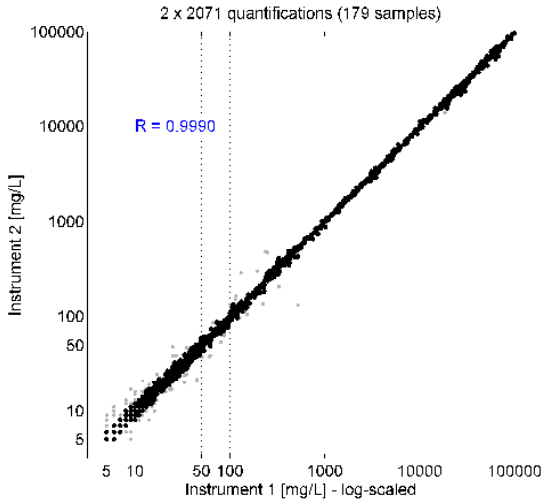
Advantage of NMR
Highest Reproducibility

- Highest reproducibility → allows to detect even the smallest variations in concentration of all relevant compounds



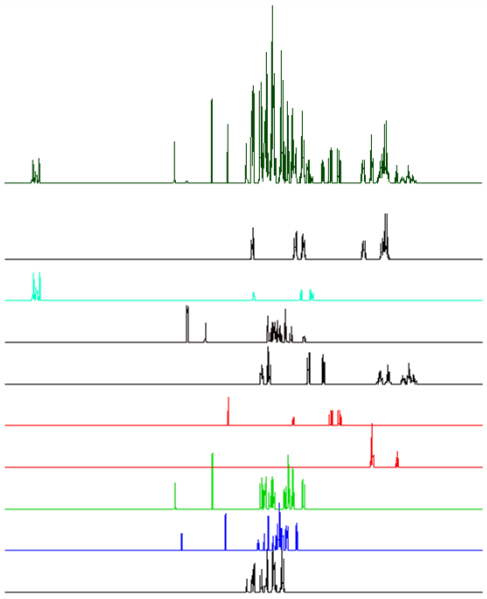
- Inter-instrumental reproducibility

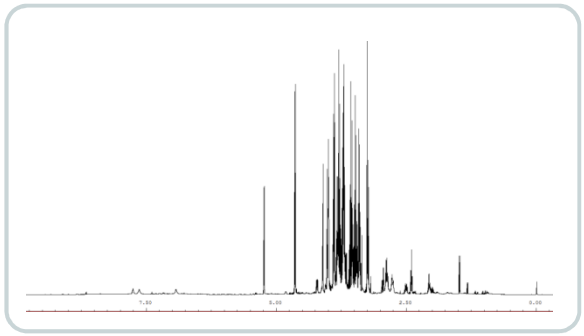
(at same magnetic field strenght)



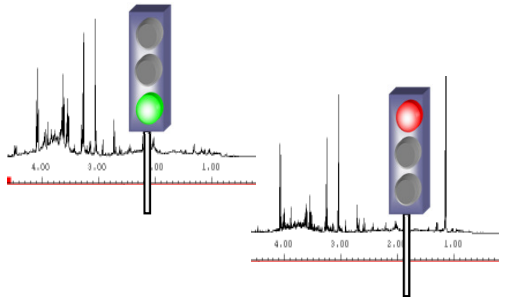
Comparison of quantification results of 179 samples (2523 resultts) measured on 2 equivalent NMR systems.

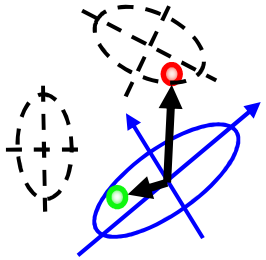
Applications of 1H NMR screening





Fingerprint/profil





Targeted

Identification & quantification of targeted compounds

Non targeted

Statistical models for classification and verification of samples

NMR as Pre-Screening Method

Each Sample:

NMR Profiling

Results Summary

Type of Analysis	Analysis ID	Result	Values	Status
Classification Models				
Similarity Analysis	1000/2	OS-KS-BRS[1]	–	–
Global Model	1500/3	OS/MN[2]	0.86	–
Orange / Mandarin	2101/3	OS[3]	0.91	–
Orange Concentrate/Direct	2102/3	OS-K[4]	0.97	–
Orange Origin	2103/3	Brazil[5]	0.77	–
Validation Models				
Univariate Analysis (OS-K)	5002/1	Off-Model	–	●
SIMCA (OS-K)	7002/1	Off-Model	–	●
Fruit Content (Orange)	10001/1	–	95%	–
Quantification	Q/1	–	–	●

Results Summary

Type of Analysis	Analysis ID	Result	Values	Status
Classification Models				
Similarity Analysis	1000/2	AS-KS-VRC[1]	–	–
Global Model	1500/3	AS[2]	0.93	–
Apple Concentrate/Direct	2201/3	AS-K[3]	0.99	–
Apple Origin	2202/4	AS-VRC[4]	0.84	–
Validation Models				
Univariate Analysis (AS-K-VRC)	5007/1	In-Model	–	●
SIMCA (AS-K-VRC)	7007/1	In-Model	–	●
Fruit Content (Apple)	10000/1	–	100%	–
Quantification	Q/1	–	–	●

Sample not OK

Sample OK

Confirmation by conventional analyses

OK, no further effort needed

(except e.g. pesticides, minerals)

Standard Operation Procedures SOPs

SOPs are one of the most important features for comparable spectra and correct quantification

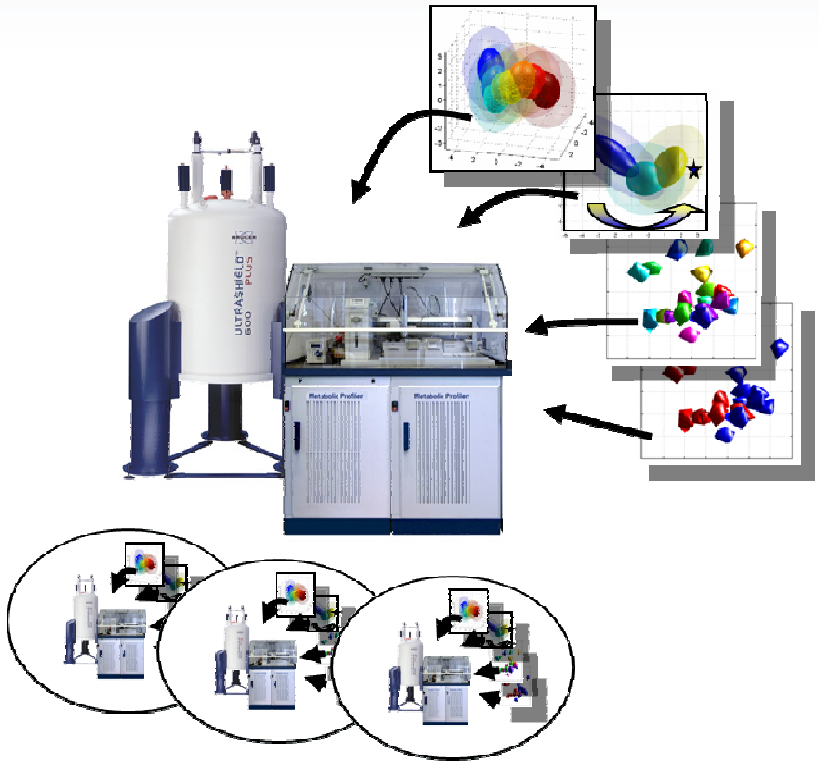
- Sample collection
- Sample storage
- Sample preparation
- Measurement
  - Temperature equilibration
    - Automatic tuning and matching
      - Lock
        - Shim
          - Lockphase optimization
            - Pulse-calibration, Adjustment of all power levels
              - Generation of shape pulse in case of multiple suppression
                - 1D acquisition
                  - fast 2D-J-Resolved acquisition
- Processing
- Post processing

Transferability of statistical models

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

Models need to be applicable to data generated :

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



Essentiel for the participation to:

- Long-term studies
- Multi-center studies

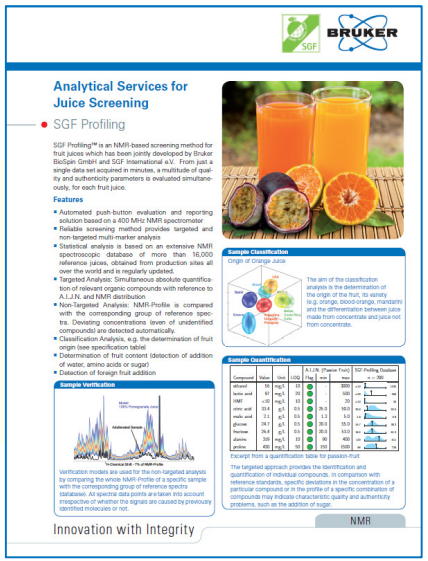
Bruker-SGF Fruit Juice Screener

The first
NMR push button system

from

order input to final report

providing rapid response



400 MHz system
5mm Probe
Z-Grad, Automatic Tune/Match

1H-NMR profiling features

- Full Automation (Acquisition → Reporting)
- Only one measurement (~ **20 minutes**)
- Semi automatic preparation
- Targeted Analysis
  - Quantification of tens of compounds
- Non-Targeted Analysis (Statistics)
  - Check of authenticity
  - Check of quality
  - Detection of frauds
- Summary of all results in PDF file

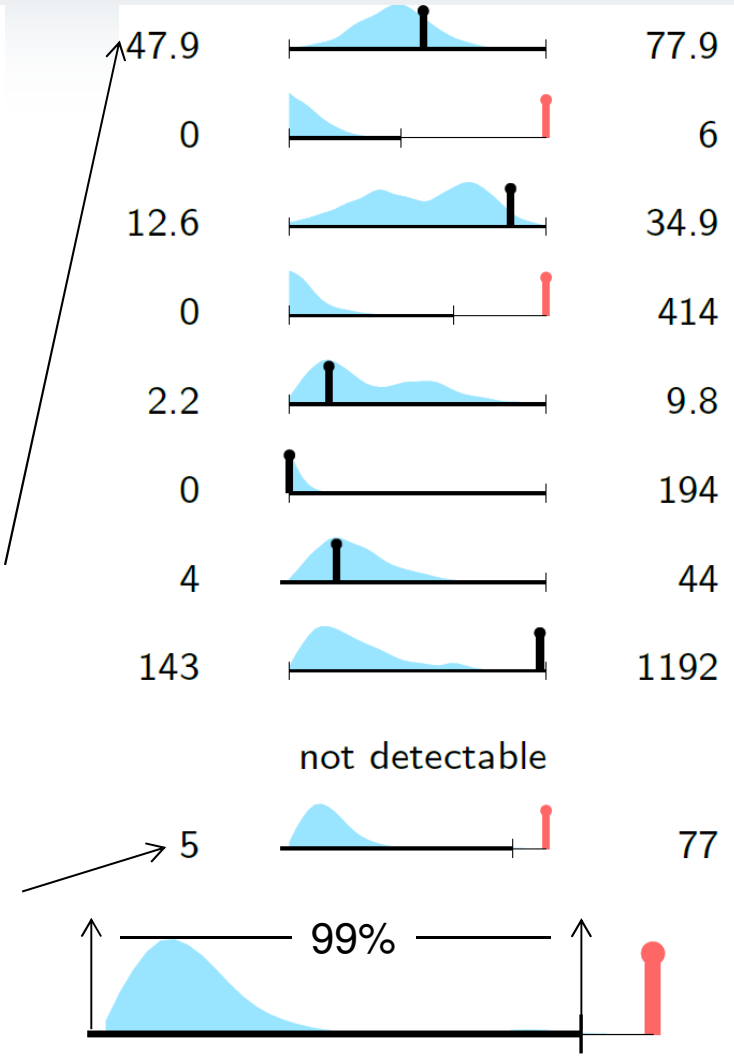
Proof-of-Principle

Same Methodology for other Areas of Mixture Analysis

- Food
  - Wine
  - Honey
  - Edible oil
  - Soft-Drinks
  - Milk, Milk-Powder
- Biofluids (600 MHz)
  - Urine
  - Plasma

Automatic Report Fruit juice-Screener

Compound	Result	Unit	Flag	A.I.J.N. min	A.I.J.N. max	SGF-Profiling n =2733
5-hydroxymethylfurfural	N/Q	mg/l	●	-	20	0 – 16
D-galacturonic acid	410	mg/l	○	-	-	0 – 1950
acetaldehyde	<5	mg/l	○	-	-	not detectable
acetoine	N/Q	mg/l	○	-	-	not detectable
alanine	43	mg/l	●	1	50	10 – 53
arbutin	N/Q	mg/l	○	-	-	not detectable
benzaldehyde	N/Q	mg/l	○	-	-	not detectable
benzoic acid	N/Q	mg/l	○	-	-	not detectable
chlorogenic acid	69	mg/l	○	-	-	0 – 227
citramalic acid	34	mg/l	○	-	-	3 – 114
citric acid	N/Q	g/l	●	-	0.15	not detectable
ethanol	14	mg/l	●	-	3000	0 – 619
formic acid	<5	mg/l	○	-	-	0 – 17
fructose	63.6	g/l	●	45.0	85.0	47.9 – 77.9
fumaric acid	13	mg/l	●↑	-	5	0 – 6
glucose	31.8	g/l	●	15.0	35.0	12.6 – 34.9
lactic acid	650	mg/l	●↑	-	500	0 – 414
malic acid	3.4	g/l	●	3.0	-	2.2 – 9.8
methanol	N/Q	mg/l	○	-	-	0 – 194
pyruvic acid	11	mg/l	○	-	-	4 – 44
quinic acid	1166	mg/l	○	-	-	143 – 1192
sorbic acid	N/Q	mg/l	○	-	-	not detectable
succinic acid	88	mg/l	○	-	-	5 – 77
sucrose	8.7	g/l	●	5.0	30.0	6.5 – 39.6

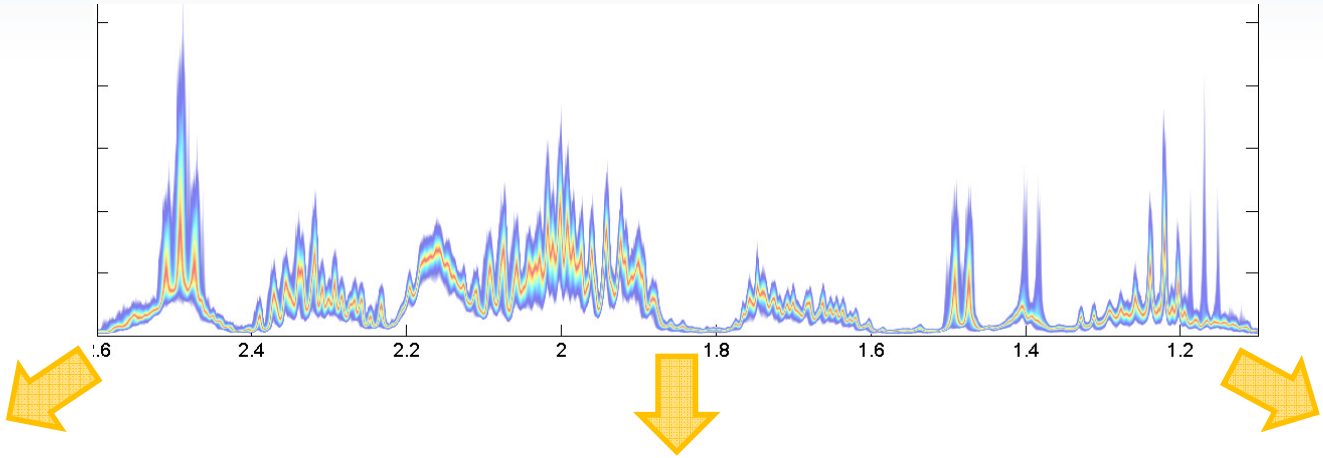


Report of a non-conformous apple juice

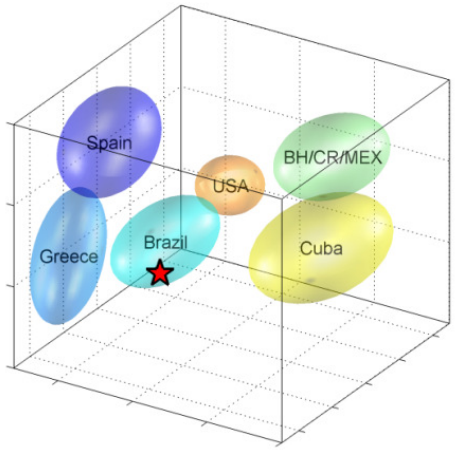
Conclusions made by Quantification

- **Sugar profile** ⇨ Addition of sugar
- **Acids profile** ⇨ Addition of acids
  - e.g.: Citric acid, malic acid in apple Juice
- **Ratio Malic acid / Quinic acid**
  - Ripeness of the fruit
- **Ratio Citric acid / Iso-citric acid** in lemon juice
  - Addition of citric acid
- **Concentration of galacturonic acid**
  - Enzymatic treatment of the fruits (e.g. in apple juice)
- **Concentration of Phlorin in citrus fruit**
  - Usage of peels in the juice
- **Concentration of lactic acid, fumaric acid, formic acid, ethanol, HMF**
  - Quality parameters
- **Addition of another fruit**
  - e.g. pear in apple juice (marker: arbutin)

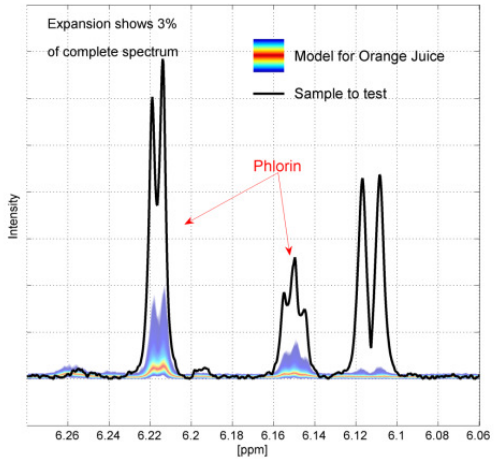
Applications of statistical analysis



Classification



Verification



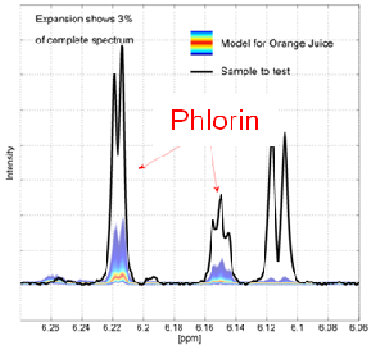
Indirect Quantification of componants by Regression

Parameter	Result	Unit
Total Acid pH 7	112	meq/l
Total Acid pH 8.1	118	meq/l
Total Acid (pH 7, tartaric acid)	8.4	g/l
Total Acid (pH 7, malic acid)	7.5	g/l
Total Acid (pH 8.1, citric acid)	7.6	g/l
Potassium	1808	mg/l
Magnesium	103	mg/l

Verification of samples

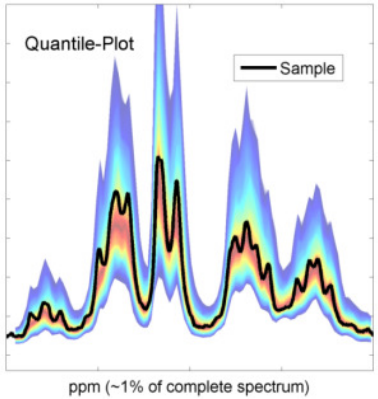
NMR profile is compared with the corresponding group of reference spectra

Reference Spectral database > 20.000 samples

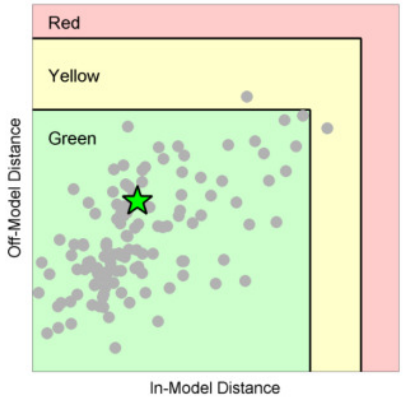


Verification models include uni-variate and multivariate models

Univariate analysis

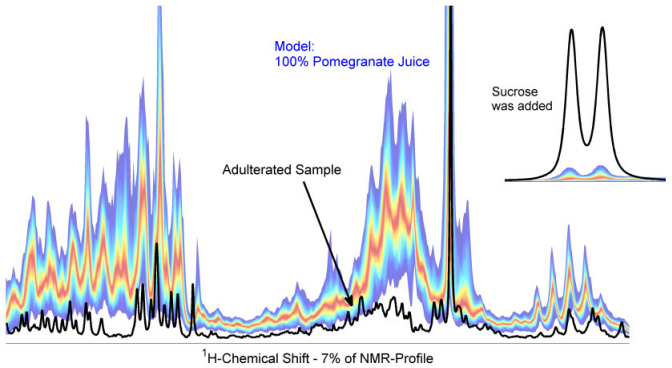


Multivariate analysis

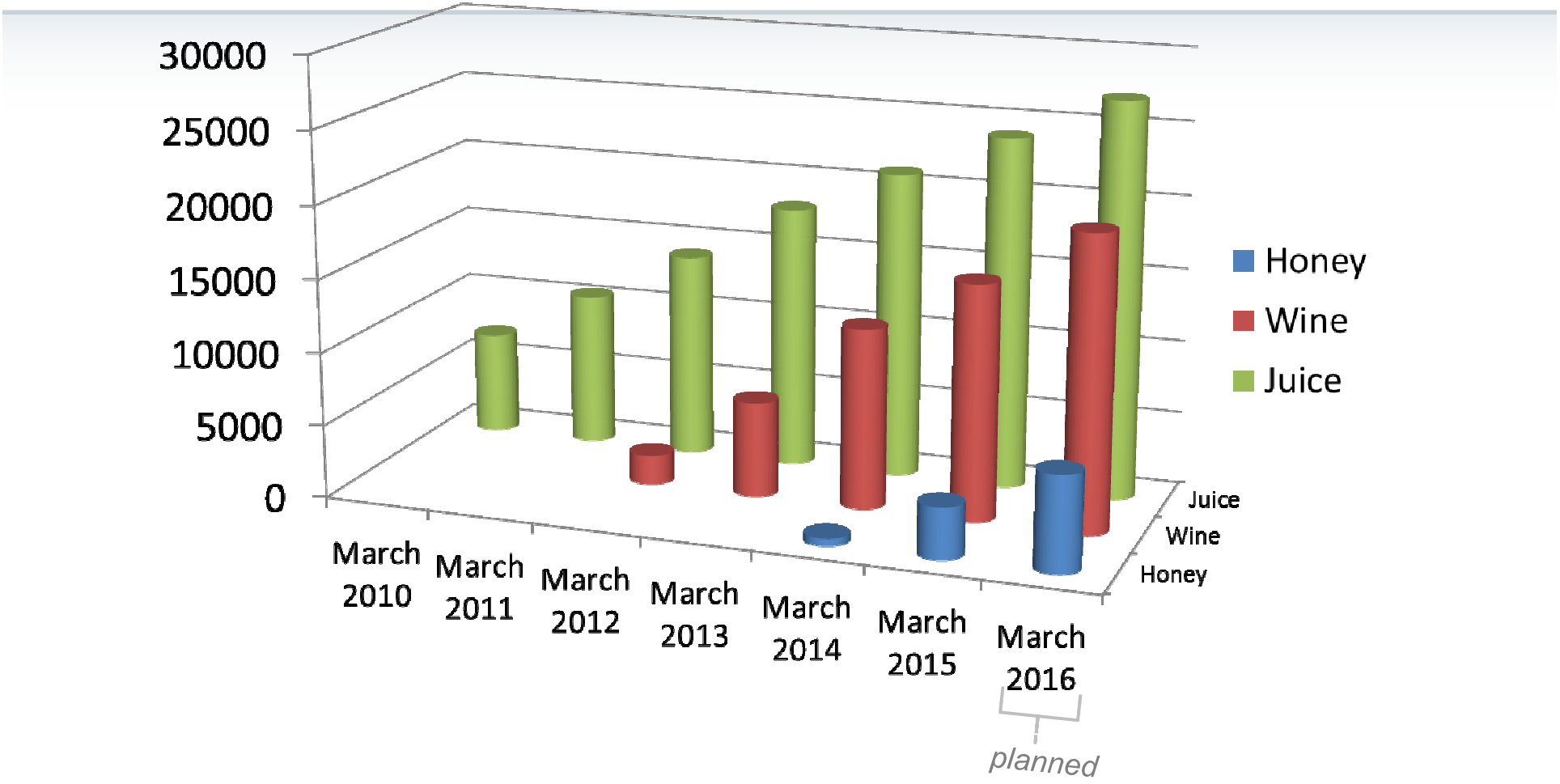


Possibility to detect atypical deviations, unexpected frauds

Examples in juices : falsifications, adulteration, illegal production process, fruit blending, lower fruit content…

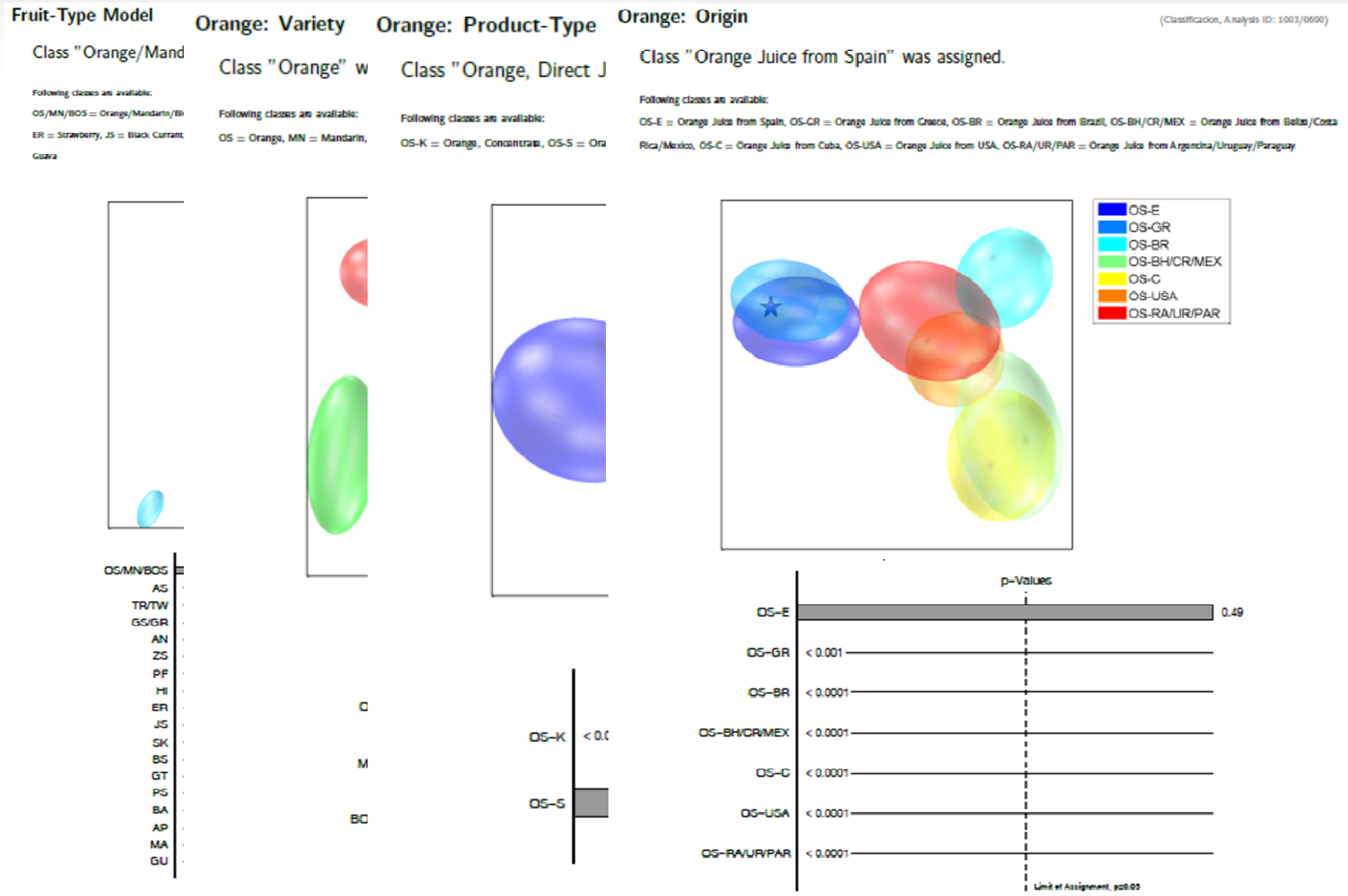


Authentic samples databases



For every matrix: 3000 – 4000 samples per year

PDF report : classification models



PDF report : verification results

Verification Models

Applied Model: Orange from Europe

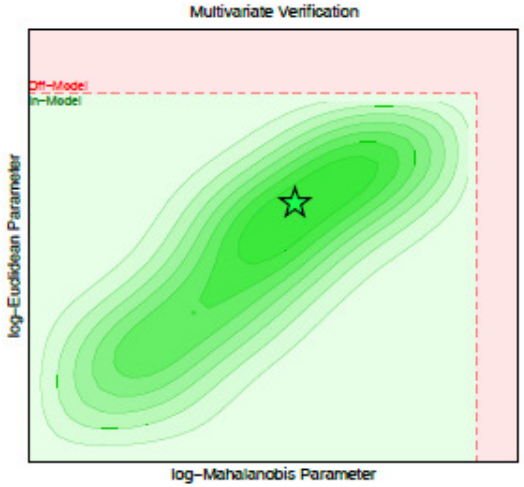
Univariate Verification (Verification, A

Result: Deviating signals were found at following chemical shifts:

1.387^{up} 1.404^{up}

Multivariate Verification (Verification, A

Result: No deviation was detected in multivariate verification (In-Model).



Fruit Content

Applied Model: Orange, Spain

Fruit Content is consistent compared to 100%: Yes

Blend Citrus Sinensis/Reticulata

Model: Model could not be applied.

Due to detected deviations in verification analysis, fruit blending models have not been applied.

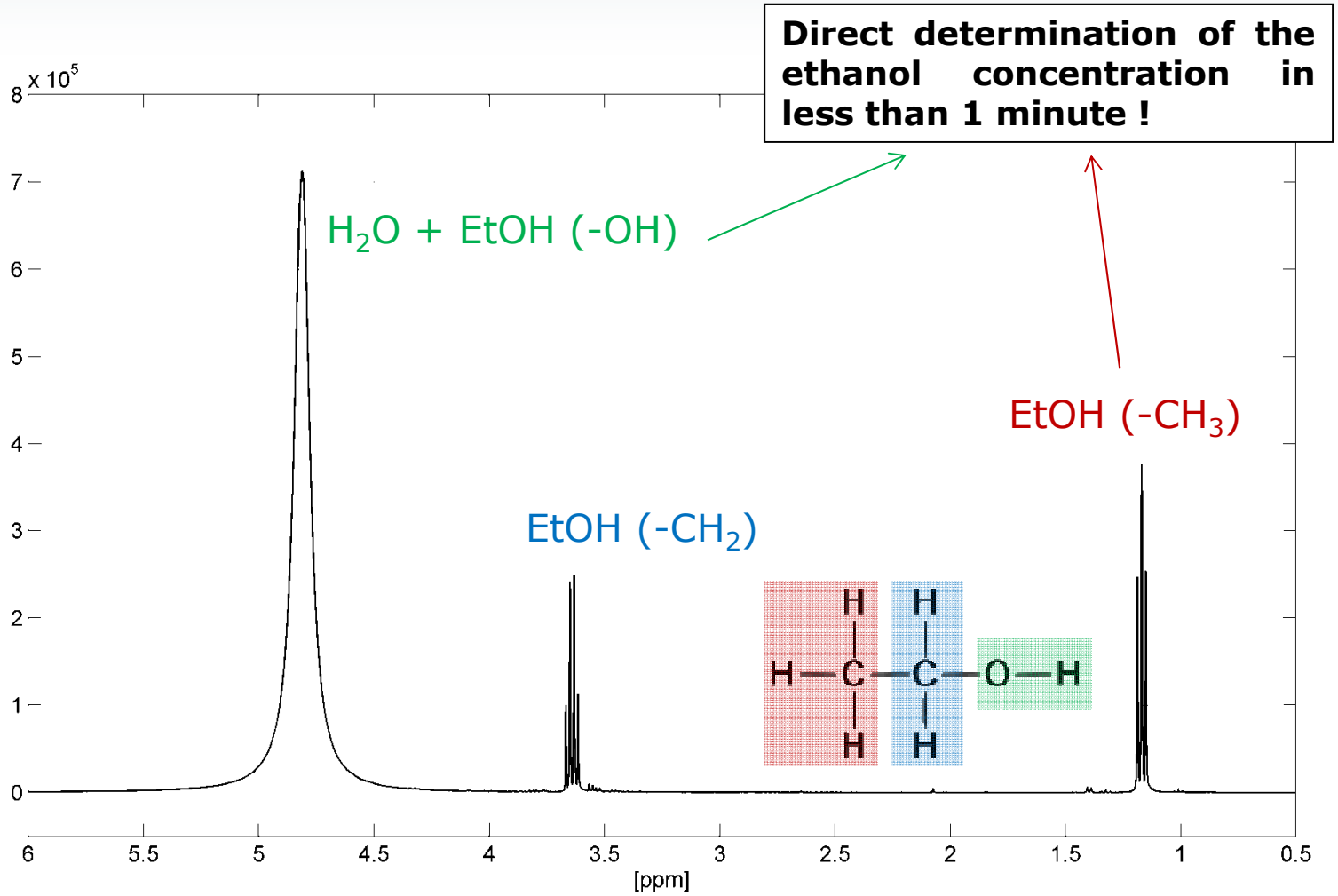
Wine Analysis and Requests

Wine by NMR:

- Replaces conventional, targeted wine analysis

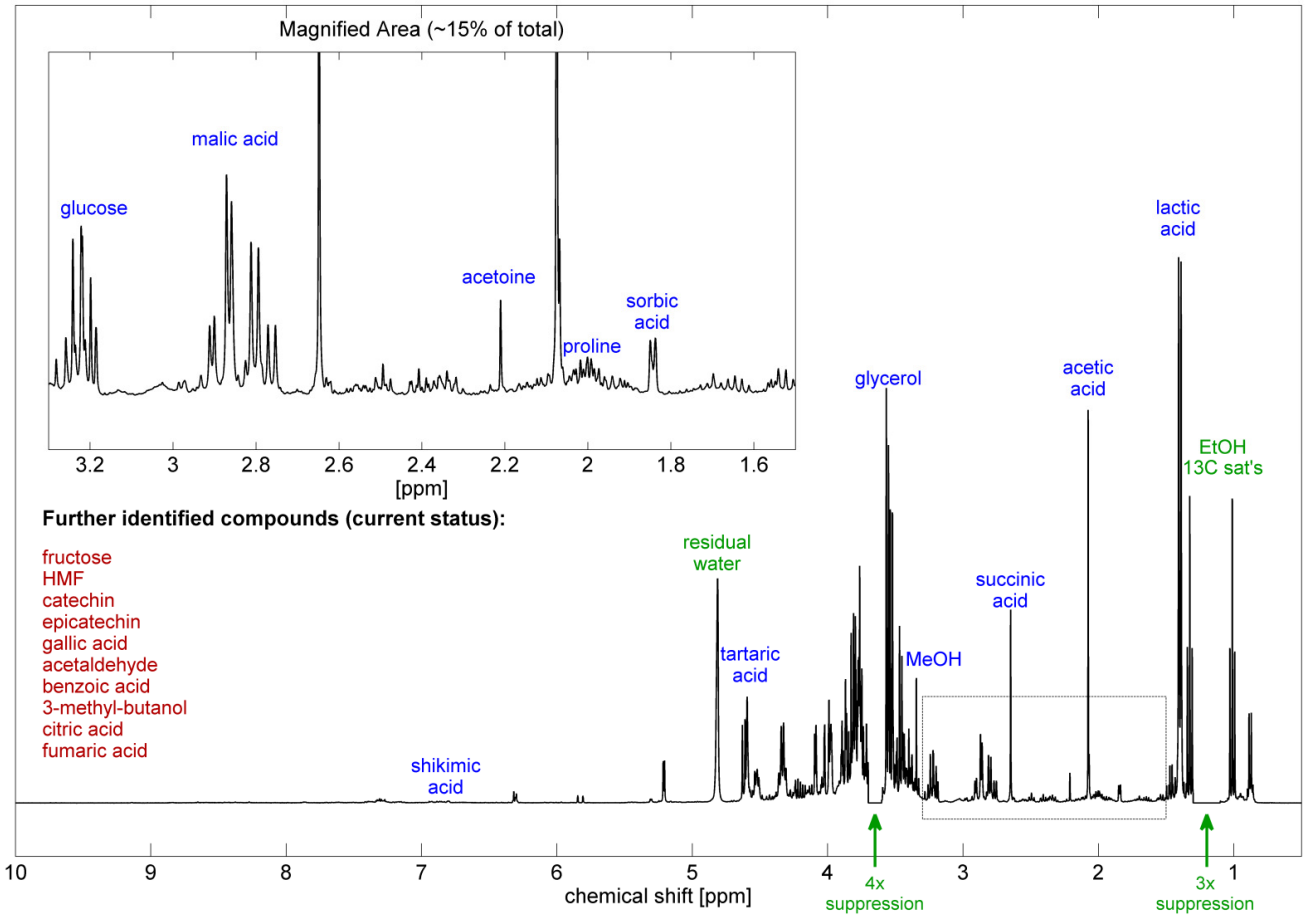
  (currently ~ 60 compounds quantified)
- Determination of grape variety
- Geographical origin for selected regions
- Company product profile NMR
- Detection of irregularities of any kind

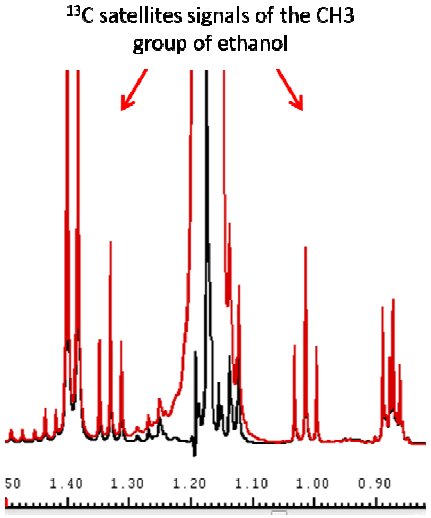
Proton spectrum of wine: Direct measurement



Multi-suppression on ethanol and water

Multiple Suppression with 8 irradiation bands





Automated quantification and reporting

Targeted Analysis

In the following tables the results of the quantitative analysis are given. Parameters labelled with * are calculated parameters. Please refer to the additional remarks for quantified parameters, flags and reference values on page 8. The displayed distributions of the Wine-Profiling™ NMR reference database refer to group *Tempranillo*.

Standard Parameters:

Compound	Value	Unit	LOQ	Flag	Official Ref. min	Official Ref. 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	<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

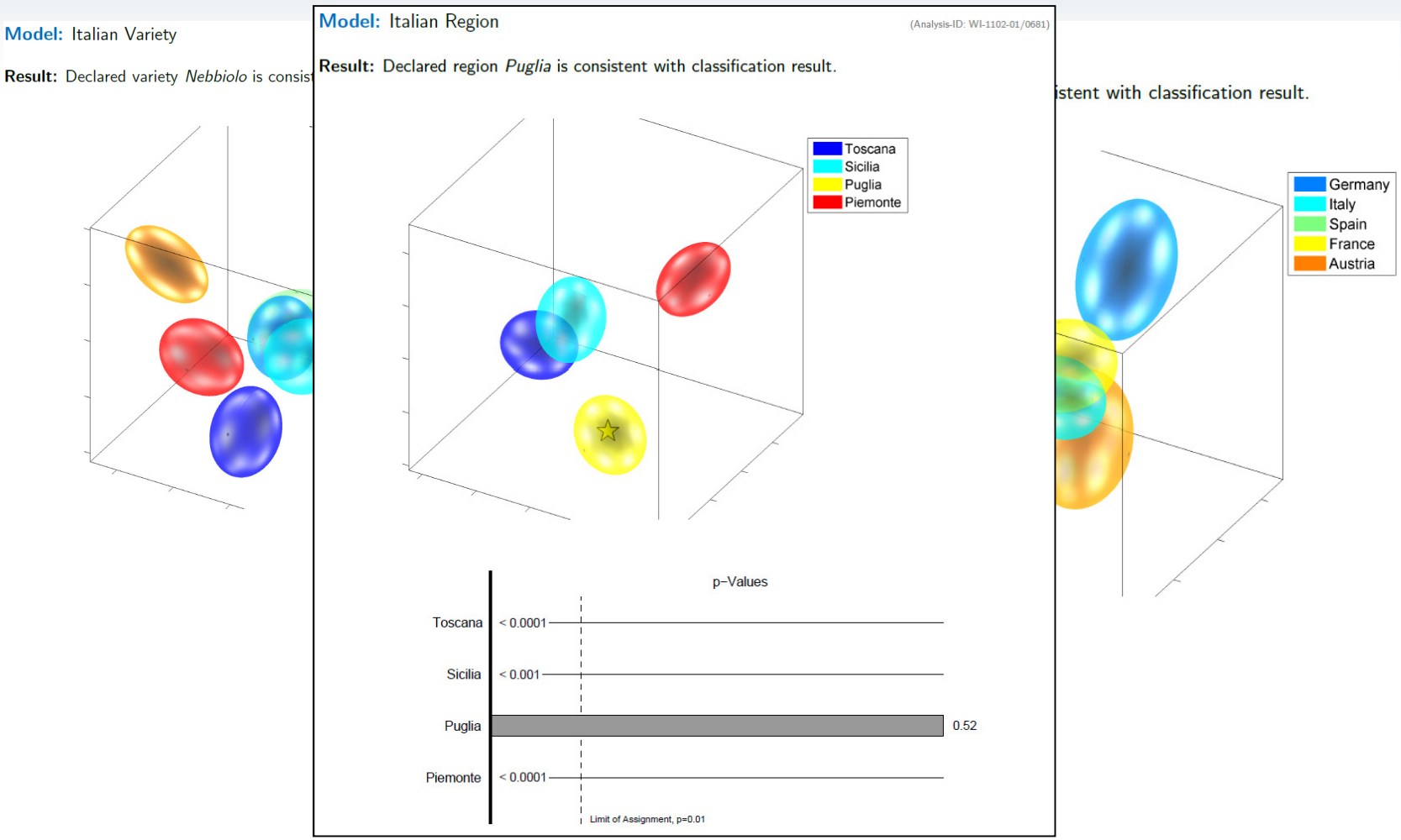
Degradation Parameters:

Compound	Value	Unit	LOQ	Flag	Official Ref. min	Official Ref. max	Wine-Profiling™ NMR reference database
acetic acid	733	mg/L	100	○	-	-	355 – 970
acetoine	12	mg/L	10	○	-	-	<10 – 64
ethylacetate	169	mg/L	50	○	-	-	<50 – 212
ethyllactate	325	mg/L	150	○	-	-	<150 – 450
formic acid	9	mg/L	5	○	-	-	<5 – 13
fumaric acid	<5	mg/L	5	○	-	-	<5 mg/L in reference set
gluconic acid	<400	mg/L	400	●	-	-	<400 mg/L in reference set
putrescine	<50	mg/L	50	○	-	-	<50 – 75
cadaverine	<50	mg/L	50	○	-	-	<50 mg/L in reference set
HMF	<5	mg/L	5	●	-	-	<5 mg/L in reference set
furfural	<2	mg/L	2	○	-	-	<2 mg/L in reference set

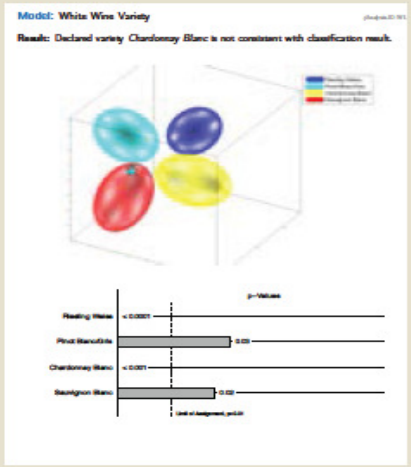
Higher Alcohols / Fermentation Products:

Compound	Value	Unit	LOQ	Flag	Official Ref. min	Official Ref. max	Wine-Profiling™ NMR reference database
methanol	190	mg/L	30	●	-	400	<30 – 191
1,3-propanediol	<40	mg/L	40	○	-	-	<40 – 359
2,3-butanediol	727	mg/L	100	○	-	-	265 – 1100
2-methyl-propanol	<70	mg/L	70	○	-	-	<70 – 80
2-phenylethanol	61	mg/L	25	○	-	-	<25 – 73
3-methyl-butanol	237	mg/L	100	○	-	-	143 – 282
acetaldehyde	<10	mg/L	10	○	-	-	<10 – 39
pyruvic acid	<20	mg/L	20	●	-	-	<20 mg/L in reference set
galacturonic acid	1.3	g/L	0.2	○	-	-	0.2 – 1.3
succinic acid	965	mg/L	50	○	-	-	490 – 1100
glycerol/ethanol*	8.3	%	-	●	-	-	4.3 – 9.4

Non targeted analysis

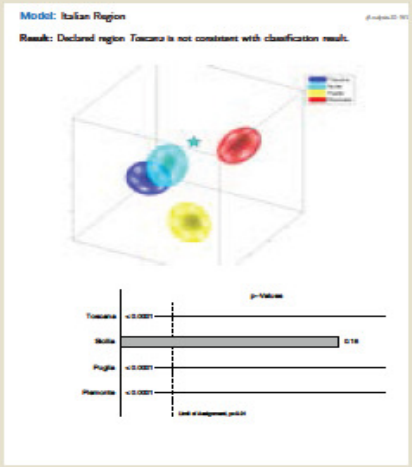


QC in German Supermarkets using Wine-Profiling



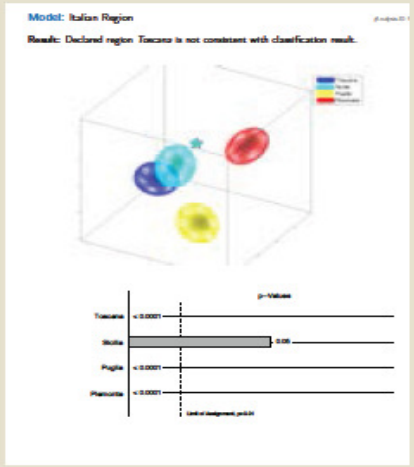
Dieser »Chardonnay« besteht aus Pinot Grigio und Sauvignon Blanc

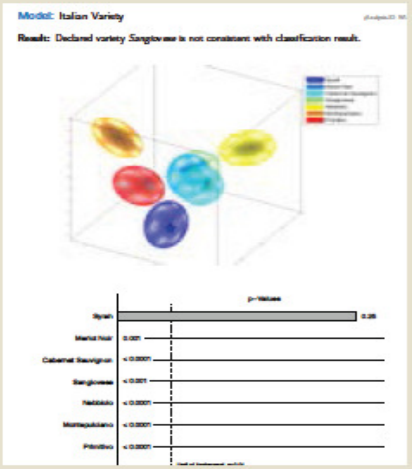
Chardonnay really is mix of Pinot Grigio + Sauvignon blanc



Dieser Wein stammt nicht aus der Toskana, sondern aus Sizillien

Wine not from Tuscany as labeled, but from Sicily





Doppelt daneben: Dieser Wein, angeblich ein Sangiovese aus der Toskana, ist ein Syrah aus Sizilien

Double fraud, labeled as Sangiovese from Tuscany really is a Syrah from Sicily

Honey frauds

If pollen are removed, conventional analysis can not detect origin or type of honey, while NMR still can.

The following syrups show a marker to identify addition to honey:

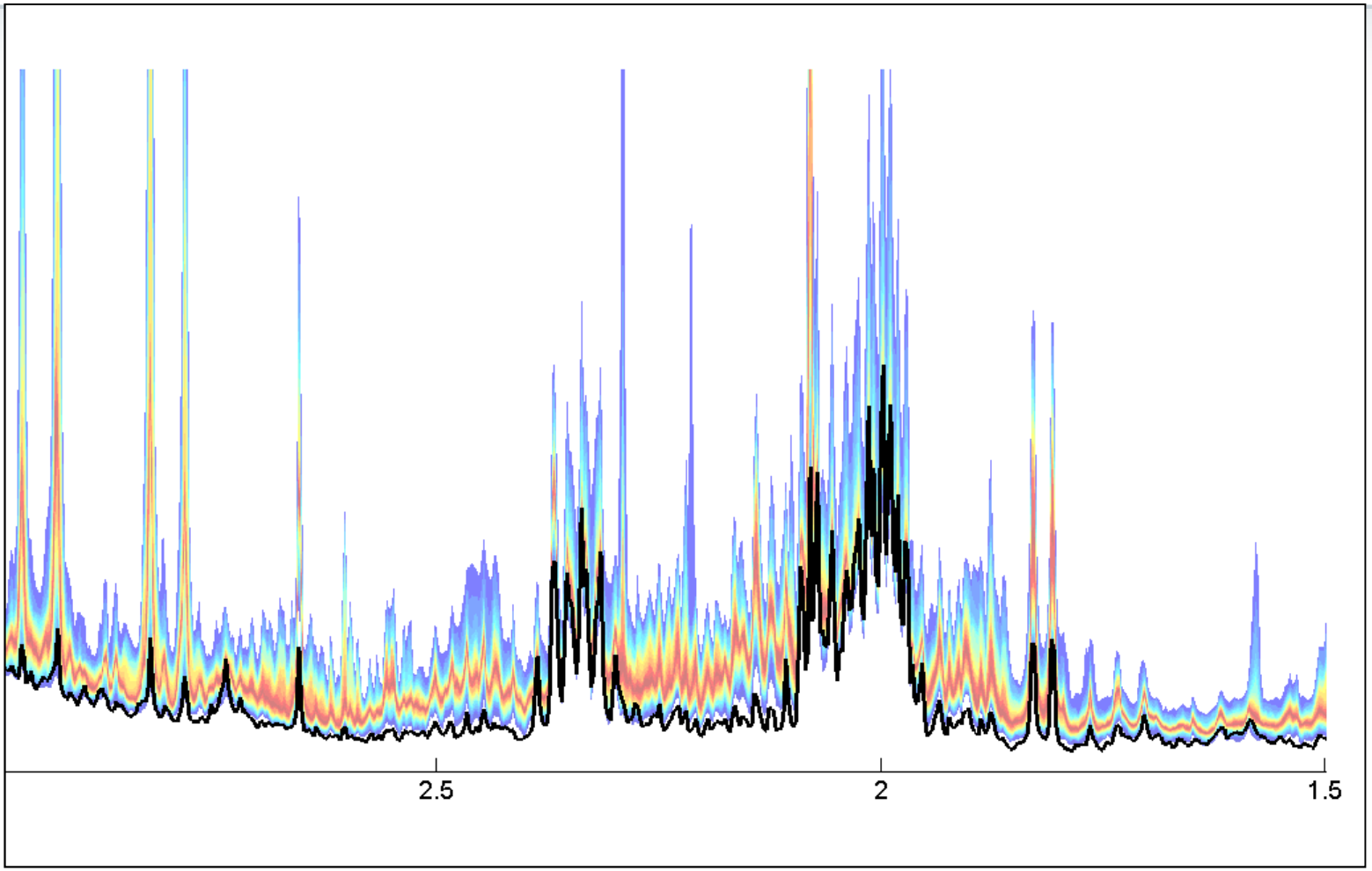
- Some Fructose/Glucose syrups available large scale on the market derived from corn
- Agave Sirups and products thereof (in Germany Schneekoppe, …)

The marker is not found in the following syrups tested so far:

- Rice
- Sugar-cane
- Sugar-beet
- Bee-feed (sirup or dough)

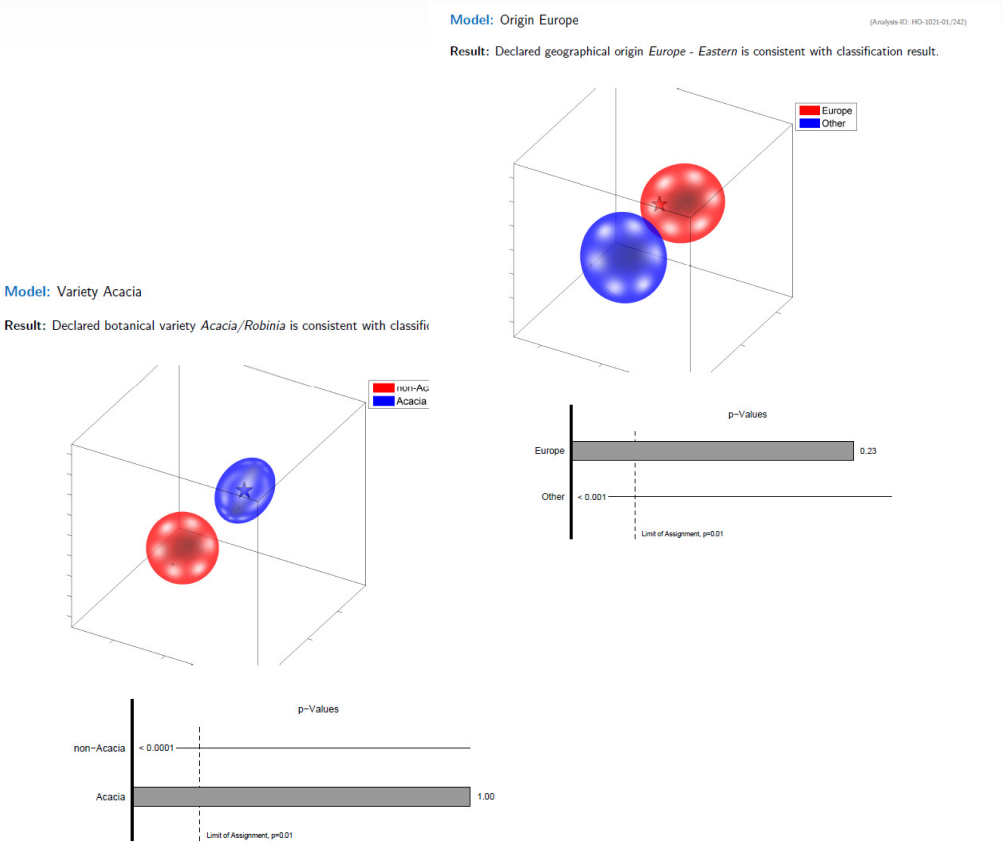
Without marker only dilution detection can be used:
needs NMR and well populated authentic honey database

Honey frauds



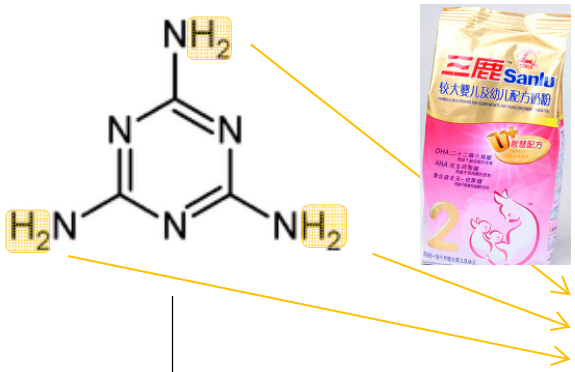
Normal Model Blossom Honey + new sample with sugar syrup addition

Honey profiling

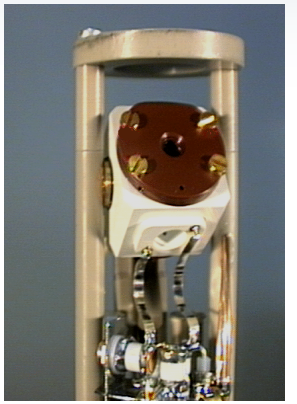


Compound	Value	Unit	NMR Distribution
5-hydroxymethylfurfural	44	mg/kg	<5 71
L-pyroglutamic acid	<30	mg/kg	<30 380
acetic acid	17	mg/kg	7 140
acetoin	<5	mg/kg	<5 10
alanine	<5	mg/kg	<5 120
citric acid	<100	mg/kg	<100 834
ethanol	28	mg/kg	<10 1025
formic acid	12	mg/kg	<5 226
fructose	40.2	g/100g	28.5 40.8
fructose / glucose	1.58	-	0.93 1.59
glucose	25.4	g/100g	21.9 35.2
glucose + fructose	65.6	g/100g	51.1 72.5
isoleucine	<50	mg/kg	<50 83
leucine	<50	mg/kg	<50 113
malic acid	<100	mg/kg	<100 839
maltose	0.8	g/100g	<0.5 0.9
melezitose	1.0	g/100g	<0.2 1.6
phenylalanine	11	mg/kg	<10 813
proline	217	mg/kg	<100 877
shikimic acid	<50	mg/kg	<50 192
succinic acid	8	mg/kg	5 209
sucrose	1.5	g/100g	<0.5 2.1
trigonelline	<10	mg/kg	<10 33
tyrosine	<50	mg/kg	<50 250
valine	<10	mg/kg	<10 51

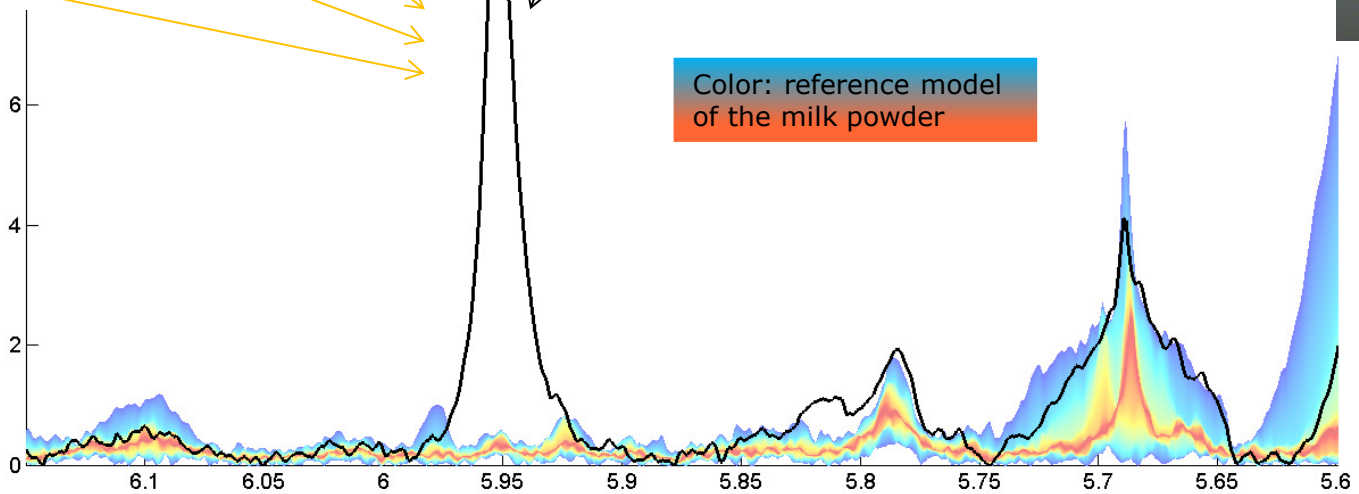
Study of milk powder in semi-solid state



San-Lu milk powder:
Melamine (460 mg/kg)



Color: reference model of the milk powder



Solvent : DMSO ; CPMG experiment

Melamine can be quantified directly
(Integral corresponds to 6 Protons, M = 126 g/mol)

Conclusions

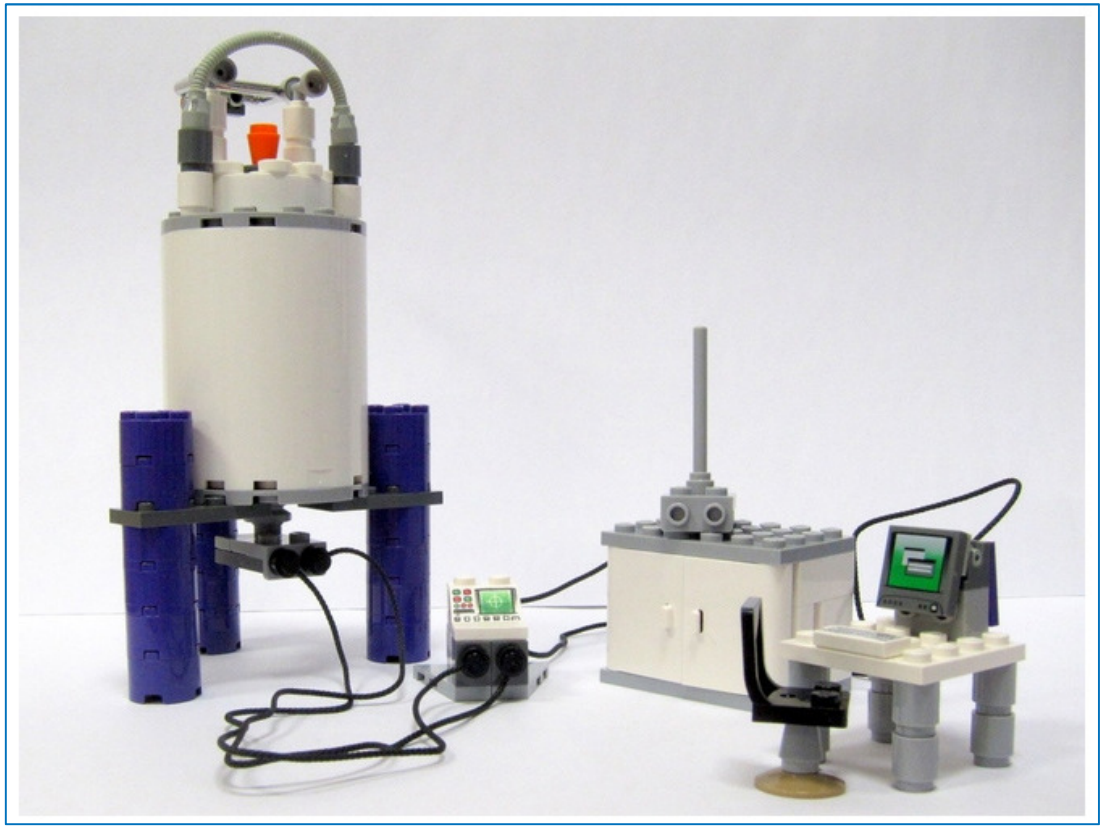
NMR in high throughput mode can generate a multitude of targeted and untargeted results in one measurement, such offering best analytical value at low price per sample.

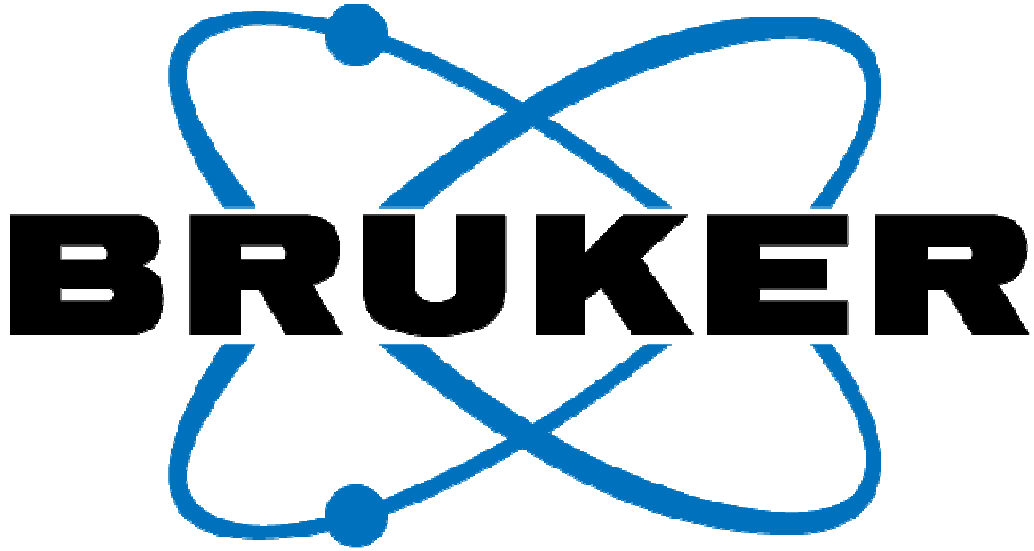
SGF-Profiling for the screening of fruit juices can be seen as **proof-of-principle** for other upcoming applications. The same workflow and underlying mathematical methods can be easily transferred to other quality control application as well as to a wide range of clinical/medical applications.

Multiple suppression helps in the detection of small signals in the presence of very large signals.

Easy sample preparation in all applications.

Thank you for your kind attention!





Innovation with Integrity