Olive Oil Profiling

on FoodScreener 400 and Fourier-80 Benchtop

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Outline

- Introduction
- Sample Calibration & Sample preparation
- Oil-Profiling at the Food Screener (400 MHz) & F80
- Features
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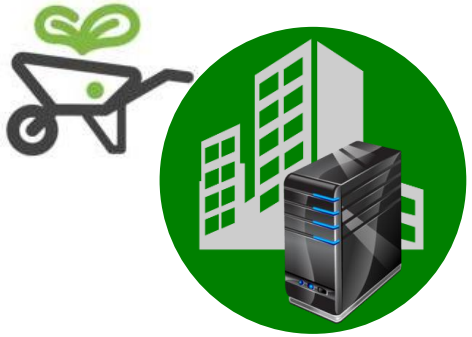
- Products
- Targeted Market Segments
- Configuration
- Pricing and business models
- Q&A

Introduction

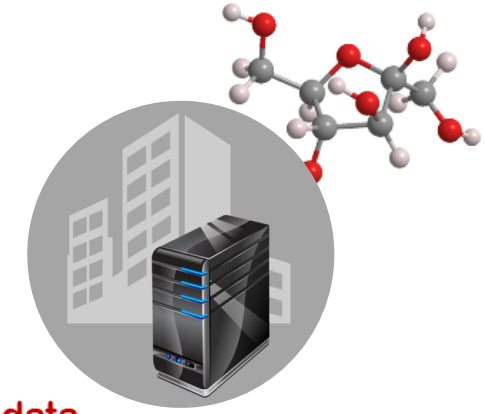
The process behind food profiling solutions

specialist in NMR spectroscopy
and instrumentation

expertise in food regulation
fraud investigation



access to authentic samples
partner in global food chains



access to metadata
expertise in quality parameters

Edible Oil Profiling - from 400 MHz to 80 MHz

The development story began with 400 MHz, and it continues with 80 MHz

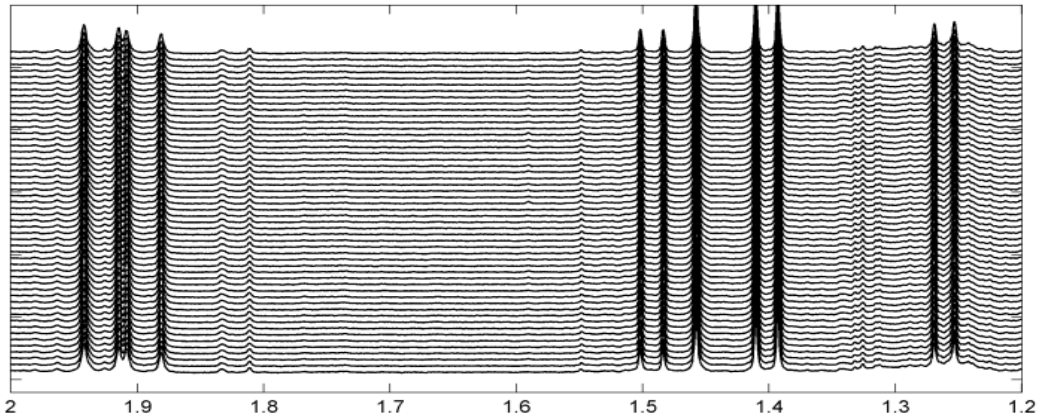
FoodScreener
400 MHz

F80 Benchtop NMR
80 MHz

Why using NMR for food profiling?

Reproducibility

- 50 replicate measurements, including sample preparation



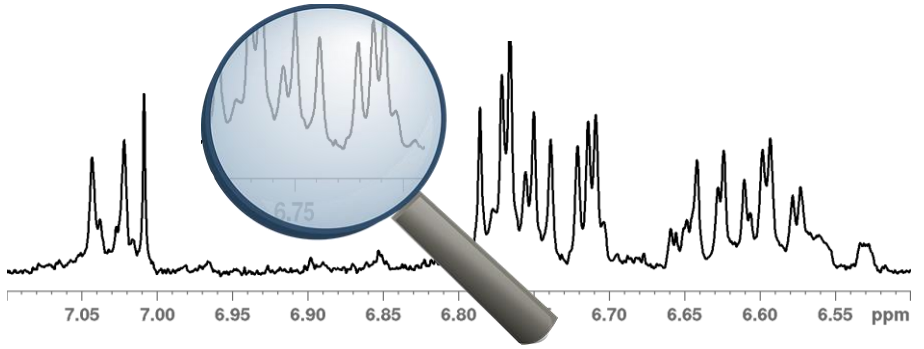
- databases can be built up and combined using raw data generated wherever an Oil-Profiling equipment is available

One quantification reference for all oil samples and oil parameters

- NMR is intrinsically quantitative

Raw data re-analysis

- whenever a so far irrelevant oil component comes into the focus of interest, it is already in the NMR raw data, and can be analyzed retrospectively



Highly efficient holistic method

- the sample preparation for NMR analysis leaves the oils' component "fingerprint" unchanged
- the whole oil profile - from "matrix effects" down to single components - is deciphered in one run

Why using "comparably insensitive" NMR for food profiling?

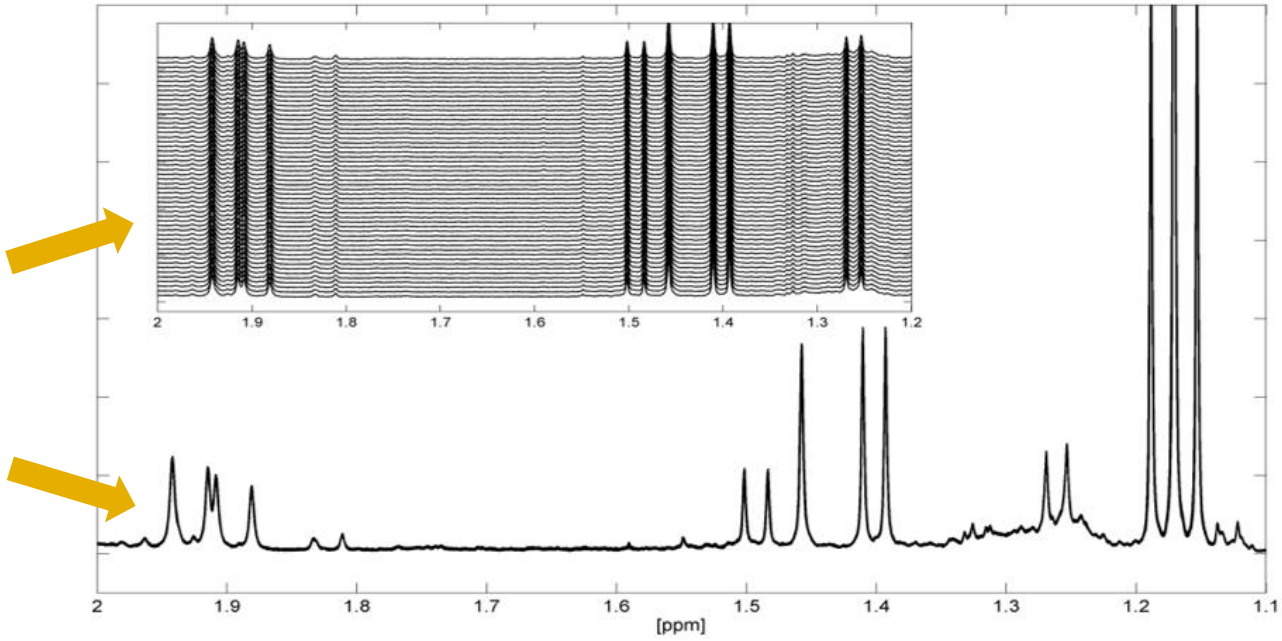
Detecting tiny variations by data comparison requires **reproducibility**, both of the sample preparation process and the instrumentation!
This is prerequisite for database build-up and global spectra comparison, independent from time, persons, instruments, and facilities.

NMR spectroscopy ...

- is extremely reproducible

30 replicate NMR measurements, including sample preparation

When superimposed, they seem to be only one single spectrum

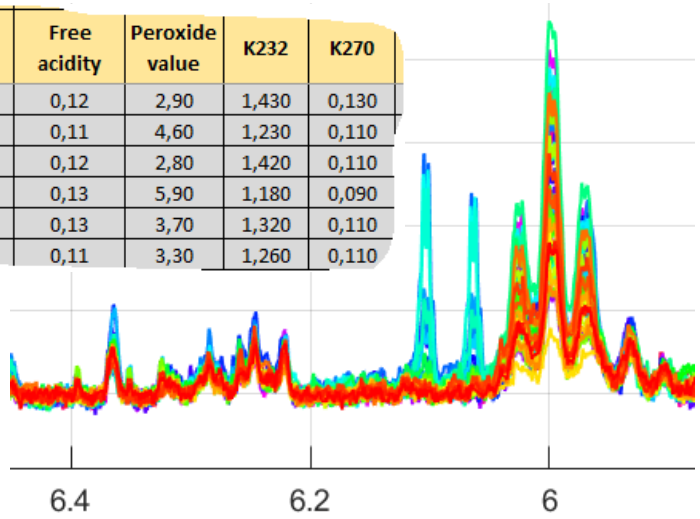


- enables to detect what you are looking for (targeted), and to find something you did not look for, or even what is unknown (non-targeted)

Regression by NMR

Quantification by multivariate regression is applied (instead of direct quantification = integration of a compound's signal). Main Advantage of using linear Regression: statistical models can be applied and concentrations interpolated. This can be done even for NMR peaks that are hidden in the forest of NMR signals and cannot be integrated directly.

Sample-ID	Harvest period	Quality grade	Free acidity	Peroxide value	K232	K270
3269_210324	2020-2021	EVOO	0,12	2,90	1,430	0,130
3270_210324	2020-2021	EVOO	0,11	4,60	1,230	0,110
3271_210324	2020-2021	EVOO	0,12	2,80	1,420	0,110
3272_210324	2020-2021	EVOO	0,13	5,90	1,180	0,090
3273_210324	2020-2021	EVOO	0,13	3,70	1,320	0,110
3274_210324	2020-2021	EVOO	0,11	3,30	1,260	0,110



Parameter	Unit	Value	EVOO Reference (IOC) min	max	Flag
Free acidity	%w/w as oleic acid	0.21	-	0.80	●
Peroxide value	mEq O2/kg	7.4	-	20.0	●
K270	-	0.11	-	0.22	●
K232	-	1.8	-	2.5	●
Delta K	-	0.0078	-	0.0100	●
Total polyphenols	mg/kg	170	-	-	○
Linoleic acid	%m/m methyl esters	7.9	2.5	21.0	●

The fundament of the quantification of single components or sum parameters by regression is a database, containing

- as many as possible NMR spectra (both 400 and 80 MHz)
- sample-assigned values of all parameters to be quantified, determined by orthogonal (in our case non-NMR) analytical methods (like wet chemistry, HPLC, titration, ...)

Statistical processing of NMR data then generates regression models for each parameter by correlating specific variations with metadata values.

Then, these regression models are used to quantify corresponding parameters in samples to be analyzed.

Introduction

Main instrumental features

- method installation via an Installer (analogue to all other FoodScreening methods)
- experiments/reports fully automated for both platforms (SampleTrack)
  - sample setup via Oil Easy Dialog menu
  - F80 - sample-ID by SampleTrack
  - FoodScreener - barcode labels
  - automated Analysis Report generation

- F80/FoodScreener usable with or without Sample Changer
- Standardized Work Instructions for oil sample preparation available
- Oil-Profiling 1.0 Solution Manuals will be available soon

The Oil-Profiling experimental set

80 MHz platform

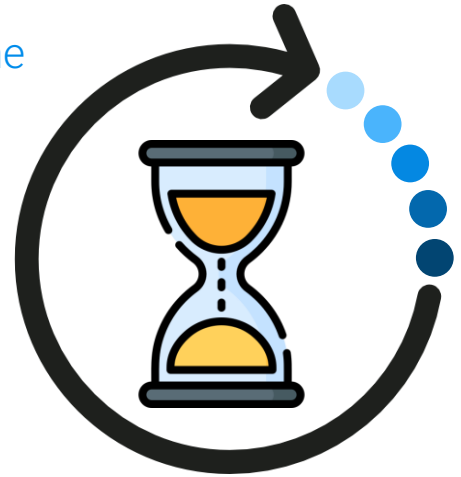
- standard ^{1}H-NMR spectrum, with parameters optimized for oil samples ("zg" pulse sequence)

400 MHz platform

- standard ("zg") ^{1}H-NMR spectrum, with parameters optimized for oil samples
- ^{1}H-NMR spectrum where intense lipid signals are suppressed - a "noesy" pulse sequence containing a shaped pulse usable for signal suppression is used
- ^{1}H-JRES experiment for further spectral information (e.g. easier access to signal multiplicity in regions with overlapping signals)

total experimental time

12 minutes



25 minutes (including sample temperature stabilization, atma, lock, shim, and pulsecal)

Pipette Calibration

Sample Preparation

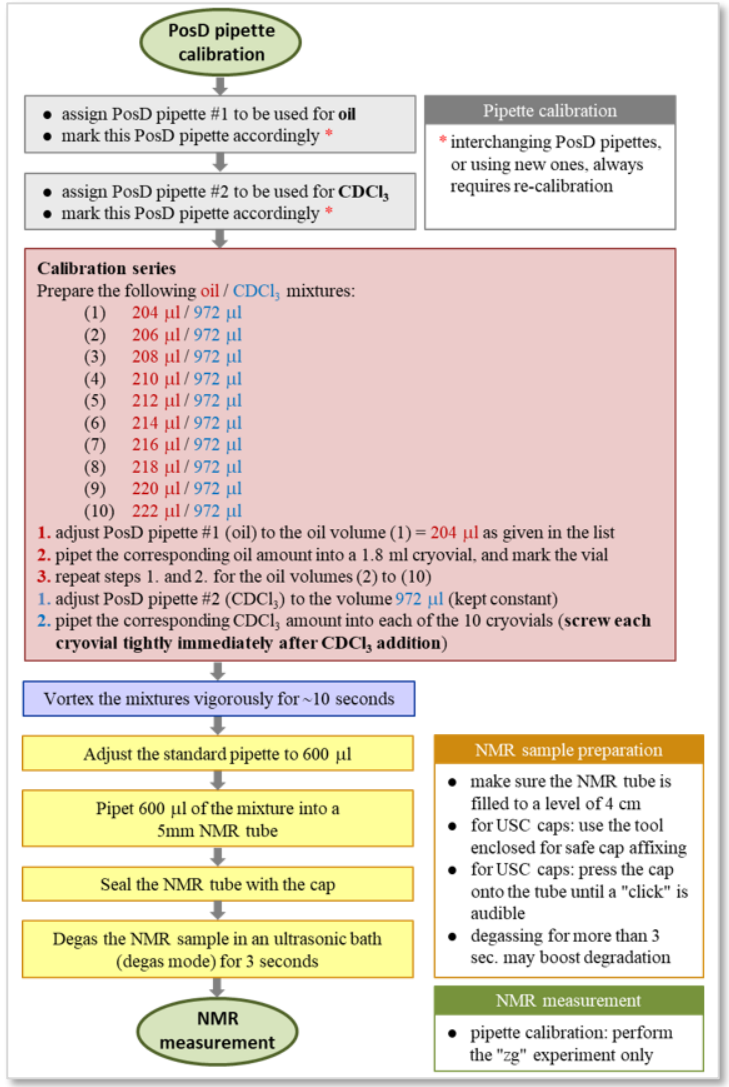
Pipette Calibration

- The use of positive displacement pipettes (PosD) is necessary because of the low viscosity of the solvent CDCl3
- Pipettes are initially calibrated according to Bruker Work Instructions. Calibration is done by identifying the correct Oil/CDCl3 ratio. This is done automatically by NMR. Typically the volume of oil to be pipetted will be ~214 uL
- The use of USC caps for the NMR tubes (the purple ones) is necessary to prevent solvent evaporation

Positive Displacement Pipette

olive oil samples in $CDCl_3$ situation 14 day after sample preparation

Pipette Calibration Instructions



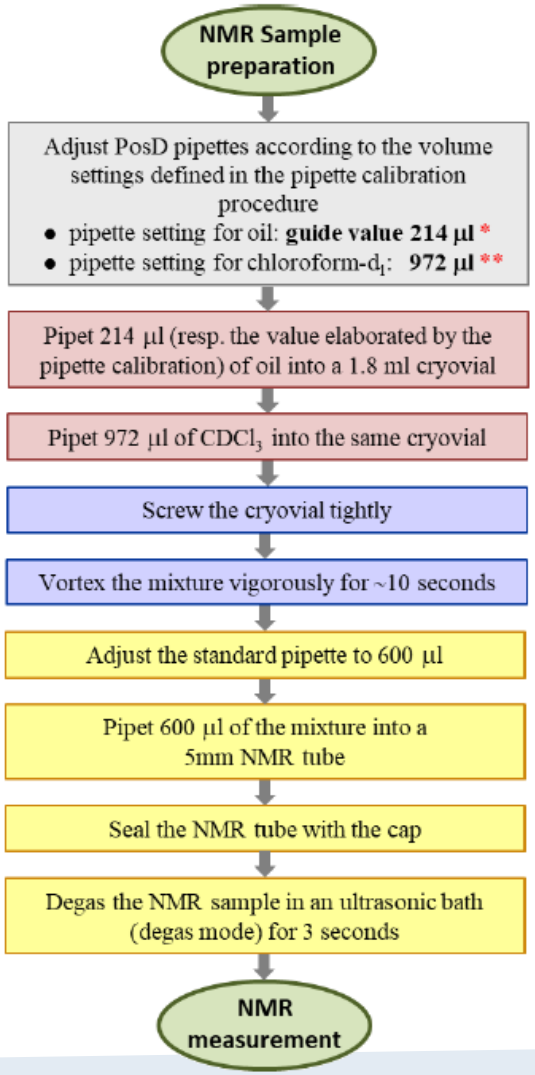
Sample Preparation & Quality Control Measures

- Sample preparation is the same for the F80 and the 400 MHz Food Screener & according to the Bruker Instructions (Video at the end of the presentation)
- Quality Control is done with reference samples from Bruker (not Oil) and with one Oil sample from the customer (can be any oil sample chosen by the customer).
- 400 MHz: 3 reference samples required + 1 Oil reference sample
- 80 MHz: 1 reference samples required + 1 Oil reference sample

AV400 Reference samples	Produced/prepared by	Further informations or explanations
QuantRef	Bruker BioSpin GmbH (Germany)	validated solution for quantification
Sucrose	Bruker BioSpin AG (Switzerland)	certified* product "2mM sucrose in the NMR tube"
TempCal	Bruker BioSpin AG (Switzerland)	certified* product "99.8% MeOD in the NMR tube"
F80 Benchtop Reference samples	**Produced/prepared by**	**Further informations or explanations**
QuantRefB	User (prepare according to flow-chart in Chapter 9.3)	validated solution for quality control and quantifi-cation
Instrument-independent Reference sample	**Produced/prepared by**	**Further informations or explanations**
OilRef	User (prepare according to flow-chart in Chapter 7, as any other oil sample)	• define ~200 ml of any olive oil as "oil for Oil-Ref", and mark it accordingly • when consumed, continue likewise with a further 200 ml portion of olive oil • prepare the OilRef sample in the course of the preparation of other oil samples • one OilRef samples for all NMR instruments

* Reference sample has to be ordered *always together* with the associated certificate

Sample Preparation Instructions



"Evolution" of the oil sample preparation method

original SOP (weight-based, manual)

EXCEL-guided weighing of oil and $CDCl_3$ amount

keep this ratio as constant as possible

Oil weight (mg)	Final weight (mg)	weight Oil / weight CDCl3	CDCl3 volume difference to ref (µl)
140,00	1176,00	13,51351351	0,00
139,60	1172,10	13,52058111	-0,36
140,90	1184,70	13,49875455	0,77

with more and more samples, manual weighing becomes a hassle ...

Quantos

"interim" SOP (weight-based, semi-automated)

"F80-compatible" low-cost solution

final SOP (volume-based, manual)

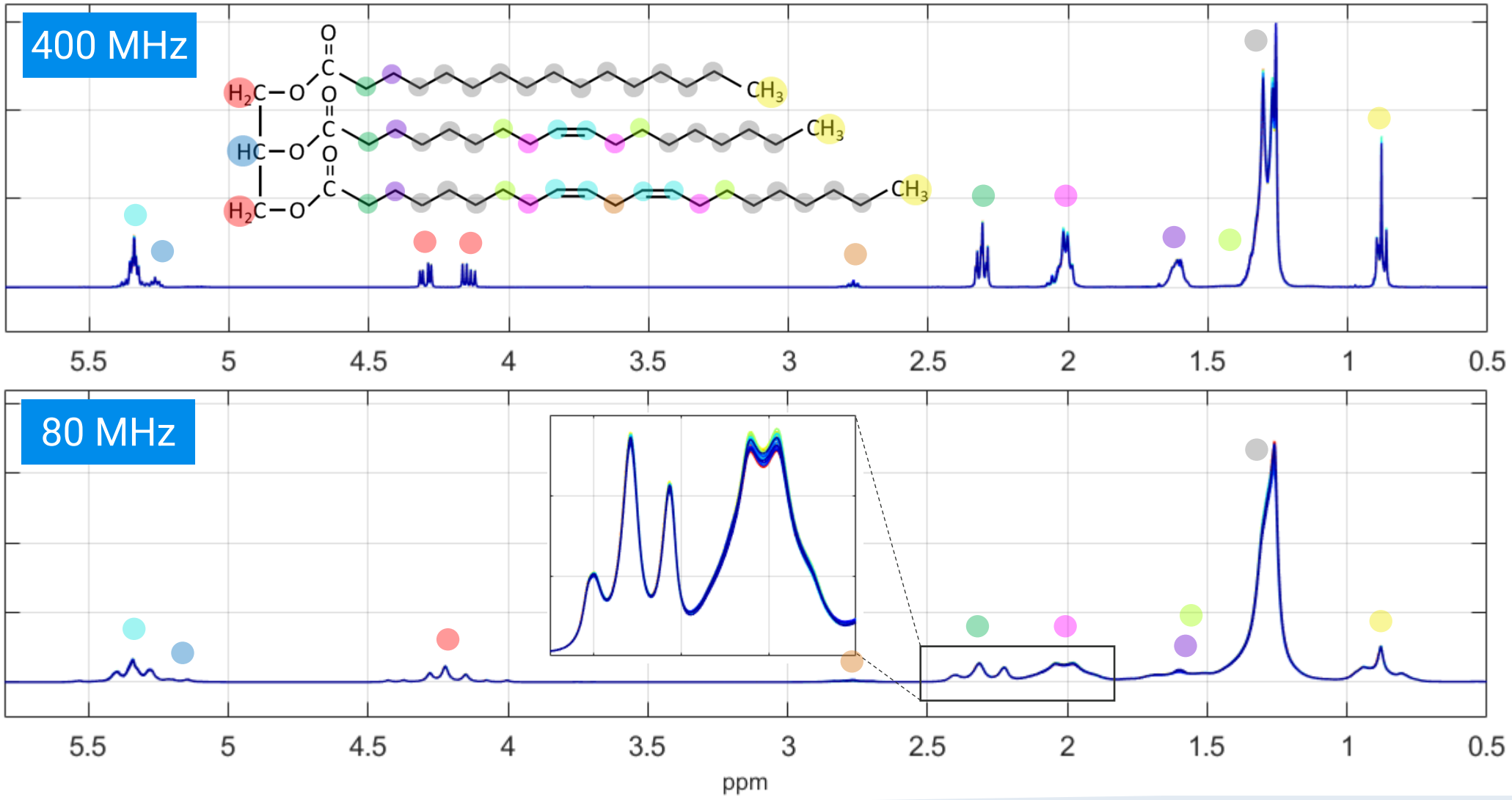
Positive Displacement pipet

Oil-Profiling at the Food Screener (400 MHz) & F80

Olive oil spectra - the lipid profile

Some signals always are quasi-identical, (even for oil types other than olive oil)

Some other signals vary within a certain range from olive oil sample to sample



Identical Reports for the F80 & the 400 MHz

Model:

- oil type (olive oil vs. others)
- country of origin (Spain, Italy, Greece)
- Extra virgin vs. other qualities

Analysis Report

Olive Oil Profiling 400 MHz™

Sample ID: 108928_210212

Information/Declaration provided by customer:

Type of Oil: Olive Oil
Country of Origin: Greece
Quality Grade: undefined
Harvest Period: undefined

Disclaimer: this information will affect the applicability and validity of analyses and results.

Measuring Date: 16-Feb-2021 16:00:07
Reporting Date: 02-Mar-2022 16:39:58, 5 pages, Version 1.0.0

Results Summary

Type of Analysis	Result	Status
Analysis of Origin		
Country Greece	Consistent	●
Quantitative Analysis	Consistent with IOC references	●
Comparison to Reference Group	Typical	●

The data analysis is performed at Bruker BioSpin GmbH (Ettlingen, Germany) according to testing method Olive Oil Profiling 400 MHz. All results solely refer to the tested sample as provided by the customer.

Quantitative Analysis

(Analysis-ID: OO1400-REG-20220302)

Parameter	Unit	Value	EVOO Reference (IOC) min	EVOO Reference (IOC) max	Flag
Free acidity	%w/w as oleic acid	0.21	-	0.80	●
Peroxide value	mEq O2/kg	7.4	-	20.0	●
K270	-	0.11	-	0.22	●
K232	-	1.8	-	2.5	●
Delta K	-	0.0078	-	0.0100	●
Total polyphenols	mg/kg	170	-	-	○
Linoleic acid	%m/m methyl esters	7.9	2.5	21.0	●
Linolenic acid	%m/m methyl esters	0.80	-	1.00	●
Oleic acid	%m/m methyl esters	75.4	55.0	83.0	●
Palmitic acid	%m/m methyl esters	11.2	7.5	20.0	●
Palmitoleic acid	%m/m methyl esters	1.0	0.3	3.5	●
Stearic acid	%m/m methyl esters	2.2	0.5	5.0	●
Wax content	mg/kg	7	-	150	●
Erythrodiol + Uvaol	% total sterols	1.9	-	4.5	●
b-Sitosterol apparent	% total sterols	94.7	93.0	-	●
Total sterols	mg/kg	1321	1000	-	●
Total MUFA	% of total fatty acids	75.9	-	-	○
Total PUFA	% of total fatty acids	8.8	-	-	○
Total TFA	% of total fatty acids	0.07	-	-	○
Total SFA	% of total fatty acids	13.7	-	-	○

MUFA mono-unsaturated fatty acids
PUFA poly-unsaturated fatty acids
TFA trans unsaturated fatty acids
SFA saturated fatty acids

Video: Oil Profiling F80 & 400 MHz

Please Watch the video starting at minute 26 from the full recorded session: LINK

Features

Features

	Olive Oil Profiling 1.0	Olive Oil Profiling 2.0
Compliance check for declared country of origin	Spain, Greece	Addition of Italy
Compliance check for Extra Virgin OO	Not available	Yes
Quantitative Analysis of IOC regulated parameters	15 parameters	15 parameters
Detection of atypical profiles	Yes	Yes

- Fully automated analysis
- Rapid analysis: 12mn/sample on Fourier 80 vs 25mn/sample on FoodScreener
- Fast sample preparation: roughly 3mn/sample.
- Under work: ISO17025 accreditation

Unique Selling Points

Unique selling points

Overview of competing techniques

	Block Chain Genetics (DNA)	Panel Test (Sensory)	Wet Chemistry	GC or LC FID/Fluores/MS	UV-VIS NIR	NMR
Authenticity	Country of Origin					Country of Origin
	Cultivar					Cultivar
				Blends/dilutions		Blends/dilutions
Quality Grade		EVOO/VOO Atributes & Defects				EVOO/VOO Screening
Quality Phys-Chemistry			Tritation Gravimetry	GC-FID for Fatty Acids E.E.	UV 270/232 NIR	Most relevant parameters

- Combine analysis of country of origin, quality grade and chemical parameters, with one single measurement !
- Screening for quality grade with NMR reduces the number of samples to be tested by panel test (sensory), as this requires experts, and can not exceed 10 tests/day to be reliable.
- Fully automated and easy to use methods
- Fast analysis
- No specialist required to operate the system.
- Ready to use method: no method development required !

Products

Product families

Fourier 80 with automation

FoodScreener

Targeted Market Segments and Value Proposition

Targeted Market Segments

		Preferred solution*	
		Fourier 80	**FoodScreener**
Olive Oil Bottlers			
Commercial Service Providers	Main Hub		
	(International) Subsidiaries		
	Laboratories specialized only in OO		
Governmental laboratories			

* Preferred / recommended product in green. Nevertheless, upon need and request of customer, both products can be sold without restriction to all market segments.

Value Proposition: Olive Oil Bottlers & Valorizers

= A compact, benchtop solution, easy to implement and to operate !

= Saving time and money in insourcing analysis (Fast ROI)

= Replace other techniques like e.g. NIR

= Quality & authenticity control of olive oil at both purchase and selling side

= Brand protection

= Use NMR as **marketing tool** to sell premium Olive Oils with tested origin and quality

Value Proposition: Commercial Testing Labs

= Expand product portfolio & sales revenues with innovative new method

= Differentiate from competitors

= Big labs can equip subsidiaries with a benchtop Fourier 80 system

= Fast ROI, low TCO

Value Proposition: Public & Government Accounts
Official Control, Customs & Border Protection, Regulatory Bodies

= Check if olive oils are compliant with label information & regulation

= Fight fraud on origin, ensure fair competition

= New business model (Flat Rate) offer more flexibility and allow to measure more samples

= Possibilities of cooperation to further extend the method

= Possibility to access method details for validation purposes or in case of prosecution.

Configuration

Configuration

	FoodScreener		Fourier 80	
	Options	Price (EUR)*	Options	Price (EUR)*
Platform	AV4400FOOD 1H inverse probehead, Boss3 shimsystem, BCU, TopSpin and SampleTrack.. MSASC400SBAIC magnet AH0070 transfer line	456,898 151,768 3,755	UH0085 1H/13C system Sample temperature of 25°C TopSpin and SampleTrack Bench PC, touchscreen, cleaning kit, etc. Installation Support included (2 hours of phone)	83,104
Sample changer	Included in platform 60 pos		FH0040 Optional (60 pos)	30,962 (ACA) 32,954 (IND)
Barcode reading	Automatical Samples can be put on any place on the sample changer		Manual Samples need to be placed on a specific position on the sample changer	
Olive Oil Module	AH0604	13,433	UH0085OL	10,000
	Remark: no pH adj.			

* Prices are indicative and may change from time to time, as well as region-dependent. Current prices can be found in SAP

Pricing and business models

Olive Oil Profiling Analyses Options

	FoodScreener		Fourier 80	
	Options	Segment	Options	Segment
Pay-per-use	Not available		Not available	
Pre-paid bundles	500 bundle LYOO_0500 (13000€)	ALL	500 bundle, 12 months LYOO_0500_80 (10000€)	ALL
	1000 bundle LYOO_1000 (22000€)	ALL	1000 bundle, 12 months LYOO_1000_80 (16000€)	ALL
Annual Flatrates	Capped to 10000, all food LY_FR1Y_GOV (45000€)	GOV only	Capped to 1000/y LYOO_FR1Y_GOV (8000€)	GOV only
	Capped to 600, all food LY_600_1Y_GOV (20000€)	GOV only	Capped to 1000/y LYOO_FR1Y_BOT (8000€)	Bottlers only
			Quant only LYOO_QFR1Y_BOT (4000€)	Bottlers only

* Prices are indicative and may change from time to time, as well as region-dependent. Current prices can be found in SAP

Olive Oil Profiling Analyses Options

- **Adjusted Business Model for Gov Accounts (FoodScreener)**

  - Gov accounts = official control laboratories, customs laboratories and regulatory bodies only. Not valid for universities and other ACA customers, even if the lab is owned by government.
  - The annual Flat Rate Model is valid for any food matrix
  - The annual Flat Rate models, offer more flexibility to Government accounts and incentive to test more (the more samples analyzed, the cheaper the cost per sample)
  - The flatrates are valid for 1 calendar year
  - With the purchase of the first flatrate, the customer can order AH0610_GOV, which offers the following items complimentary:
    - Installation of parameter sets for any missing food module(s); e.g. Olive Oil
    - Free analyses till the end of the calendar year (limited to 600 max)

Q&A

Q&A

Q: How is the Quantification done (e.g. linear regression)?
A: We do not quantify any oil component directly at the moment. All the components are quantified using linear regression. In other words, for every component we quantify, we have a collection of corresponding concentration ranges obtained by non-NMR methods (e.g. HPLC, wet chemical analysis, etc.) to which we apply a linear regression method in order to get the concentration of a given oil component for a given oil sample.

Q: How is peak deconvolution done? A: It is not done so far.

Q: How are the limit ranges (LOQ) obtained?
A: LOQ was not determined so far because the limit of detection (LOD) was not determined so far. Typically, LOQ= LODx2/3σ. In order to obtain LOD a dilution series is performed. Spiking experiments can also be performed.

Q: Can Total Cholesterol quantification be performed?
A: not automatically with the current version of the profiler. However, if the signal(s) are sufficiently isolated, quantification could be easily done by performing manual integration and using the Eretic method. If the signal is overlapping with other signals, quantification could still be possible to some degree by performing signal deconvolution by peak fitting.

Q: Can Total Fatty Acids and not only Olive Oils be quantified by this method?
A: This is possible but not without adjustments to the current method: so far we quantify by linear regression and this method always requires a database in the background. Presently we have only Olive Oils in the database and regression models for Greek, Spanish & Italian. Direct quantification of any component always requires a database. Direct quantification is a difficult process to establish for fatty acids. Which often have overlapping signals at 400 MHz. Another way is to perform lineshape analysis but this was also not implemented so far.

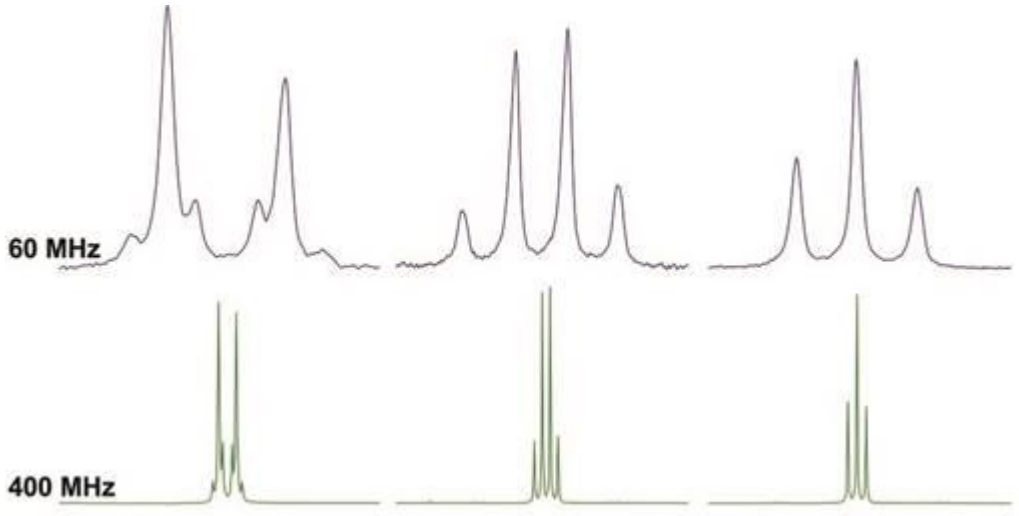
Q&A

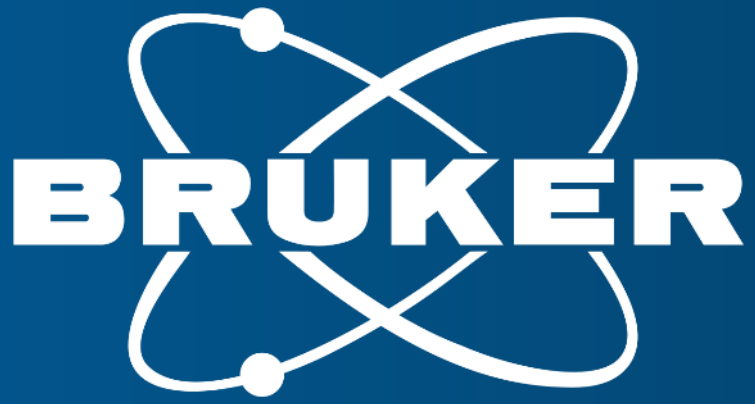
Q: What is the difference between the F80 and the 400 MHz? Will the F80 deliver less accurate results?

The first difference between both is the sensitivity: if all the conditions are the same (same sample concentration, quality, etc) you gain a factor of 11 in S/N ratio by going from the 80 MHz to the 400 MHz. This is established according to the equation:

$$\frac{S/N_{80}}{S/N_{400}} = \left(\frac{400}{80}\right)^{3/2} = 11{,}2$$

The second difference is that the resolution is reduced: signals that are well separated at 400 MHz may be partially or fully overlapping at 80 MHz. Nevertheless, most informations are available – this is not obvious when visually inspected, but "hidden" e.g. in lineshapes ... Our statistical methods can pick out them also from an 80 MHz spectrum.





Innovation with Integrity