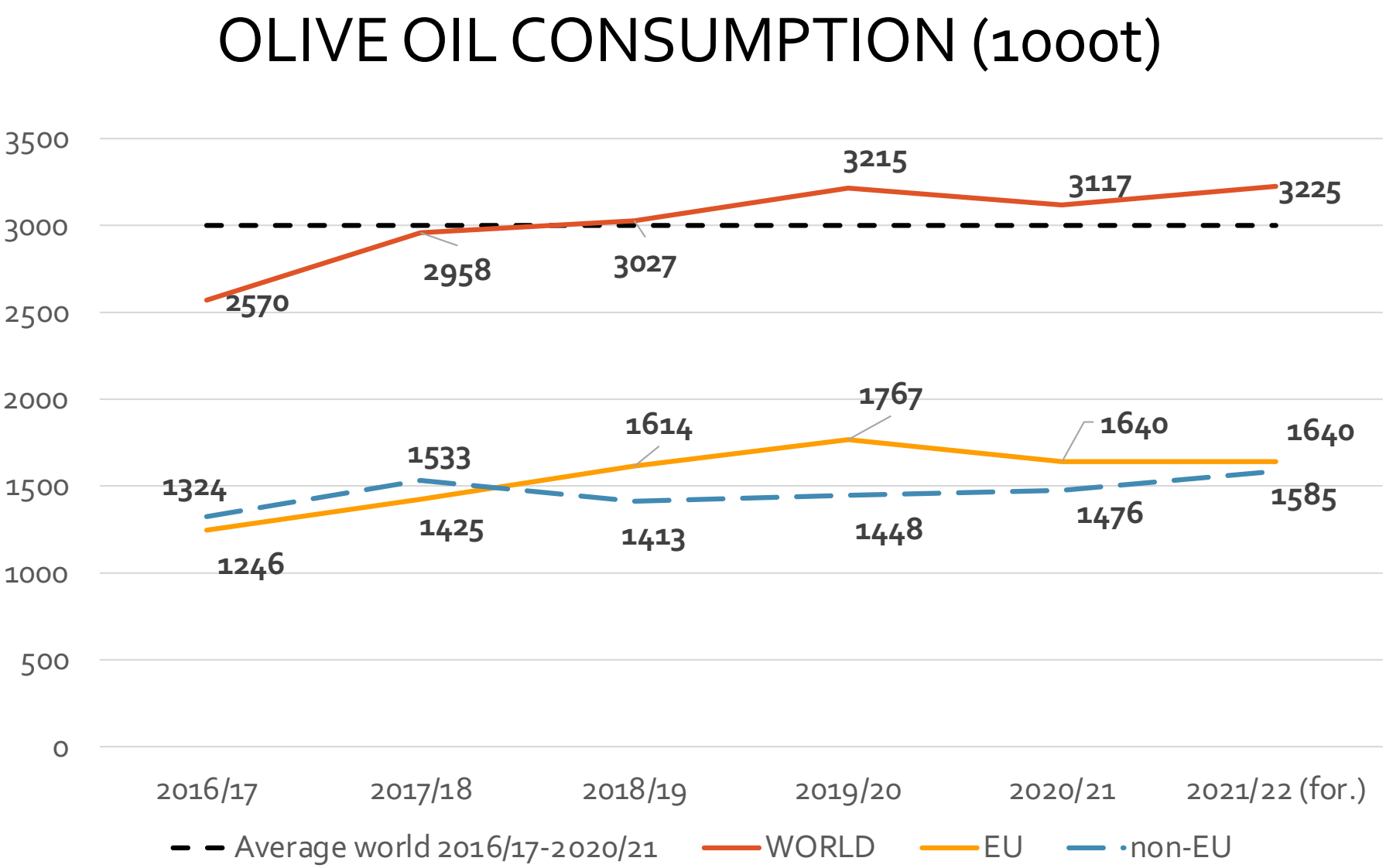
UNIVERSITÀ DI TRENTO
FONDAZIONE EDMUND MACH
C3A CENTRO AGRICOLTURA ALIMENTI AMBIENTE

# NMR spectroscopy in extra virgin olive oil authentication

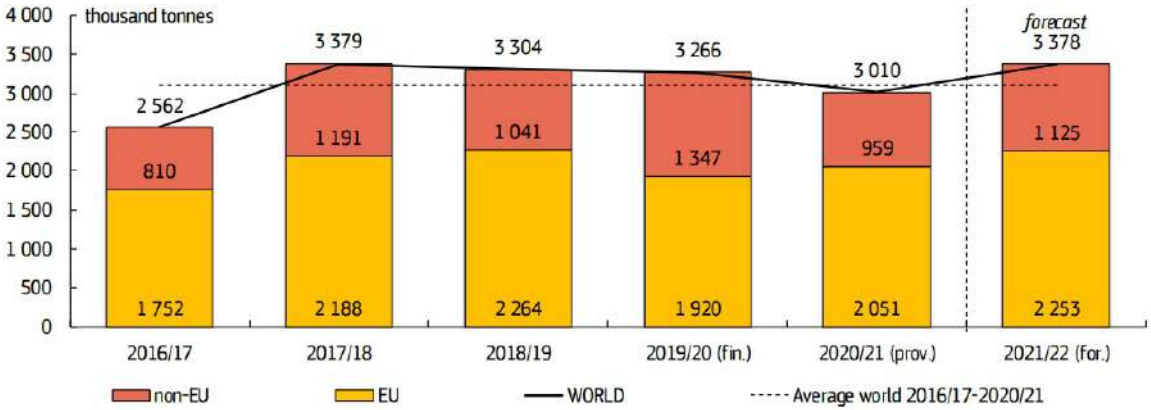
Congresso SISSG 2022, 15 – 17 giugno 2022

# Background

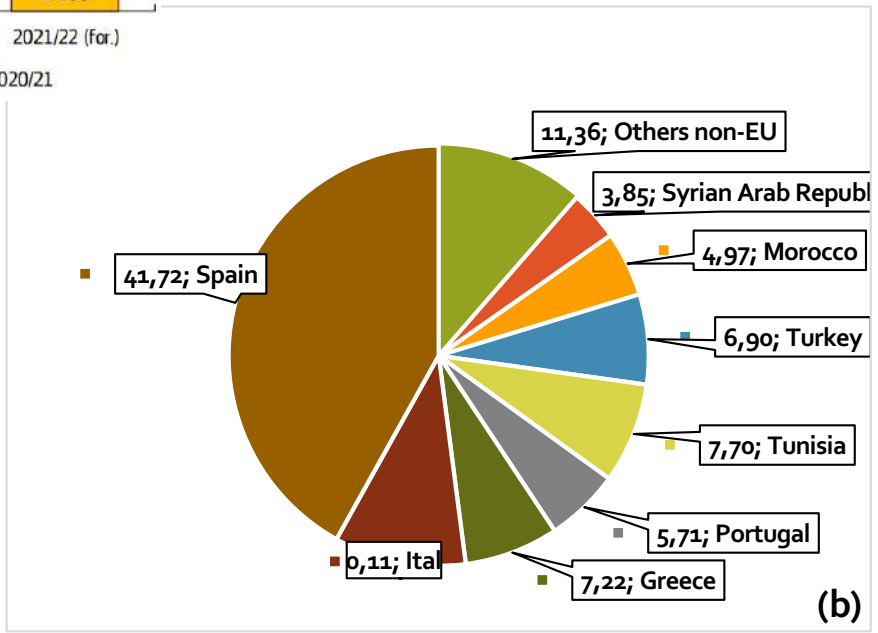



https://ec.europa.eu/info/sites/default/files/food-farmingfisheries/plants_and_plant_products/documents/olive-oil-dashboard_en.pdf

# The economic relevance of olive oil is increasing and this is highlighted by trade number statistics:




World olive oil production in the last five years and the production of olive oil in 2021/2022

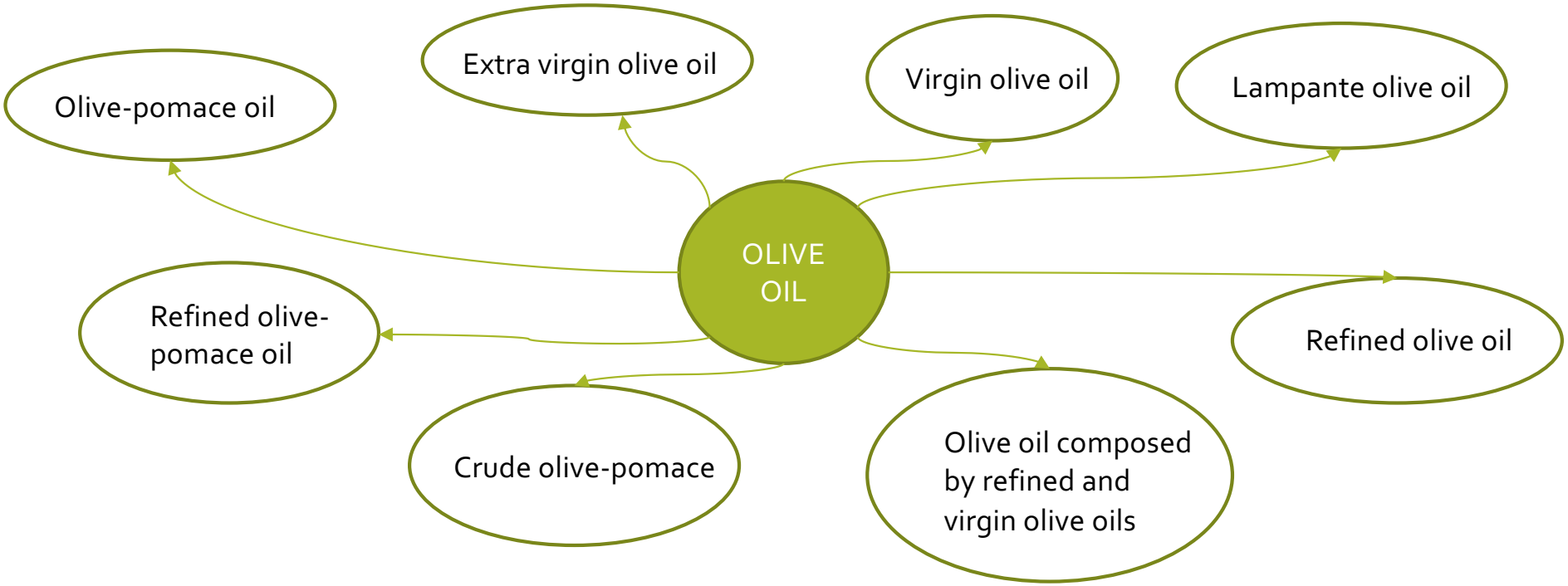


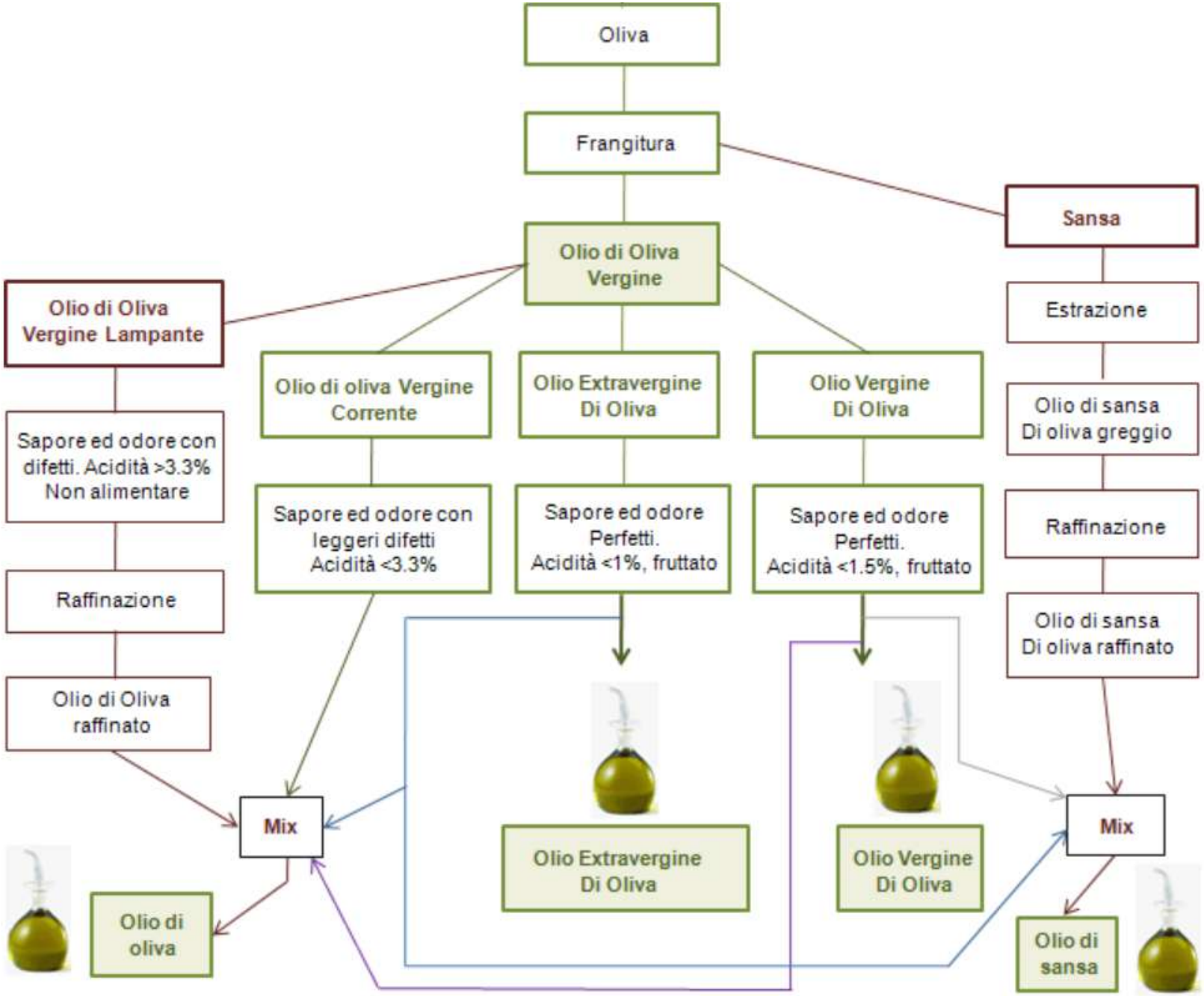

https://ec.europa.eu/info/sites/default/files/food-farming-fisheries/plants_and_plant_products/documents/market-situation-olive-oil-table-olives_en.pdf

# Extra virgin olive oil price for the most representative markets in EU


https://www.internationaloliveoil.org/wp-content/uploads/2022/01/IOC-prices-rev-0-3.html

The European Union recognizes different types of o*live oils* based on different **physico-chemical characteristics and quality standards** - Commission Regulation (EEC) No 2568/91:




Oliva
Frangitura
Sansa
Olio di Oliva Vergine
Olio di Oliva Vergine Lampante
Estrazione
Olio di oliva Vergine Corrente
Olio Extravergine Di Oliva
Olio Vergine Di Oliva
Olio di sansa Di oliva greggio
Sapore ed odore con difetti. Acidità >3.3% Non alimentare
Sapore ed odore con leggeri difetti Acidità <3.3%
Sapore ed odore Perfetti. Acidità <1%, fruttato
Sapore ed odore Perfetti. Acidità <1.5%, fruttato
Raffinazione
Raffinazione
Olio di sansa Di oliva raffinato
Olio di Oliva raffinato
Mix
Mix
Olio Extravergine Di Oliva
Olio Vergine Di Oliva
Olio di oliva
Olio di sansa


**42 PDO and 7 PGI in Italy**


## PDO and PGI - Regulation CEE 2081/92

- PDO stands for **Protected Denomination of Origin** and it is assigned by the European Union. It is meant to protect products **strictly connected** with the geographical area, so that every step of production must be placed in a specific area.

- PGI stands for **Protected Geographical Indication** and it is assigned by the European Union. This label indicates that the product was producted **at least** in one of the production steps in a given area.

https://ec.europa.eu/info/food-farming-fisheries/food-safety-and-quality/certification/quality-labels/geographical-indications-register/

## LABELLING OF ORIGIN

**Compulsory** for EVOO and VOO in 2009 (EC Regulation 182/2009)

Art. 4 of the EU Reg. 29/2012:

«*Extra virgin olive oil and virgin olive oil shall bear a designation of origin on the labelling*»,

proving the **CORRELATION BETWEEN ITS CHARACTERISTICS AND GEOGRAPHICAL ORIGIN AND PRODUCTION TECHNIQUES**.

## Olive oil typical fraud:

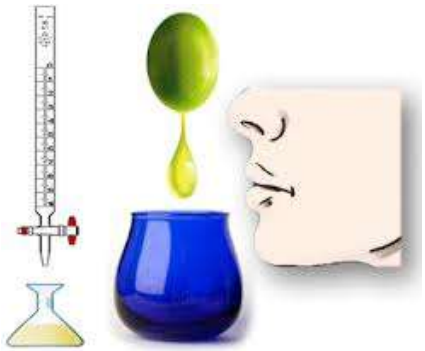
- Adulteration with cheaper oils of extra virgin olive oil without reporting this on the label
- Modification of certain characteristics (e.g. soft-deodorization, addition of chloropyll and beta-carotene)
- Mislabeling of geographical origin
- Mislabeling of varietal composition

"Fraud is so widespread that few growers can make an honest living," one expert says.
Slippery business, *The New Yorker*

# Official analysis, recognized by EU

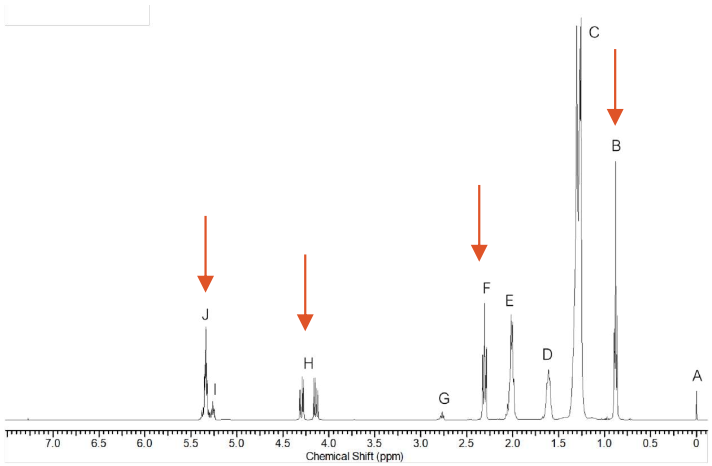
The parameters that discriminate the category of the oil are obtained with different chemical methods (Commission Regulation (EEC) No 2568/91):

- Acidity and peroxide value with titration
- Waxes, fatty acid methyl esthers and 2-glyceryl monopalmitate content with GC-FID
- Sterols with GC-UV
- Quality and preservation state with UV
- Volatile halogenated solvents with GC-ECD
- Organoleptic assessment with a Panel test

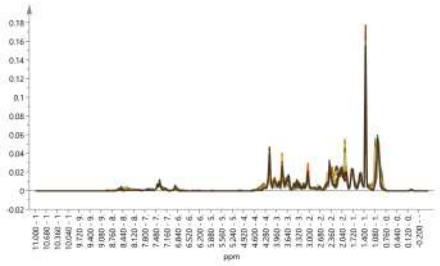


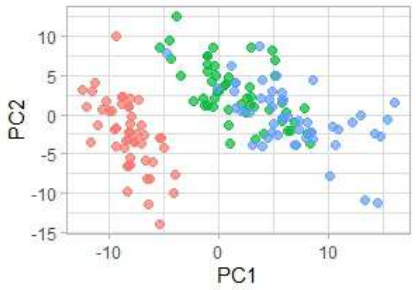
Other methods are «extensions» of the official methods that have been modified to better respond to specific requests.

**TARGETED**

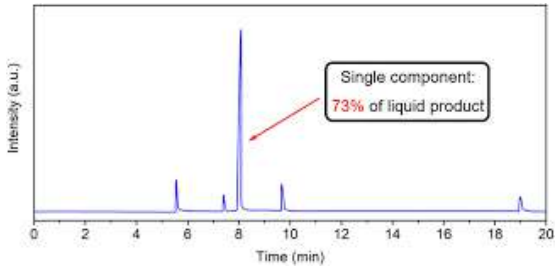



**UNTARGETED**

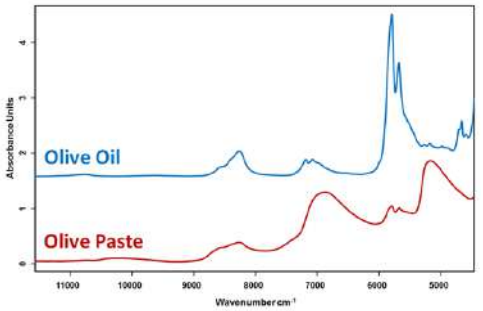




# Also other methods have been used for olive oils authenticity issues

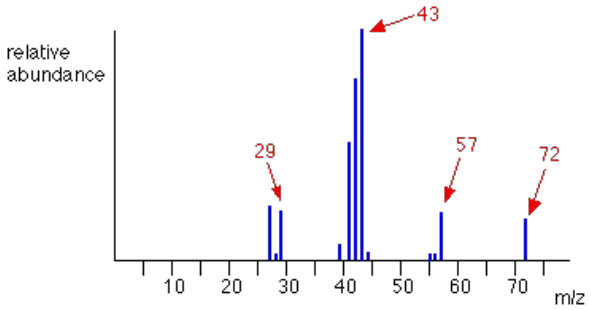



Chromatography + various detectors (FID, ECD, APCI, DAD, FLD) + statistical analysis

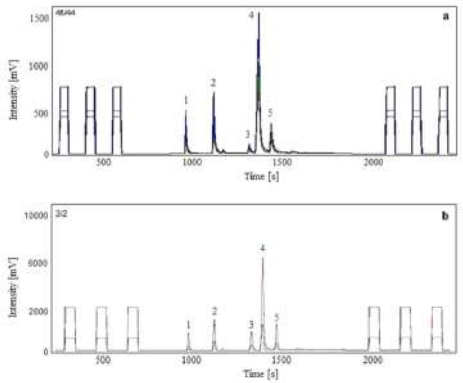



FT-IR, UV, Raman spectroscopy + statistical analysis

https://www.olive-nir.com/




Mass-spectrometry + statistical analysis




Stable isotopes analysis + (elemental analysis +) statistical analysis

# NMR for olive oil authentication

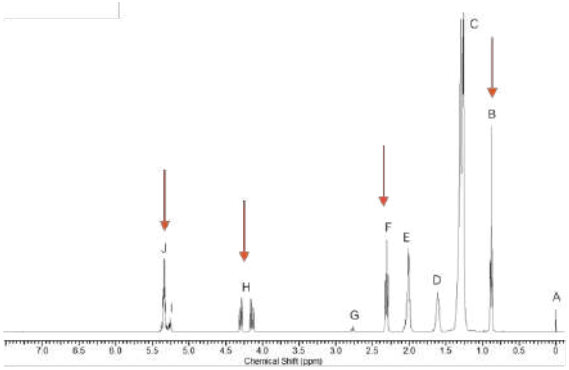
NMR spectroscopy is a powerful tool for food quality control and product authentication.

- Robust
- Reliable
- No time-consuming steps
- Fast results

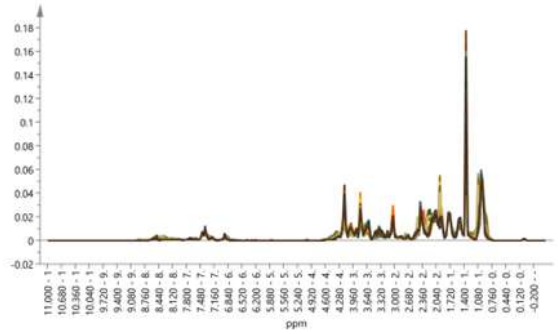
- Low intrinsic sensitivity

NMR spectroscopy

**Targeted**




**Untargeted**




## Addressed problems:

- Adulteration with cheaper oils
- Mislabeling of the cultivar
- Mislabeling of the geographical origin

# Preparation steps

For $^{1}H$ experiments: addition of **non-polar deuterated** solvents, without any other pretreatment.

100/200 mg

+

Deuterated solvent

→ 12/86 %w/w

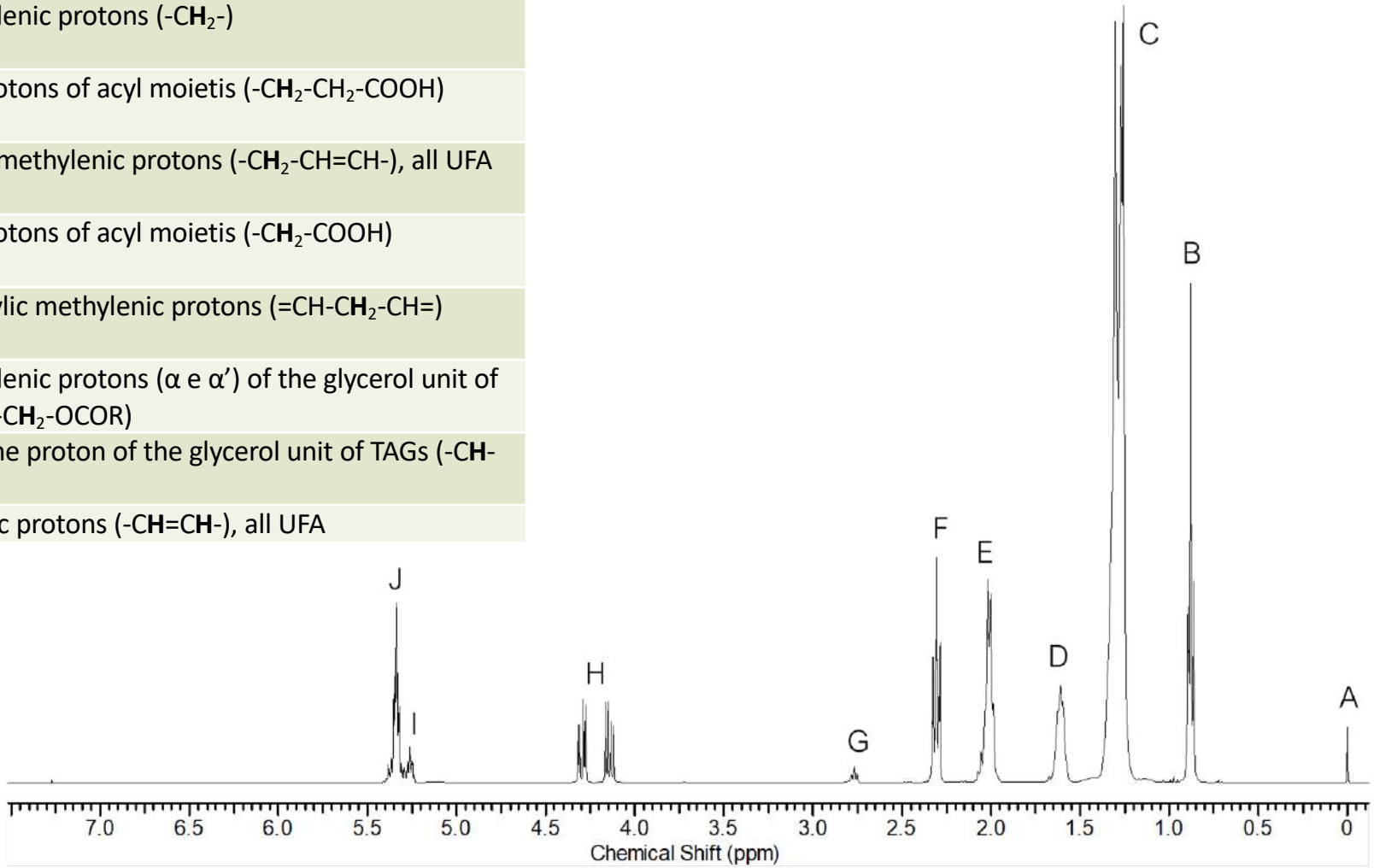
+ DMSO-d, TMS

Vortex

NMR spectrometer
Monodimensional and
NOESY experiments with
suppression of main signals

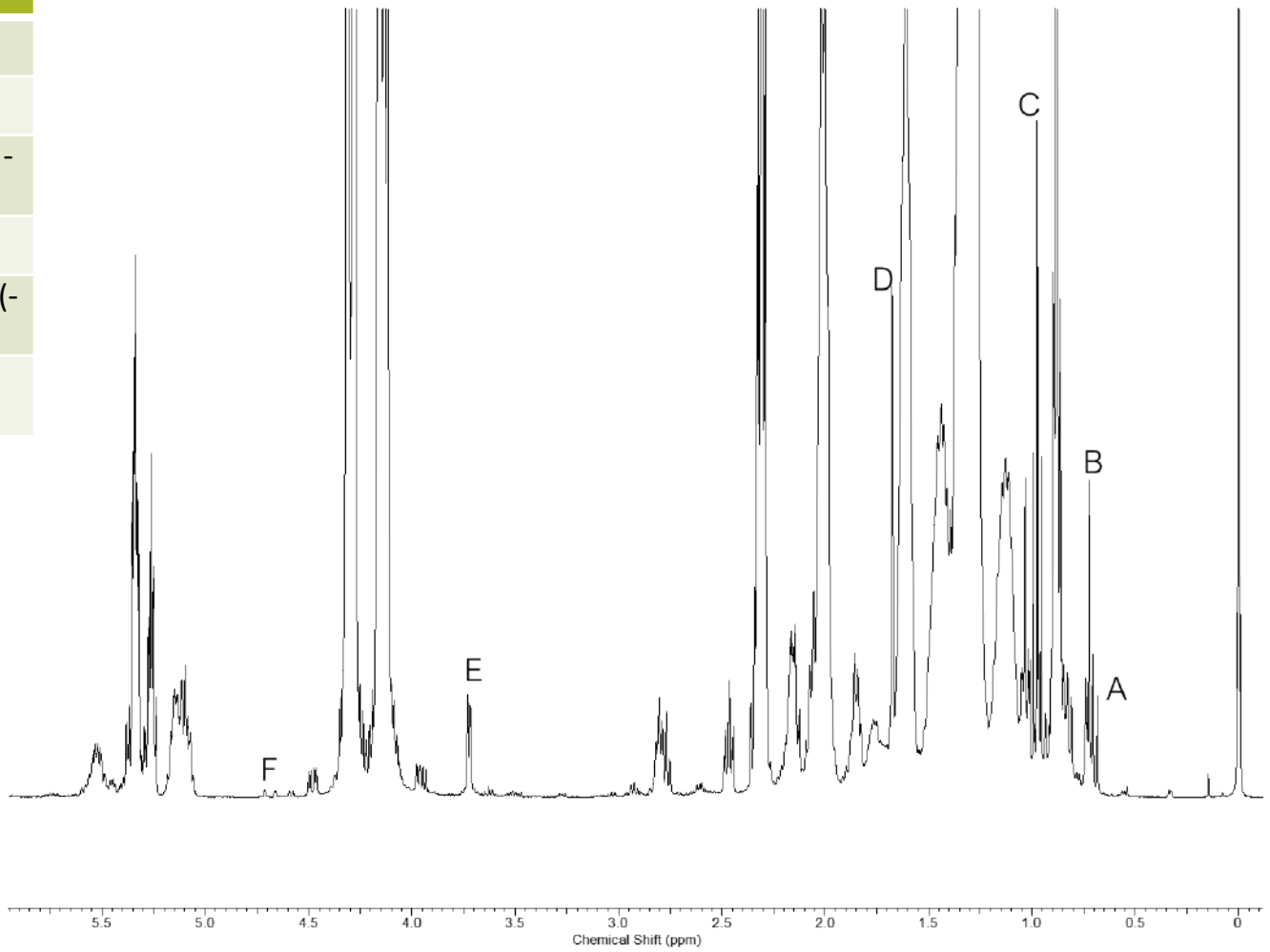
# $^1$H NMR spectra

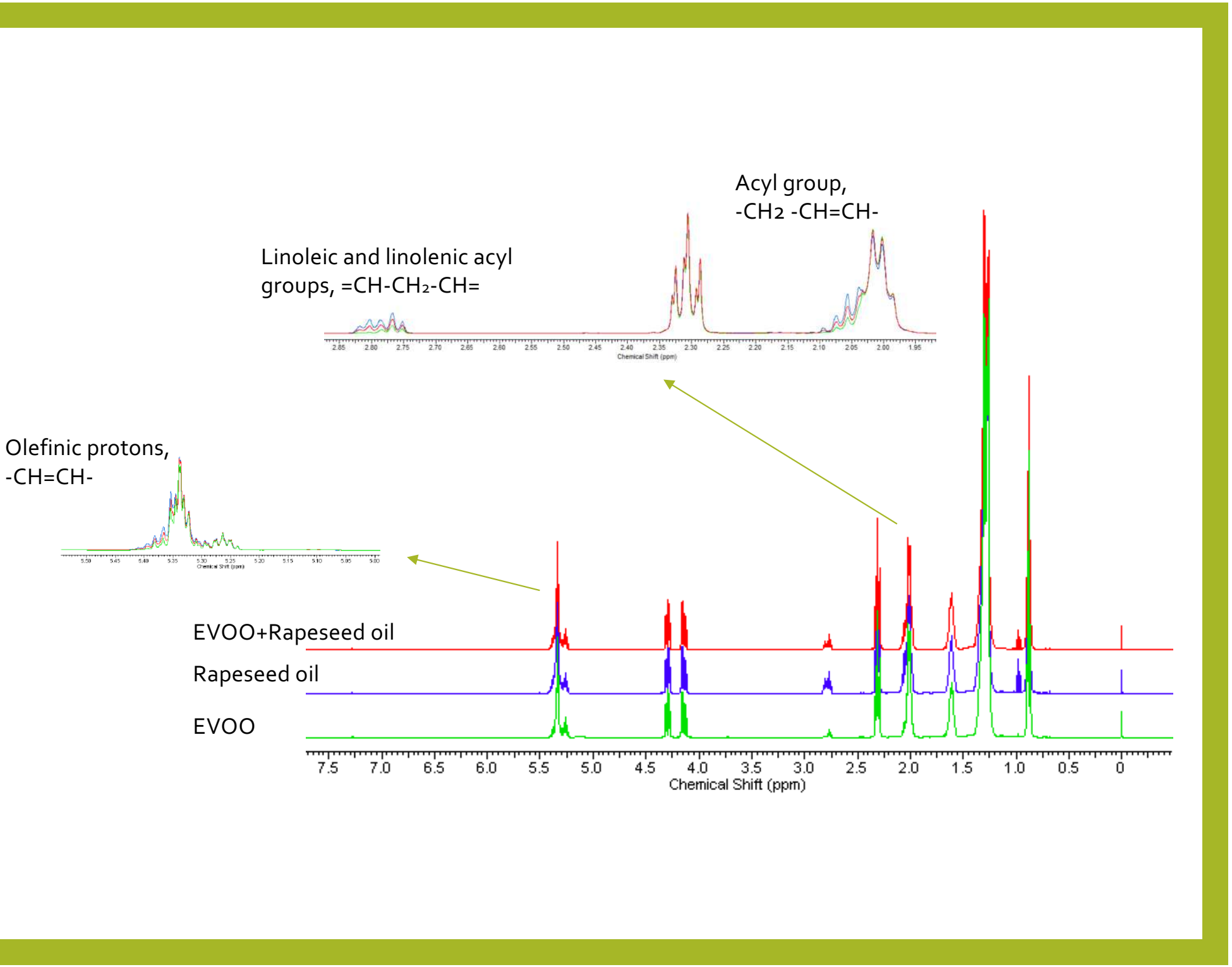
| | Peak (ppm) | Assignment |
|---|---|---|
| **A** | 0.00 ppm | TMS |
| **B** | 0.83 – 0.93 | Methylic protons (-C**H**$_3$) |
| **C** | 1.20 – 1.40 | Methylenic protons (-C**H**$_2$-) |
| **D** | 1.53 – 1.70 | H-3 protons of acyl moietis (-C**H**$_2$-CH$_2$-COOH) |
| **E** | 1.90 – 2.10 | Allylic methylenic protons (-C**H**$_2$-CH=CH-), all UFA |
| **F** | 2.25 – 2.35 | H-2 protons of acyl moietis (-C**H**$_2$-COOH) |
| **G** | 2.75 – 2.80 | Bis-allylic methylenic protons (=CH-C**H**$_2$-CH=) |
| **H** | 4.10 – 4.35 | Methylenic protons (α e α') of the glycerol unit of TAGs (-C**H**$_2$-OCOR) |
| **I** | 5.30 | Methine proton of the glycerol unit of TAGs (-C**H**-OCOR) |
| **J** | 5.35 | Olefinic protons (-C**H**=C**H**-), all UFA |



# $^1$H-NOESY NMR spectra

| | Peak (ppm) | Assignment |
|---|---|---|
| **A** | 0.68 | Sitosterol (18-C**H**$_3$) |
| **B** | 0.69 | Stigmasterol (-C**H**$_3$) |
| **C** | 0.97 | Linolenic acid (acyl -C**H**$_3$) |
| **D** | 1.66 | Squalene (-C**H**$_3$) |
| **E** | 3.69 – 3.73 | sn 1,2 diglycerides (-C**H**$_2$-OH) |
| **F** | 4.56 – 4.90 | Terpenes |



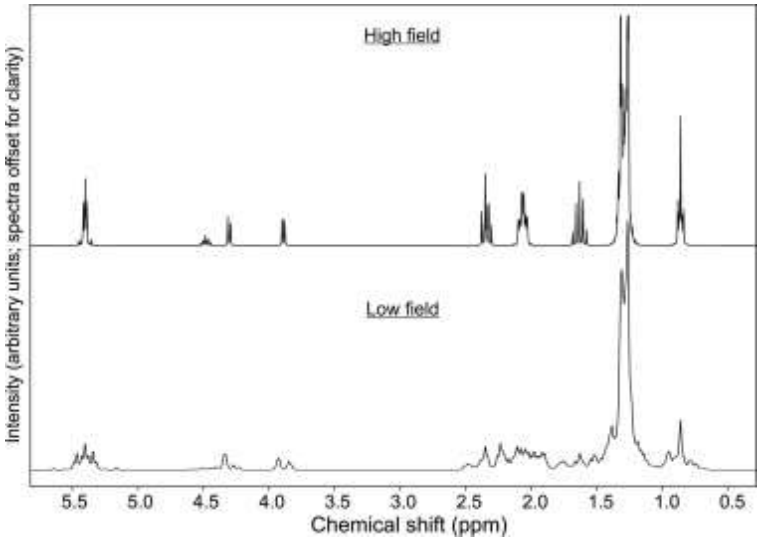

Acyl group,
-CH2 -CH=CH-
Linoleic and linolenic acyl groups, =CH-CH2-CH=
Olefinic protons,
-CH=CH-
EVOO+Rapeseed oil
Rapeseed oil
EVOO
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0
Chemical Shift (ppm)

# 1. Adulteration of olive oil

| | | | | |
|---|---|---|---|---|
| Mostly TARGETED | • $^{1}H$ NMR/400, 500, 600, 60, 43.62, 22.5, 19.91 MHz Diffusion experiment 300 MHz<br>• $^{13}C$ NMR/100.6 MHz<br>• $^{31}P$ NMR/202.2 MHz<br>• $^{19}F$ NMR/470 MHz | • Botanical origin discrimination<br>• Blends of different oils (e.g. sunflower, hazelnut) | Quantification and comparison of fatty acids in different ways | Multivariate statistical analysis, mostly PCA and ANOVA |
| UNTARGETED | $^{1}H$ NMR/400, 500 MHz | • Botanical origin discrimination<br>• Blends of different oils | Fingerprint divided in buckets | Various supervised statistical analysis, mostly PCA and PLS-DA |

# A promising method for industries: low-field NMR

The adoption of a low-field NMR spectrometer to detect authenticity is a promising perspective.




Triolein, acquired with 300 MHz and 60 MHz

Ratio olefinic to glyceride integrated peak

Reasonable screening tool, with an LOD of hazelnut oil in olive oil of ~13%w/w

Chemometric analysis, PLS-R

Determine the level of olive oils and hazelnut oils in binary mixtures to ±11.2%w/w at 95% confidence

Parker et al., *TrAC* **57** (2014), 147-158

# 2. Cultivar discrimination

| | | | | |
|---|---|---|---|---|
| TARGETED | • $^{1}H$ NMR/400, 500, 600, 700 MHz<br>• $^{13}C$ NMR/400, 150.9 MHz<br>• $^{31}P$ NMR/202.2 MHz | Discrimination among different cultivars | Determination of selected signals or fatty acids | Various supervised statistical analysis, mostly PCA and ANOVA |
| UNTARGETED | $^{1}H$ NMR/400, 500, 600, 700 MHz | Discrimination among different cultivars | Fingerprint divided in buckets | Various supervised statistical analysis, mostly PCA and PLS-DA |

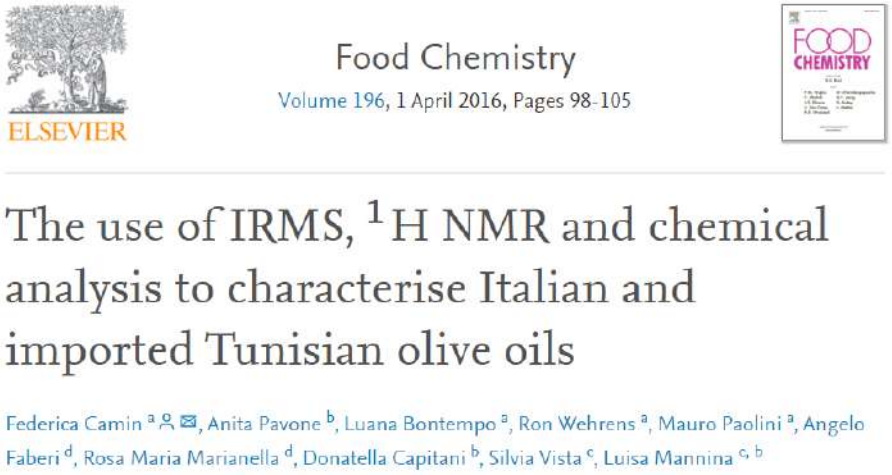
# 3. Geographical discrimination

| | | | | |
|---|---|---|---|---|
| TARGETED | • $^{1}H$ NMR/400, 500, 600, 700 MHz<br>• $^{13}C$ NMR/250 MHz | Discrimination at regional and national level | Determination of selected signals | Various supervised statistical analysis, mostly PCA and ANOVA |
| UNTARGETED | • $^{1}H$ NMR/400, 500, 600, 700 MHz<br>• $^{13}C$ NMR/250 MHz | Discrimination at PDO, regional and national level | Fingerprint divided in buckets | Various supervised statistical analysis, mostly PCA and PLS-DA |

# A promising approach

A promising approach seems to be the ***COMBINATION*** of two different techniques:

**Isotopes + NMR**



ELSEVIER

Food Chemistry

Volume 196, 1 April 2016, Pages 98-105

The use of IRMS, $^{1}$H NMR and chemical analysis to characterise Italian and imported Tunisian olive oils

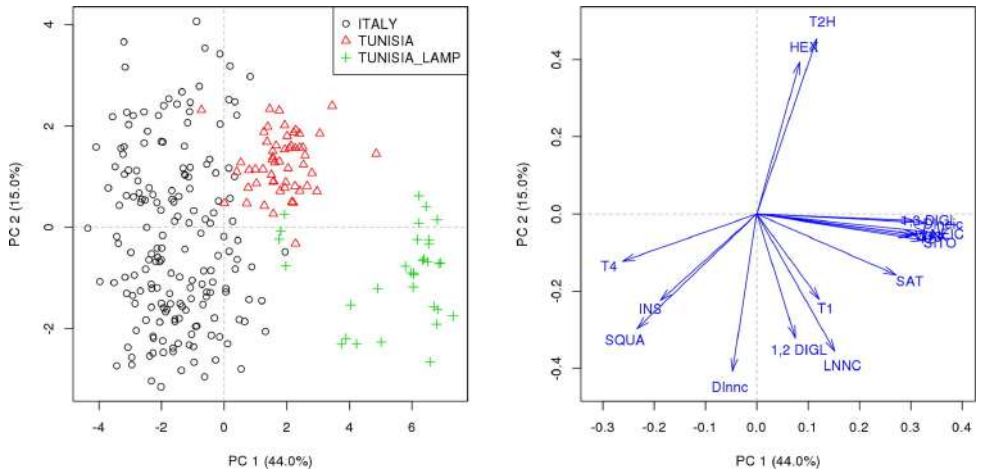
Federica Camin [a], Anita Pavone [b], Luana Bontempo [a], Ron Wehrens [a], Mauro Paolini [a], Angelo Faberi [d], Rosa Maria Marianella [d], Donatella Capitani [b], Silvia Vista [c], Luisa Mannina [c, b]

**$^{13}C/^{12}C$, $^{2}H/^{1}H$ and $^{18}O/^{16}O$** for isotopes

**17 selected signals** for NMR

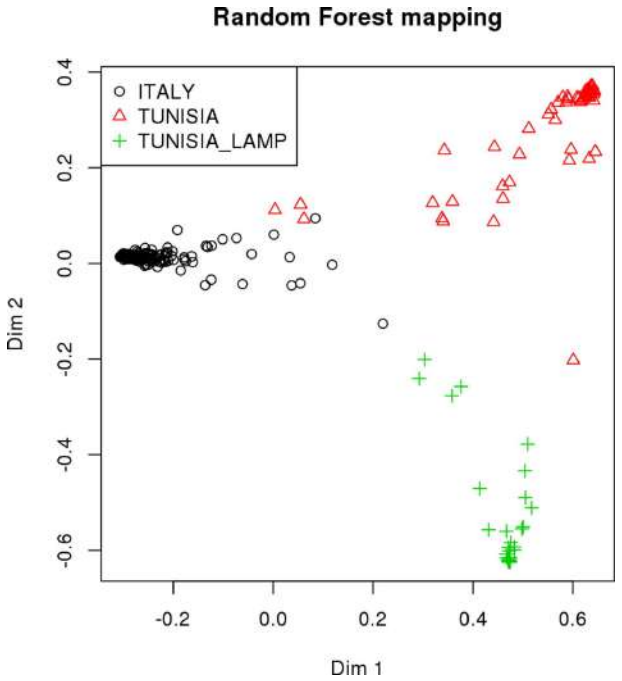
↓

Random Forest statistical analysis




PCA performed on the intesity of selected NMR variables

**98.5 % of correct classification within 3 groups and 100 % distinction between Italian and Tunisian lampante oils**




Graphic representation of multivariate RF

Camin et al., *Food Chemistry* **196** (2016), 98 - 105

# Conclusions

- NMR spectroscopy is a helpful technique for the analysis of oils
- It is highly reproducible, it has easy preparative steps and with just a single analysis the fingerprint of the sample and the quantification of the characteristic compounds can be obtained
- Used in combination with other techniques the performances are extremely high

- It has not been recognized by official authorities yet

# THANK YOU FOR YOUR KIND ATTENTION

*Acknowledgments:*

*The authors acknowledge the financial support provided by Program of the Autonomous Province of Trento (Italy) with EU co-financing (Fruitomics), Grant/Award Number: FESR 2014-2020*

Lab group: Luana Bontempo, Pavel Solovyev, Federica Camin