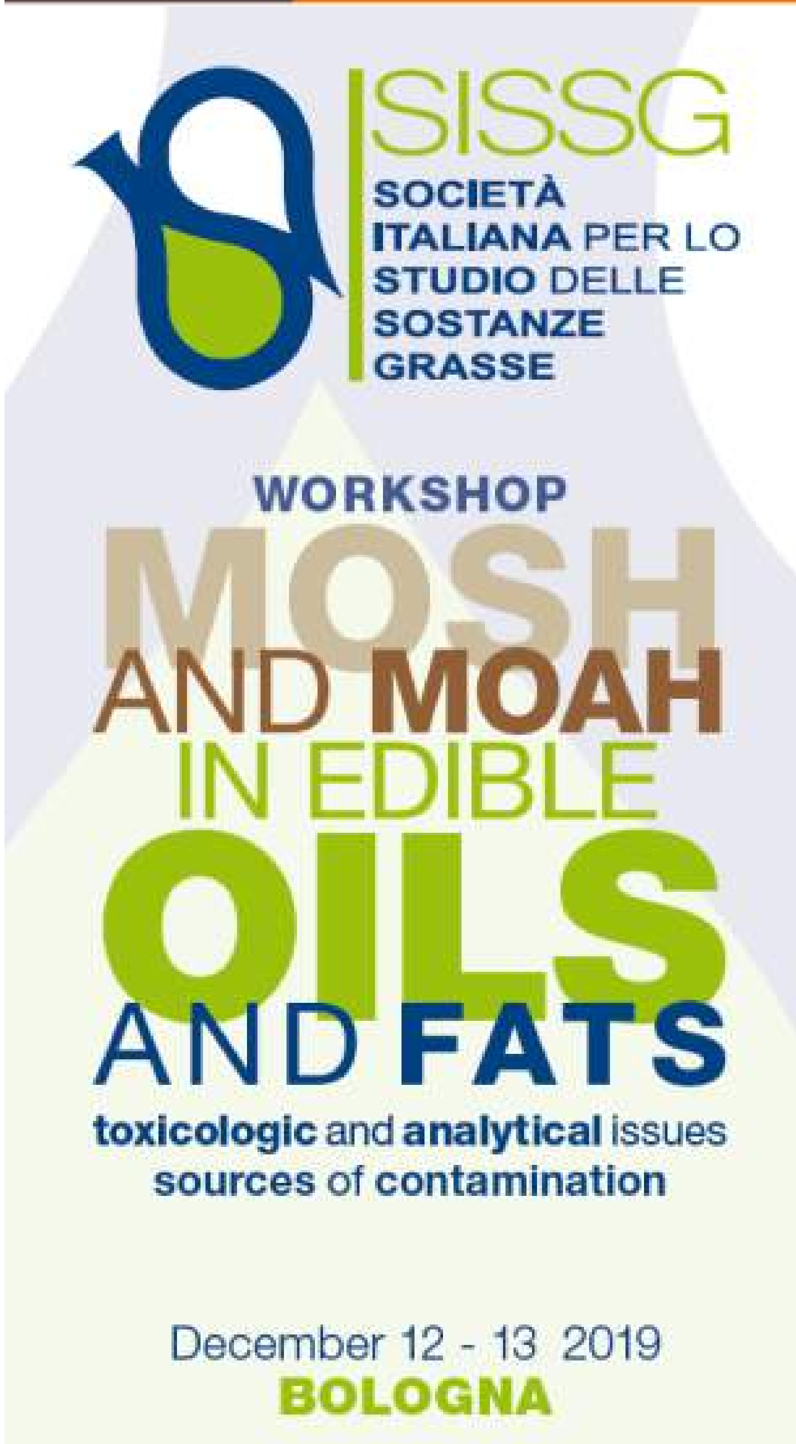

The experience of the Laboratories

Uniud lab

UNIVERSITY OF UDINE

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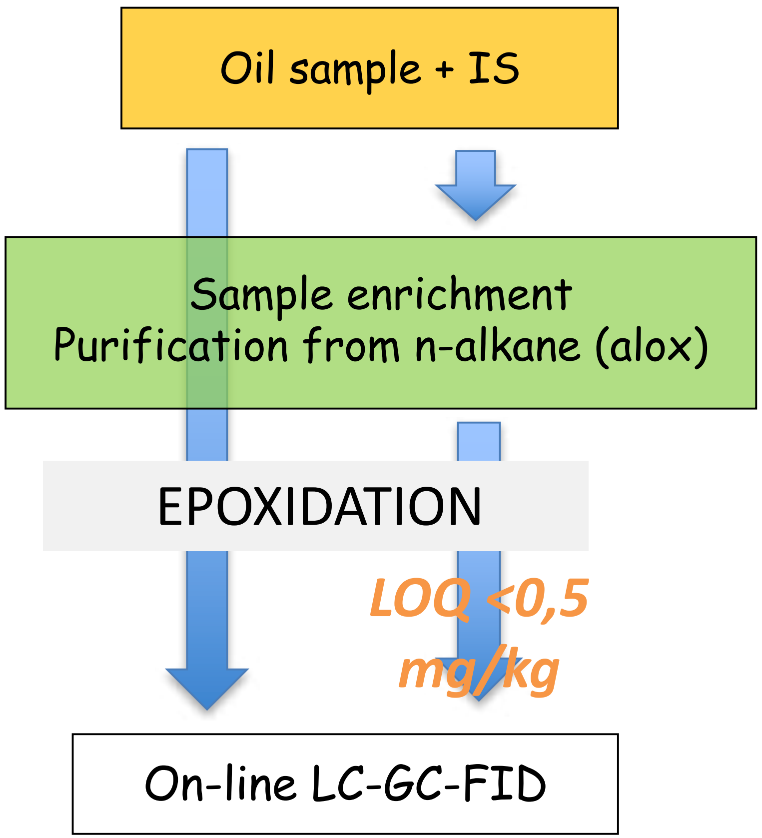
MINERAL OIL IN VEGETABLE OILS: WHAT SAMPLE PREPARATION?



- Purification from interferences (*n*-alkanes, olefins…)
  not always possible (i.e. POSH, PAO)
- Sample enrichment

<u>Positive impact</u> on method performance:

→ *improved accuracy*
→ *higher sensitivity*

To avoid <u>negative impact</u> on method performance it is important to:

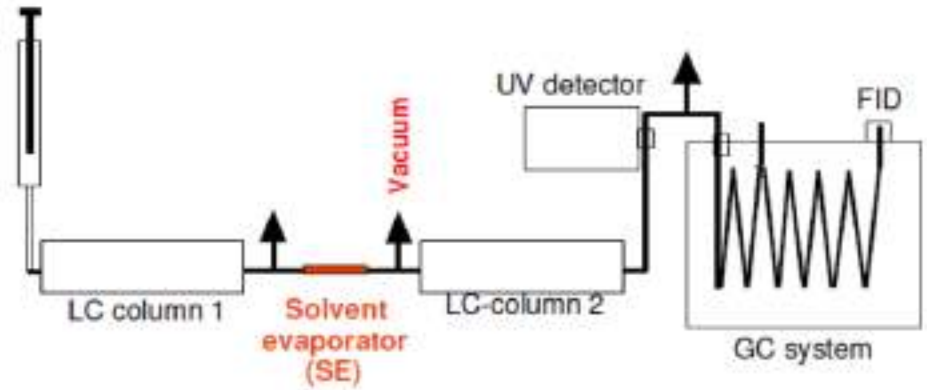
- ✓ Check for solvent purity
- ✓ Use inert and clean material
- ✓ limit solvent consumption
- ✓ limit sample manipulation

→ **BLANK ANALYSIS!**

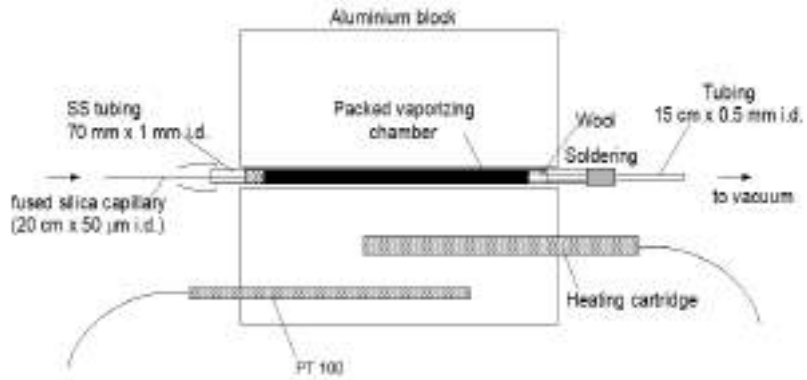
On-line LC-LC-GC for MOAH fractionation

Before 2009, mineral oil contamination was mainly investigated by analyzing the saturated fraction (MOSH). No methods for routine analysis of MOAH were available.

Our experience with mineral oil determination began in 1995, when, in collaboration with the Cantonal Laboratory of Zürich, we developed a LC-LC-GC method for analysing alkylated polycyclic aromatic hydrocarbons (MOAH) in fats and fat extracts contaminated with mineral oil.



Sabrina Moret, K. Grob, and L.S. Conte, J. Chromatogr. 750 (1996) 361-368



- The *first silica LC column retained the triglycerides, while the MOSH and the MOAH fraction were eluted*
- *The second columun was an amino phase fractioning the aromatics according to their ring number.*

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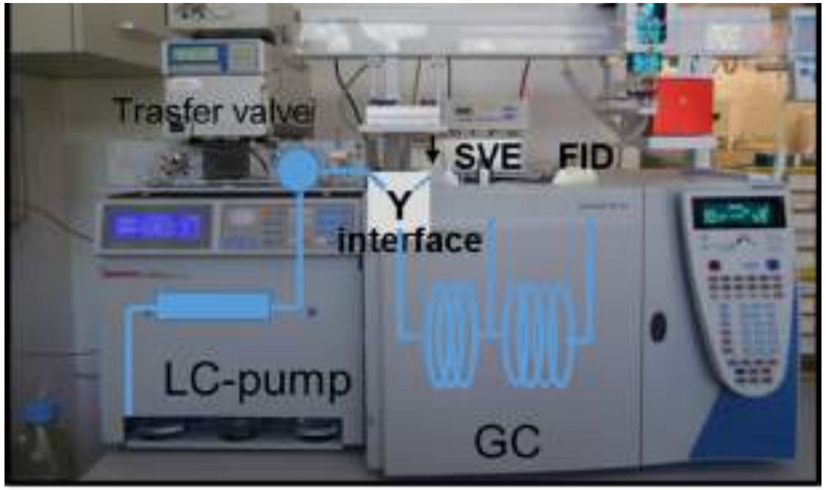
ON-LINE HPLC-GC

First method for routine analysis of MOSH and MOAH was the on-line LC-GC method developed by Biedermann et al., in 2009 (J. Agric Food Chem, 2009, 57, 8711-8721)



Barp L, Purcaro G, Moret S, Conte LS. J. Sep Sci., 2013, 36(18), 3135-9.

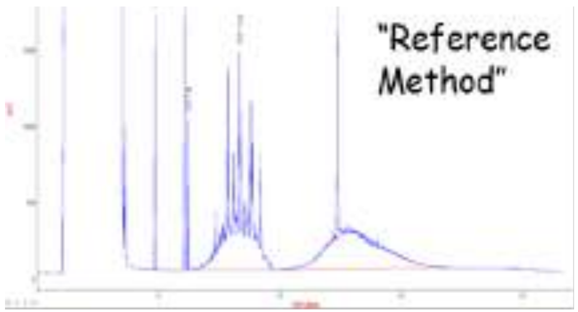
UNIUD METHOD

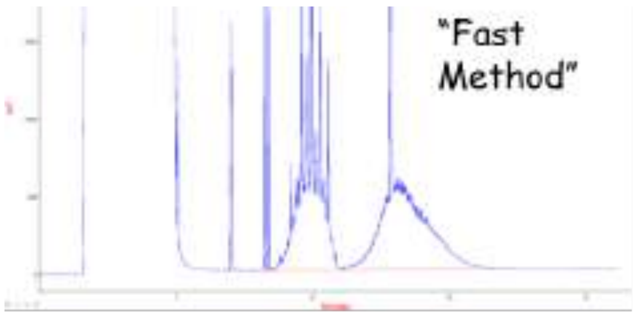
A high sample throughput LC–GC method for mineral oil determination

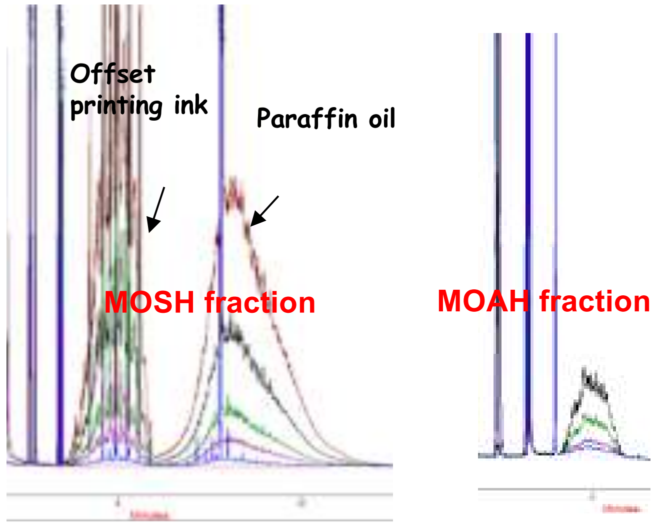
With respect to the reference method :

- rapid gradient of oven T (50 °C/min)
- Shortened reconditioning after backflush

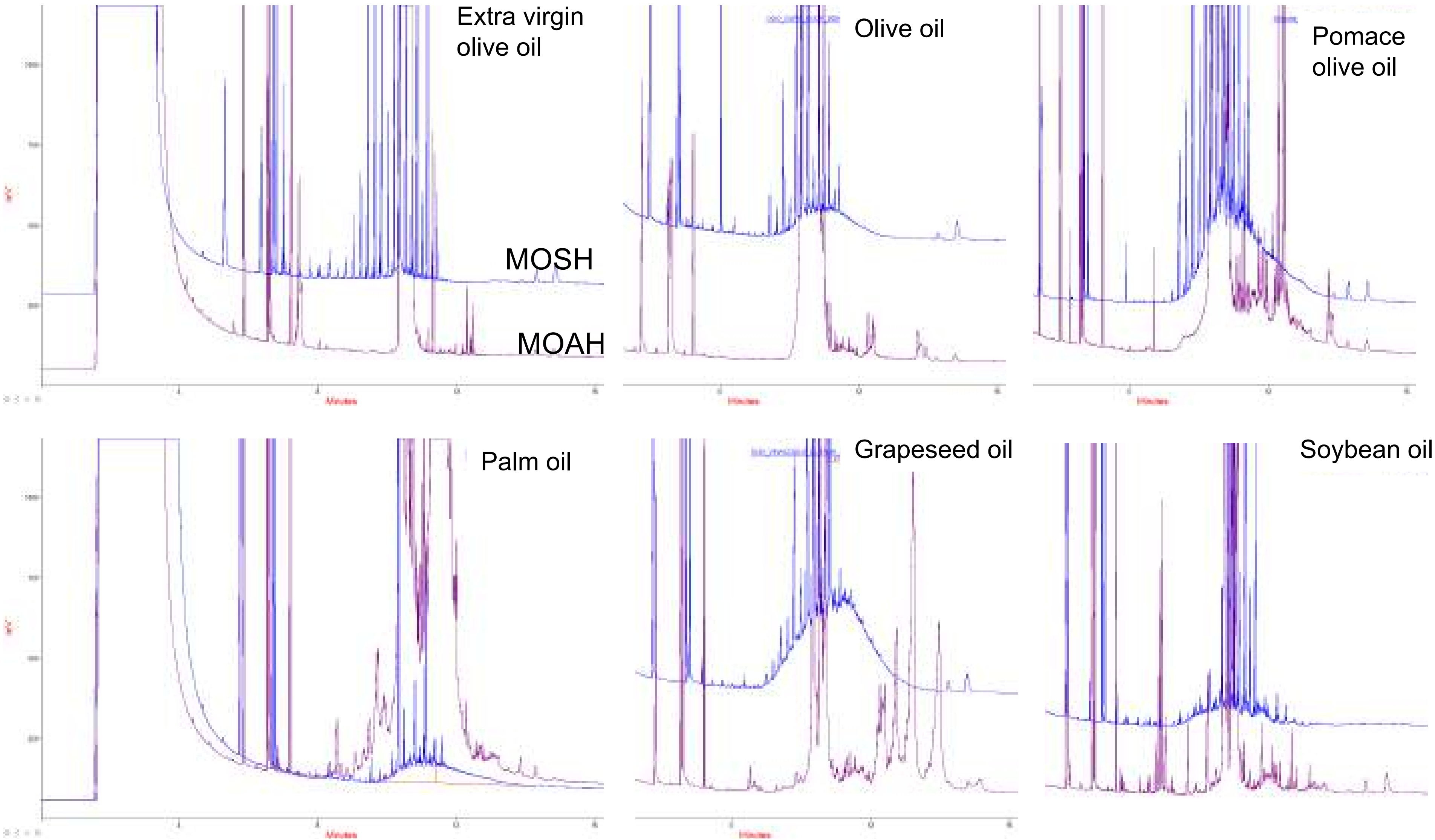
→ shorter analysis time (62 runs per day)
→ lower solvent consumption
→ Increased sensitivity







LC-GC traces of MOSH and MOAH fractions (direct analysis) of different vegetable oils



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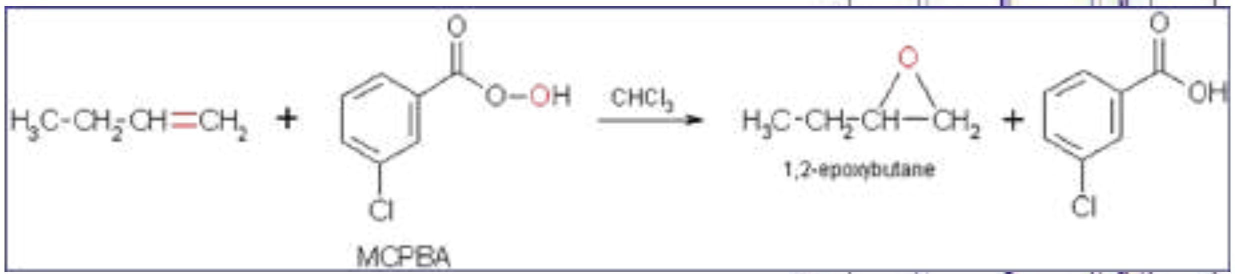
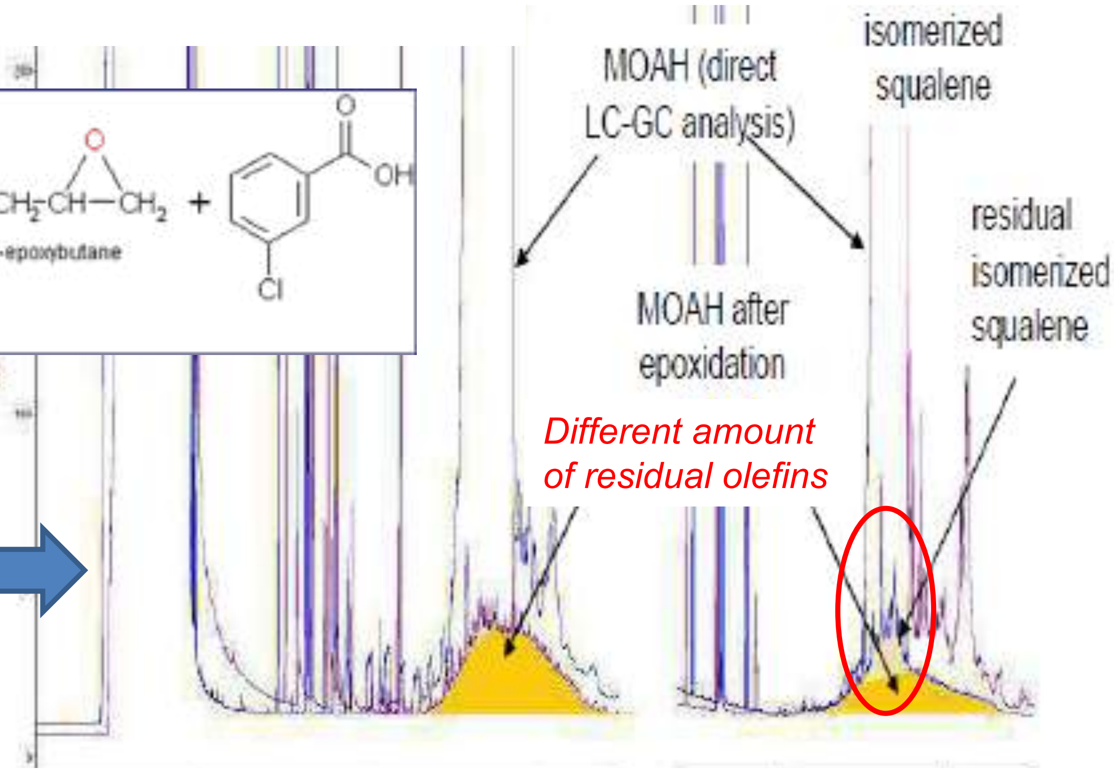
➢ **Removal of interfering olefins** (mandatory for most oils) is currently obtained by **epoxidation**





By applying the epoxidation to a number of different pomace olive oil samples under the same conditions, a ***sample-dependent behaviour*** *was observed → <u>poor robustness</u>*

Two different epoxidation protocols using different reaction solvents and conditions have been published to date:

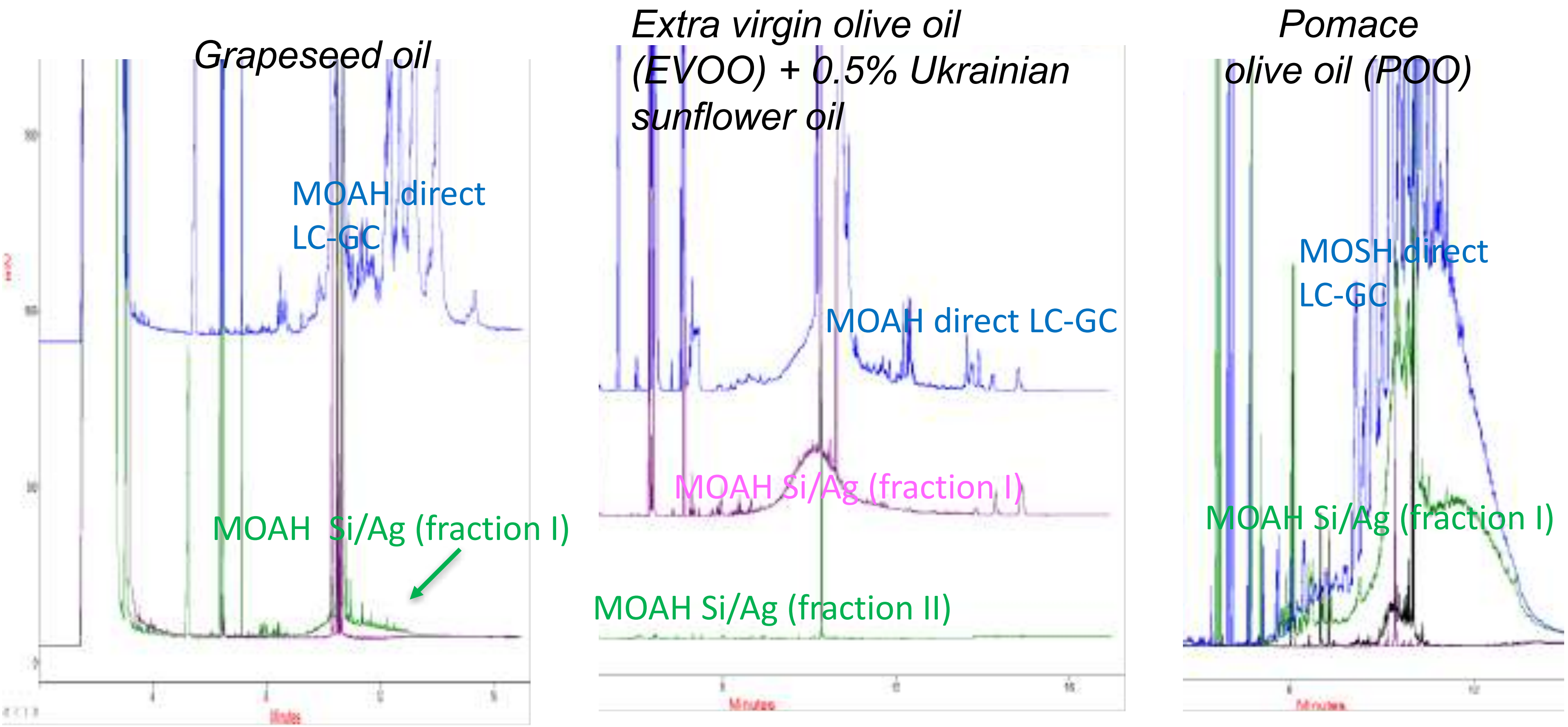
1. *Biermann, Fiselier and Grob, J. Agric. Food Chem, 2009, 57, 8711*
2. *Nestola and Schmidt, J. Chromatogr. A, 2017, 1505, 69*

Modified protocols are probably used in different laboratories

No published data on method performance (recovery, repeatability, reproducibility and method robustness); no data on comparison among different protocols.

Si/Ag as an alternative to epoxidation?

SPE on 1 g silver silica (10%) *(Moret et al., J. Chromatogr. A, 2012, 1)* was investigated as an alternative to epoxidation to eliminate/ reduce interference by olefins before on-line LC-GC.



Depending on the oil type, interfering olefins were removed completely (EVOO) or partially (POO)

Automated SPE for sample enrichment and purification

SPE on 1g Si(Ag+) → LOQ around 2-5 ppm.

To reach higher sensitivity we explored the potential of automated SPE (5 g of sorbent)

A SpeedExtractor equipped with 10 mL cells was employed and optimal conditions were found for sample enrichment and purification from interfering olefins.

Automated SPE for sample enrichment and removal of interfering olefins

CELL PREPARATION
<u>10 mL cell filled with:</u>
mixed beds
(silica gel - silver silica gel)

↓

CONDITIONING
with hexane

↓

SAMPLE LOADING
0.25-0.40 g of oil sample + IS
(in 1 mL of hexane)

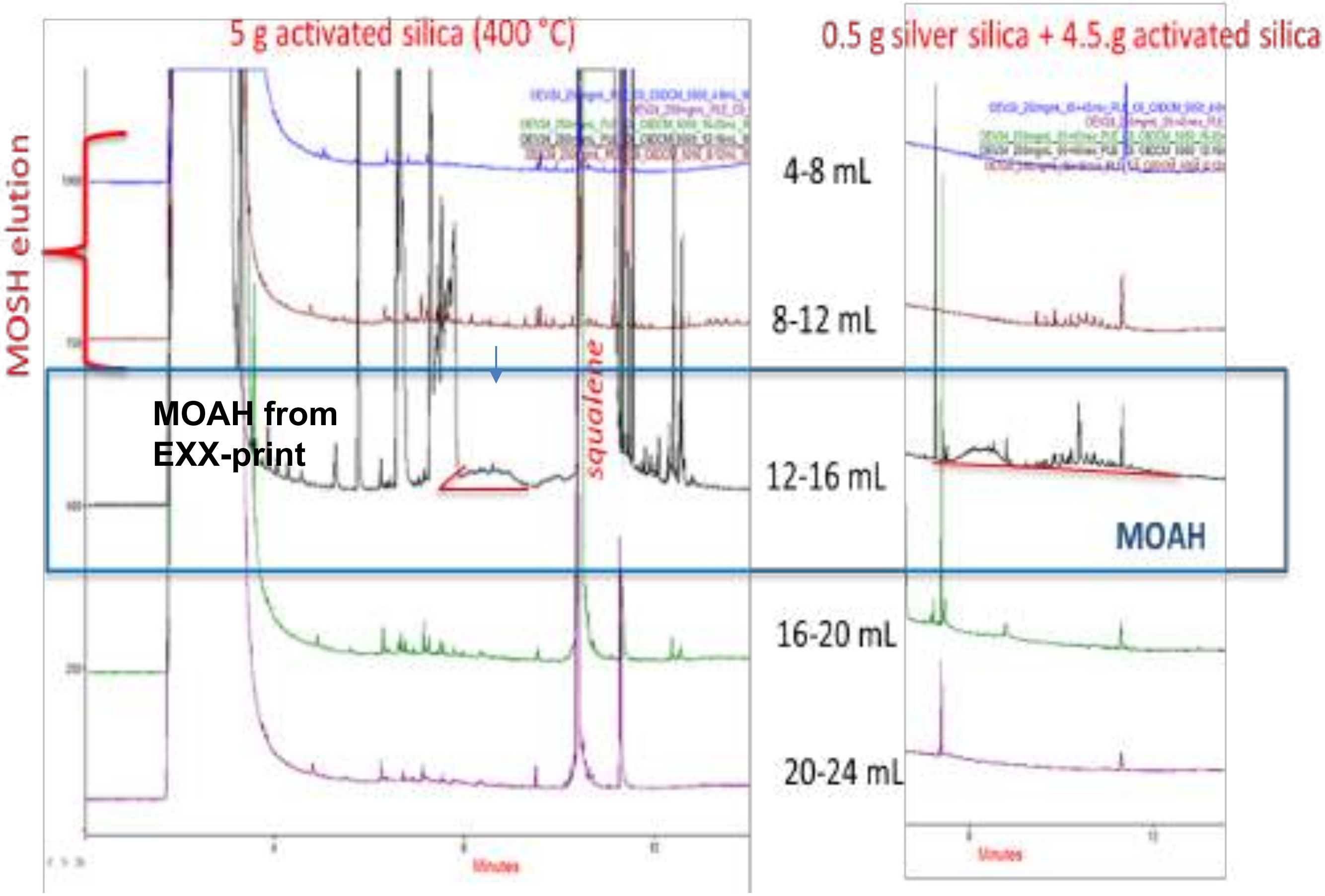
→

ELUTION with hex/CH2Cl2 1:1
using the function "WASH"
2 mL/min

↑

CONCENTRATION and
INJECTION into the LC-GC

Example of sample enrichment/purification by interfering olefins

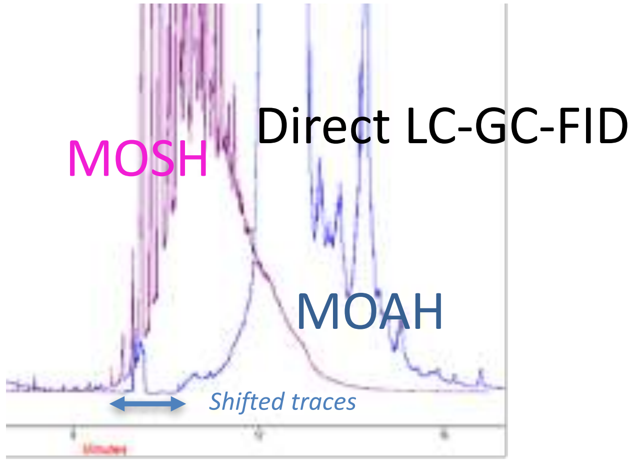


Silver silica completely retains squalene. Satisfactory results also with 0.25 g of silver silica. No differences between using mixed or stratified sorbents in the cell.

LC-GC-FID traces of extra virgin olive oil (250 mg) fortified with Exx-print (9% of MOAH) after fractionation on 5 g of activated silica and silver silica/active silica (conditioning with Hex; elution with Hex/DCM (1:1)

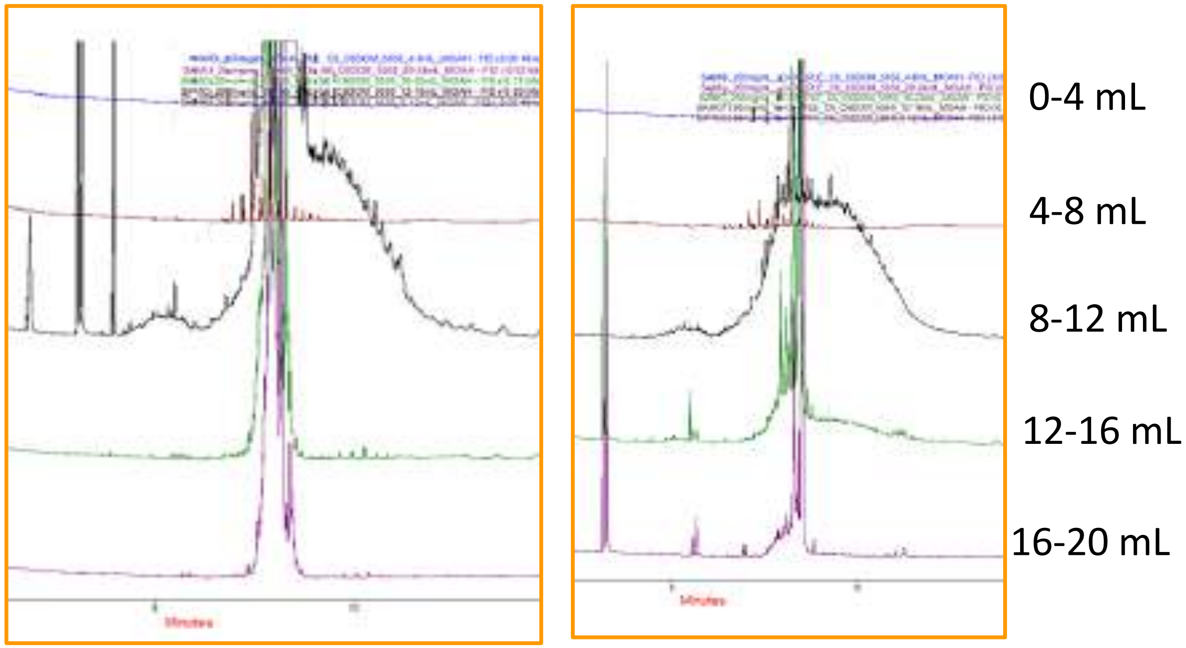
Sample:
olive pomace oil (250 mg)

Conditioning: Hex
Eluent: Hex/DCM 50:50



0.5 g Si-Ag + 4.5 g activated Si

1.5 g Si-Ag + 3.5 g activated Si



LC-GC traces after fractionating of on two-phase beds with different amounts of silver silica.

Automated SPE for sample enrichment and removal of endogenous *n*-alkanes

CELL PREPARATION
<u>10 mL cell filled with:</u>
- 10 g of activated alox (500 °C)
- Mixed bed (5 g alox + 2,5 g silica)

↓

NO CONDITIONING

SAMPLE LOADING
0.25-0.40 g of oil sample + IS
(in 1 mL of hexane)

↓

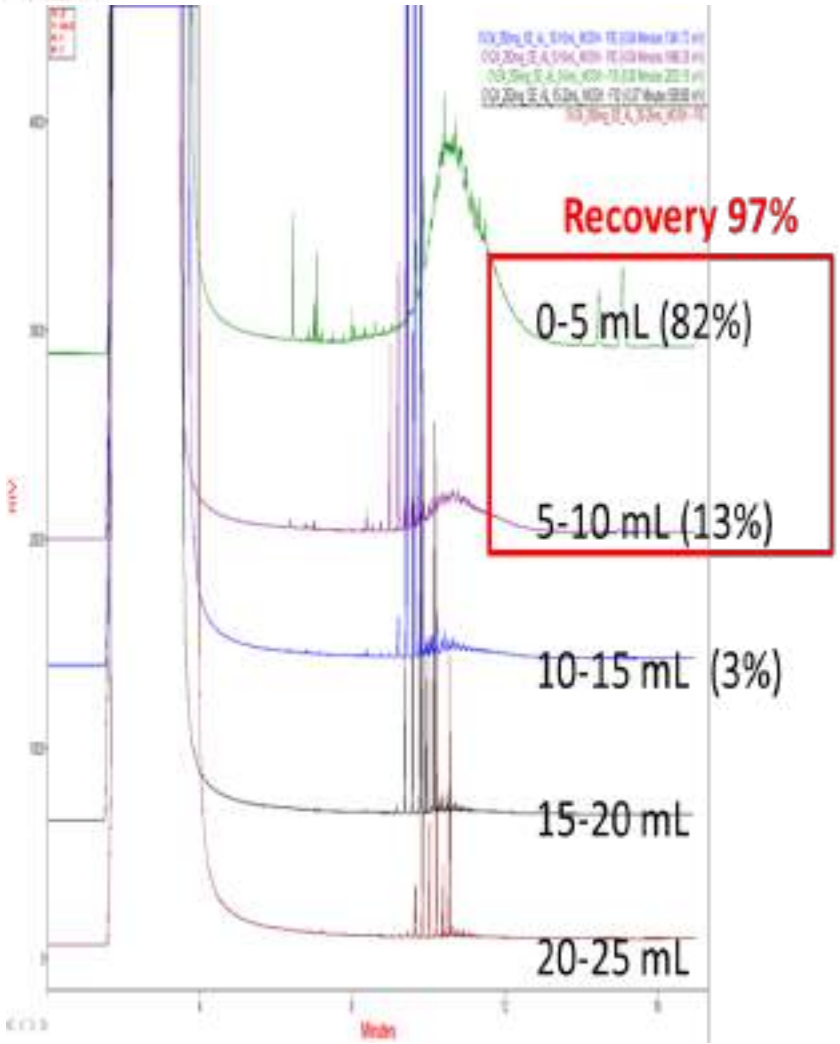
ELUTION with hexane
using the function “WASH”
2 mL/min

→

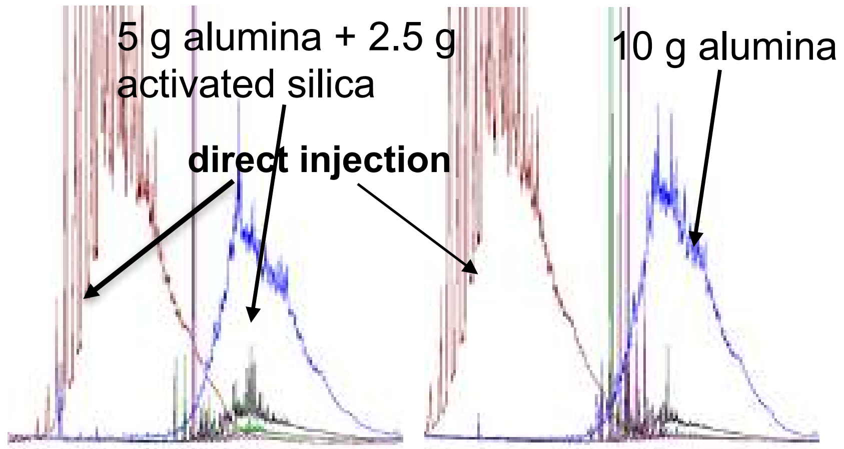
CONCENTRATION and INJECTION into the LC-GC

SpeedExtractor cells used for SPE

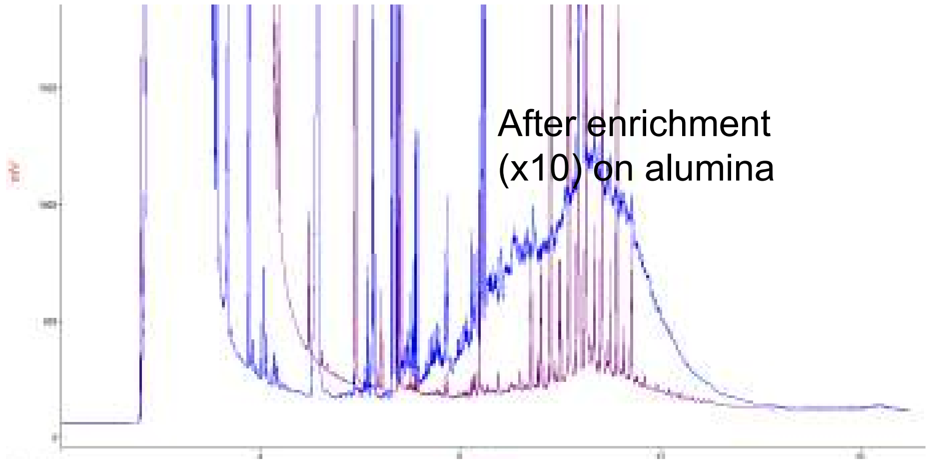
Automated SPE for *n*-alkane removal and sample enrichment



LC-GC-FID traces of an extra vergin olive oil (250 mg) fortified with paraffin oil (200 ppm), after fractionation on alumina (10 g)



LC-GC traces of the MOSH fraction of an olive pomace oil fractionated on alumina and alumina/ activated silica



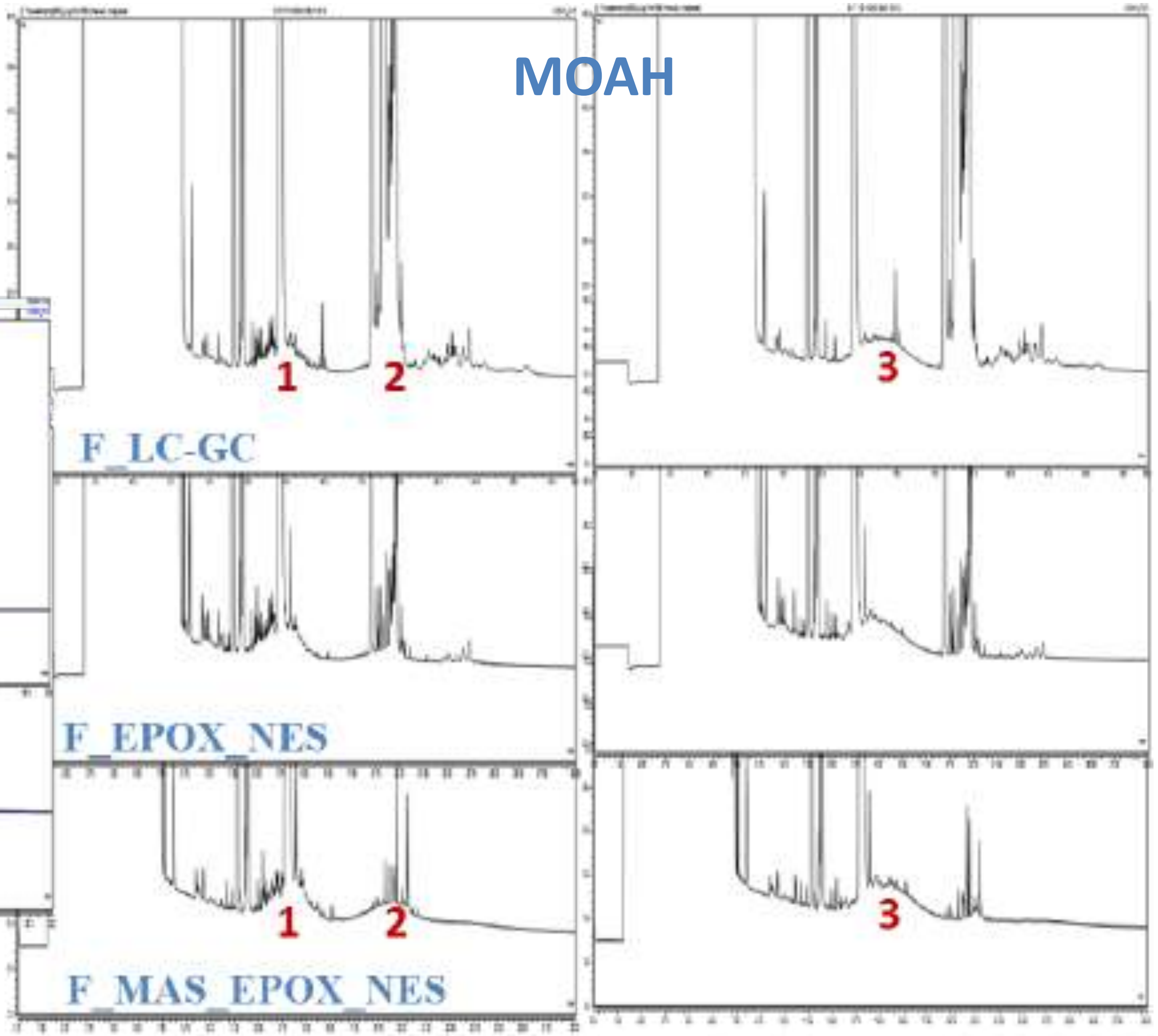
LC-GC traces of the MOSH fraction of an olive oil (contaminated with 11 mg/kg of MOSH) before (violet) and after (blu) enrichment on 10 g of allumina and reconcentration

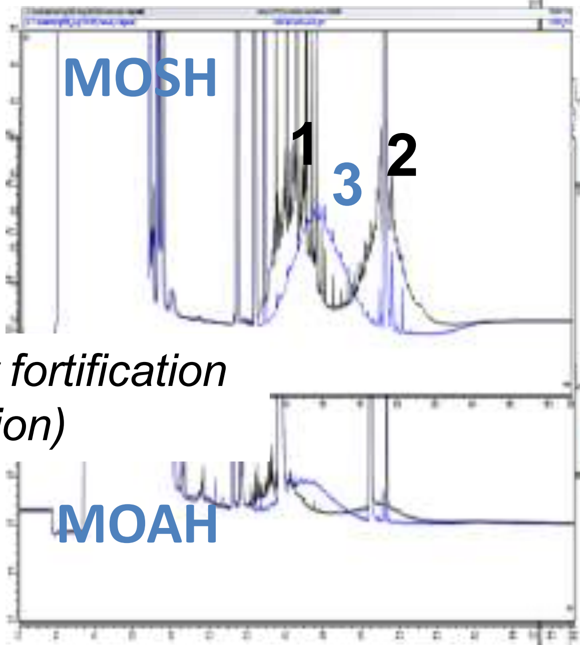
MAS followed by epoxidation prior to on-line LC-GC

MAS → EPOX → On-line HPLC-GC

MOAH traces of a sunflower oil added wih 3 different mineral oils (1= Exx-print, 2= motor oil, 3= Gravex, each at about 2-5 ppm of MOAH), injected directly, after epoxidation, and after epoxidation following MAS.

Fortified sunflower oil





MO used for fortification (direct injection)

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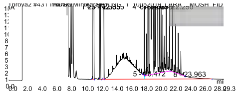
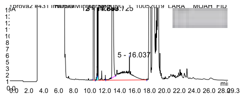
Sunflower oil Fortified with gravex

MOSH

MOAH

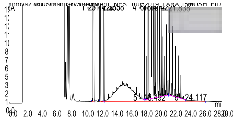


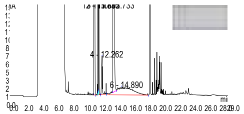


gravex

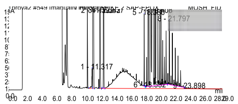
On-line LC-GC (direct)

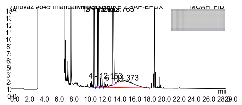
gravex





Epox - on-line LC-GC





MAS - Epox - on-line LC-GC

MAS combined with automated SPE for off-line MOH determination without epoxidation

MARS, *Microwave Accelerated Reaction System*

SpeedExtractor for automated SPE

MAS

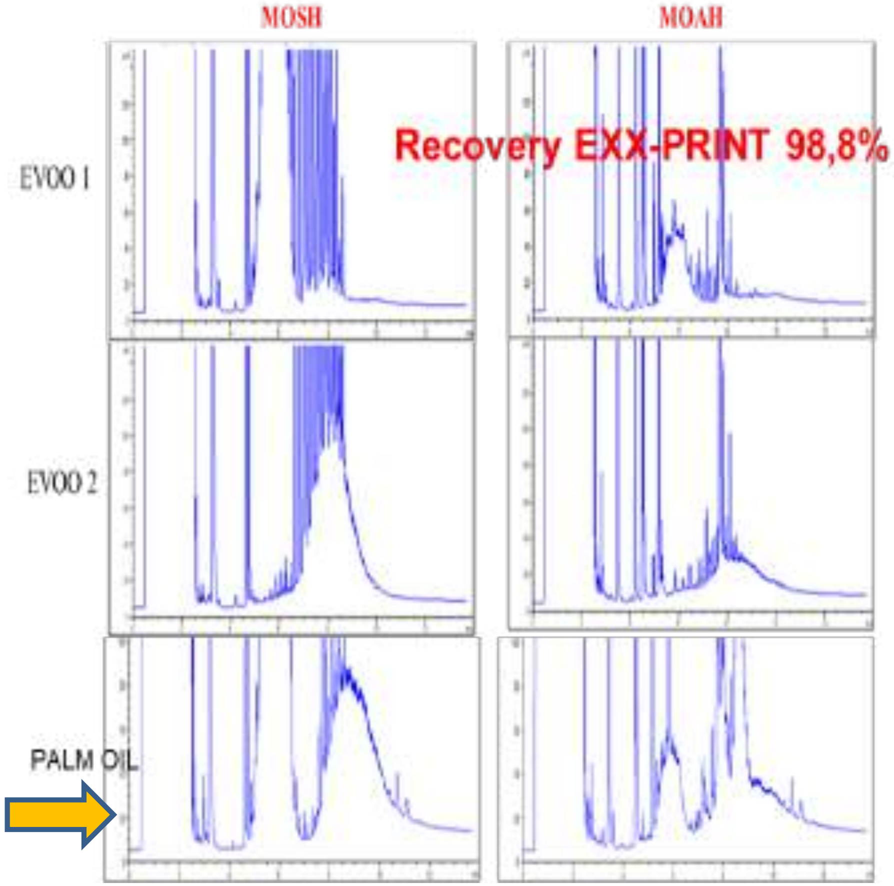
↓

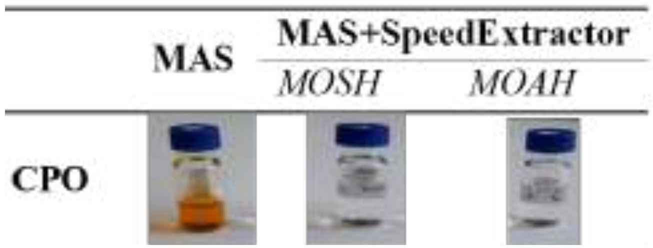
Automated SPE

Load hexane extract (concentrated to 1 mL) on a mixed bed (1 g SiAg+ 4 g SiAct), previously conditioned with hexane

Elution with hexane/dichloromethane 1:1
MOSH & MOAH collection
concentration to 200 uL – 1 mL

off-line GC-FID *(injection* 50 µL)





Some occurrence data

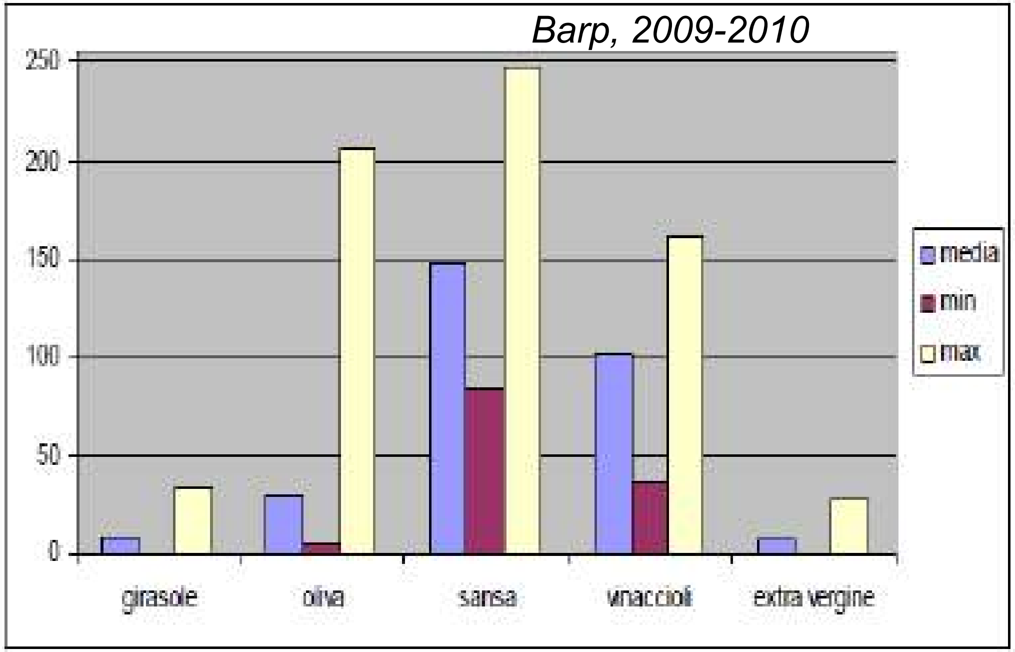
Vegetable oils from the market (2000/2005)

	n. samples	positive samples	Min	Max	Mean
soybean oil	4	2	<LQ	20	8
corn oil	8	5	<LQ	33	10
peanut oil	5	5	3	34	10
sunflower oil	10	10	5	53	12
mixseed oil	6	6	6	40	15
grapeseed oil	10	10	22	40	30
extra virgin olive oil	73	10	<LQ	120	4
olive oil	13	13	6	30	14
olive pomace oil	10	10	115	250	137
other vegetable oils	17	14	<LQ	260	37

Moret, Populin, Conte; Riv. Ital. Sost Grasse, 86, 3-14 2009

Vegetable oil from the market (2009/2010)

- Sunflower oil (13 samples)
- Grapeseed oil (5 samples)
- Olive oil (16 sample)
- Olive pomace oil (7 samples)
-Extra virgin olive oil (12 samples)



Olive oil from the market (2014-2015)

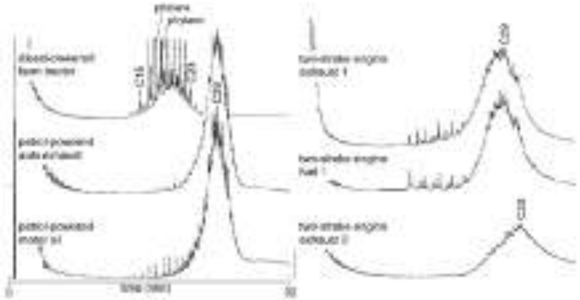
- Extra virgin olive oil (40 samples, mean MOSH 8 mg/kg,; only 2 sample with MOAH above LOQ 2 mg/kg)
- Olive oil (16 samples, mean MOSH 18 mg/kg))
- Olive pomace oil (11 samples, mean MOSH 174 mg/kg))

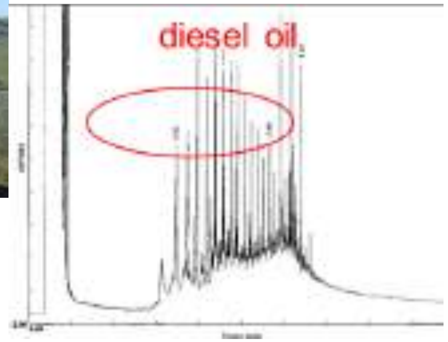
Mineral oils in vegetable oils and fats

Vegetable oils and fats are major contributors to MO dietary intake both directly and indirectly when used as ingredients (cereal based products)

Some potential sources of contamination for vegetable oils

- ➢ Environmental contamination
- ➢ Use of pesticide containing mineral oil products
- ➢ Mechanical harvesting operation
- ➢ Contamination with lubricating oils used in the extraction plant
- ➢ Contact with mineral oils used as heating oils in oil industry
- ➢ Storage and transport (seeds or olives) in jute bags
- ➢ Transport in tank containers previously used to transport mineral oils
- ➢ Contact with plastic material (→ POSH)





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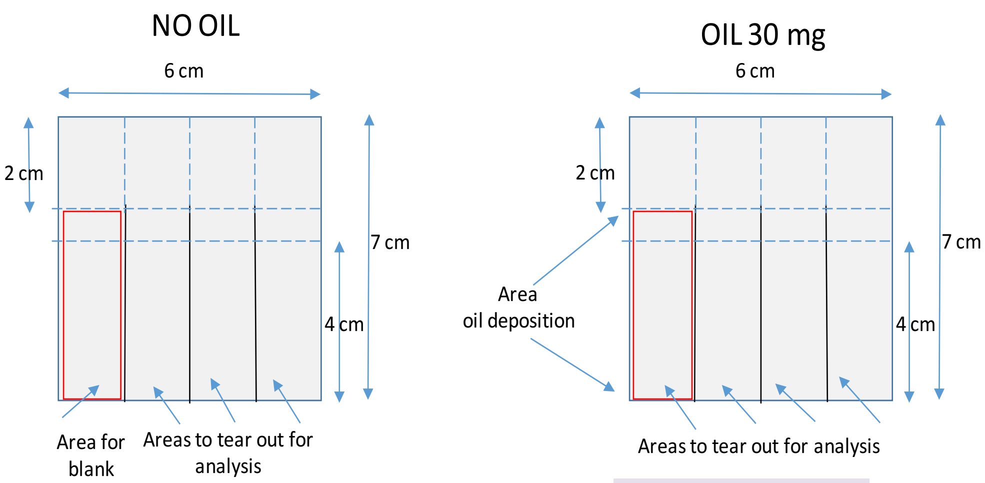
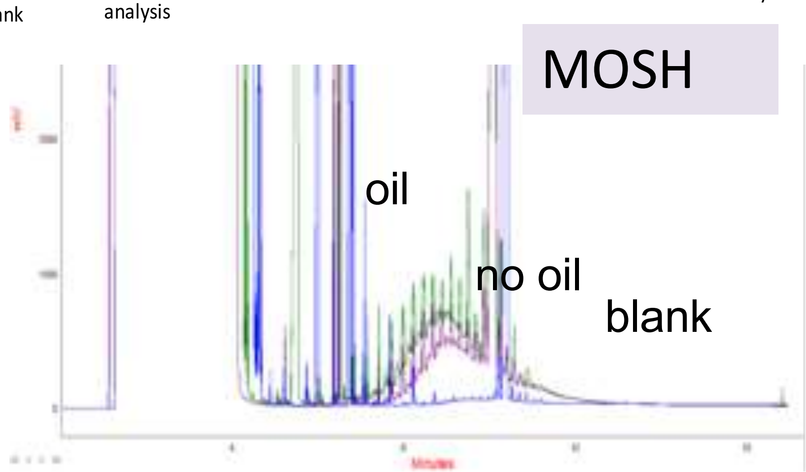
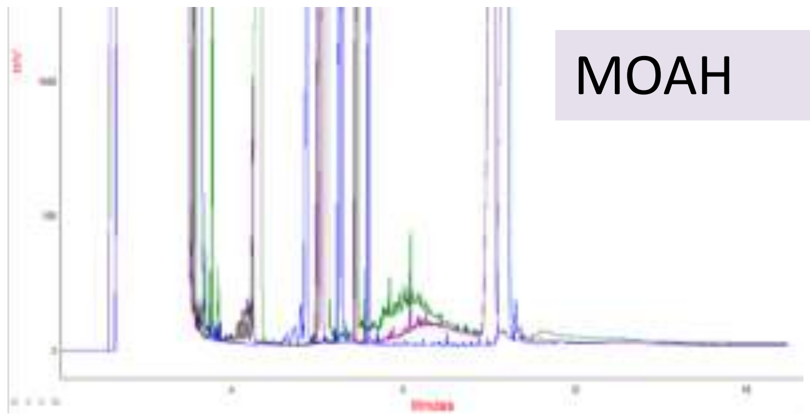
Passive traps to monitor environmental contamination

Passive traps made of paper filters (without oil and with 30 mg oil) exposed in highly trafficked road to determine the air contamination

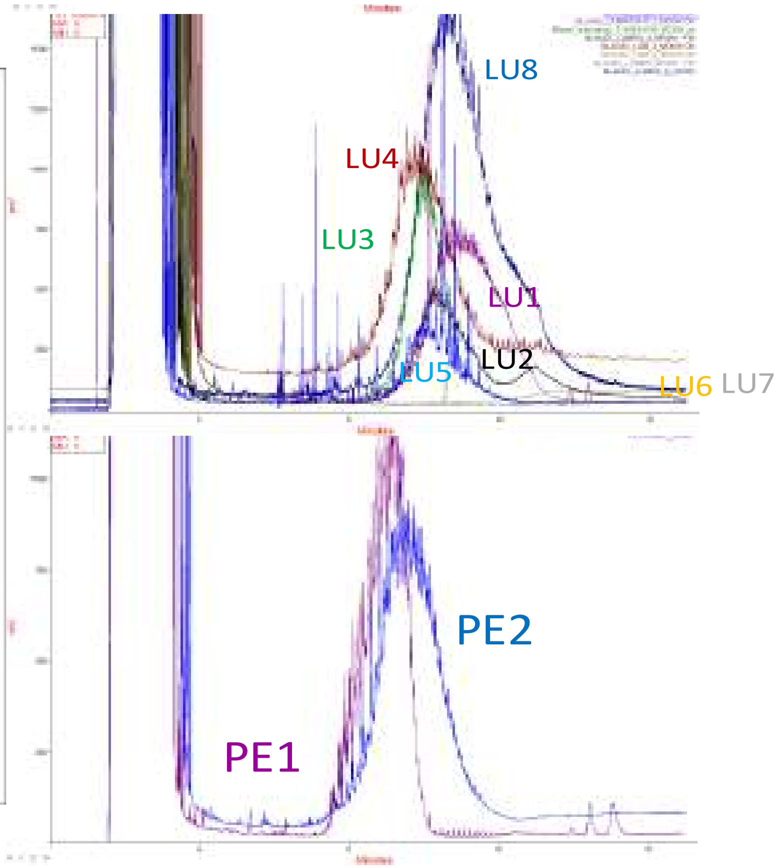






Lubricating and pesticides

	PRODUCT NAME	Code	Description and use	% on total product %MOSH	%MOAH
Lubricants	CYCLON PREMIUM, SAE30	LU1	Monograde lubricant for low performance mach	69,6	23,6
	KRONN, 75W90	LU2	Synthetic lubricant multigrade, extreme pressure; some use it as chainsaw lubricant	70,4	27,5
	SAE 5W-30, Formula F	LU3	Synthetic engine oil, used as chainsaw lubricant	80,8	0,0
	STILL HP ULTRA, BIO	LU4	Fully synthetic two-strike oil, biodegradable; added to chainsaw gasoline (5%)	6,5	1,8
	STILL BIO PLUS	LU5	Biodegradable chain lubricant; used as chainsaw	2,2	0,8
	VEGOIL Husqvarna	LU6	Pure, vegetable chain oil; used as chainsaw lubricant	0,0	0,0
	LOCTITE 8104	LU7	Food grade siliconic grease	0,0	0,0
	CENTURY, Regulus A3	LU8	Multipurpose lithium grease	5,0	2,1
			* semi-quantitative analysis since the product was not completly soluble in solvent		
Pesticides	OVIPRON TOP UPL	PE1	Mineral oil insecticide (96,5%), at low viscosity (800g/L); used also as herbicide aid; emulsionable liquid	95,9	0,0
	OLIOCIN BAYER	PE2	Mineral oil, very refined, with viscosity 696 g/L; insecticide allowed in organic agriculture	81,7	0,0



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Thank you for your kind attention

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