Mineral oils in vegetable oils: an update

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The term **mineral oil** is used to indicate products obtained from petroleum distillation and refining.

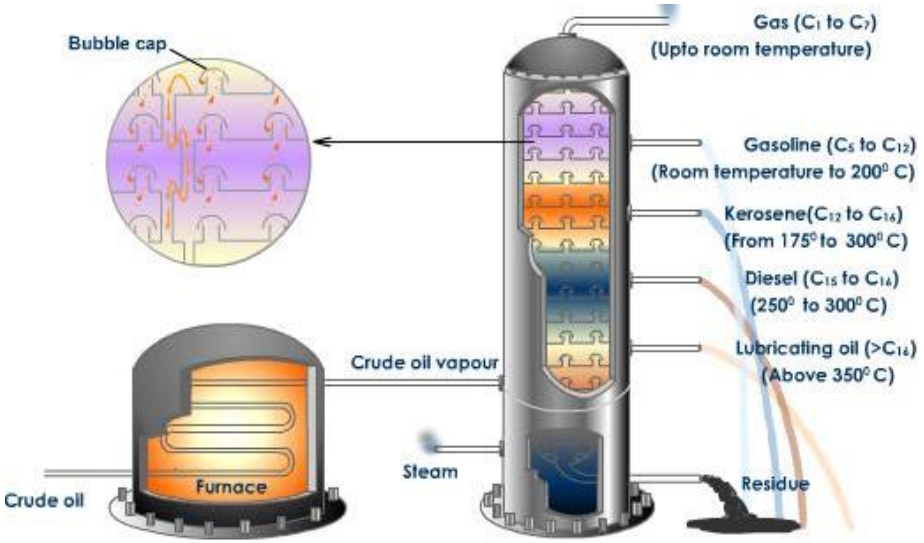
Mineral oils are complex mixtures of hydrocarbons which can not be individually separated by GC, but form “humps” of unresolved peaks.

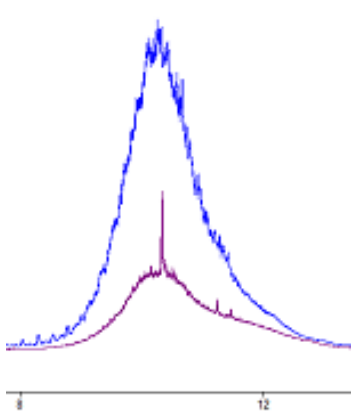
We can distinguish:

- Mineral oil saturated hydrocarbons (**MOSH)**
- Mineral oil aromatic hydrocarbons (**MOAH)**

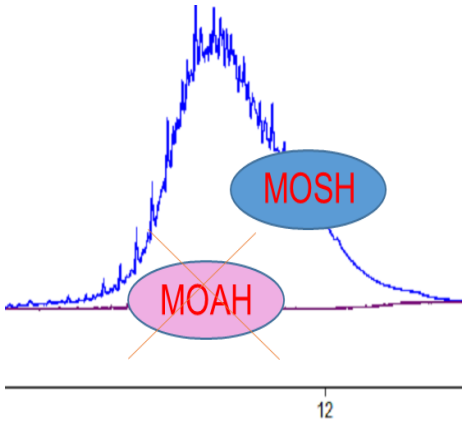
Food can be contaminated with unrefined mineral oils containing relevant amounts of MOAH, but also with “food grade” mineral oils obtained after refining (hydrogenation) to eliminate the aromatic fraction.

Since these two fractions have different toxicological relevance, they must be pre-separated before GC analysis.



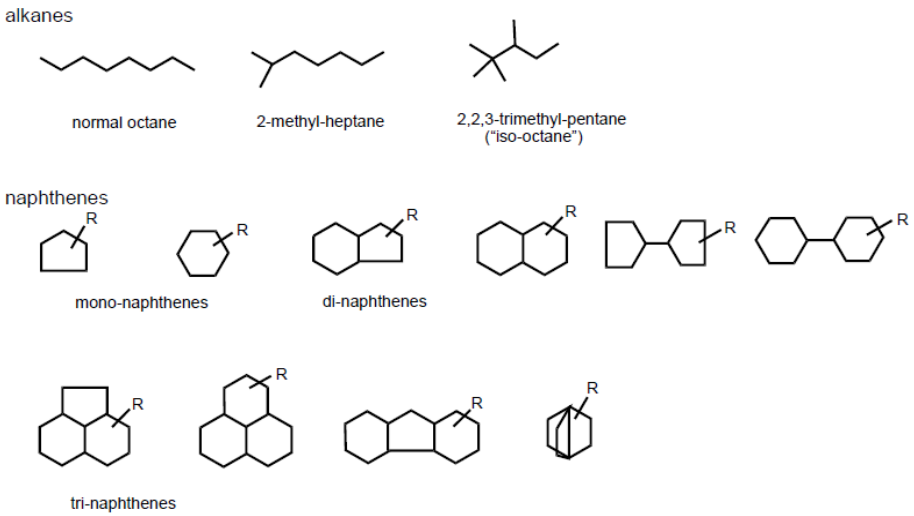


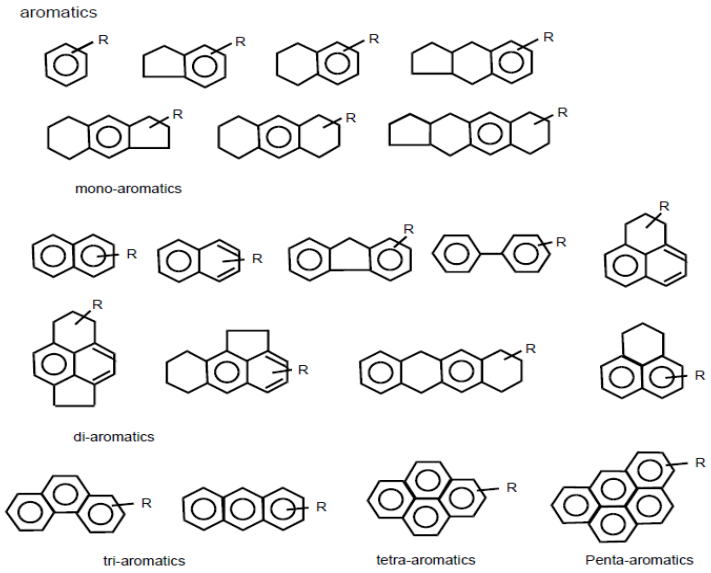
motor oil



food grade mineral oil

Composition and toxicity





MOSH

may accumulate in human tissues in lymph nodes, spleen and liver causing inflammation and microgranulomas (supported by studies on Fischer rats 344 and on human tissues)

MOAH

are genotoxic: they can be activated into chemically-reactive carcinogens such as epoxides, similar to PAH, (supported by IARC studies)
It is not possible to establish an ADI value

No EU regulations for MOSH and MOAH in FOOD!

Vegetable oils

Ukrainian sunflower oil: a legal limit of 50 mg/kg was established in 2008 and then repealed in 2014

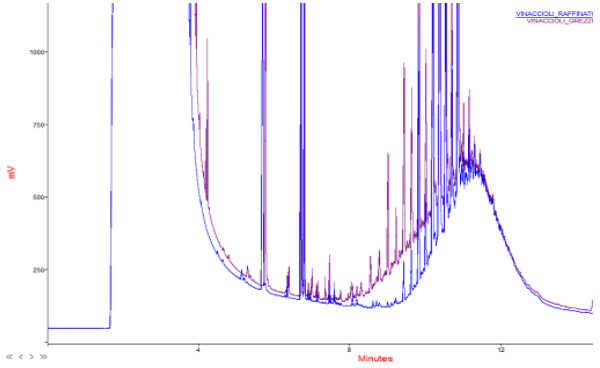
BfR (2011):
- 12 mg/kg food for MOSH C10-C16
- 4 mg/kg food for MOSH C16-C20

BEML: 4 draft ordinances (2011-2017)
The 3rd draft ordinance (2014) suggests maximum limits in food as a consequence of migration from recycled paper or paperboard:
- 0.5 mg/kg for MOAH C16-35
- 2 mg/kg for MOSH C20-35

The 4th BMEL (2017) draft ordinance suggested:
- 0.5 mg/kg limit for MOAH
- no limit for MOSH

Mineral oil in vegetable oils

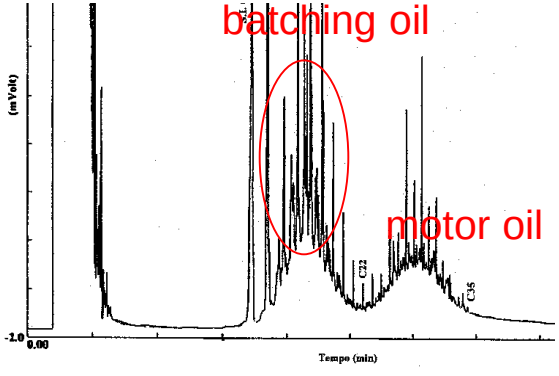
Vegetable oils are among the most important contributors of mineral oil to the total dietary intake and according to EFSA (2012) most edible oils are contaminated with detectable amount of MOSH, on average with more than 40 ppm

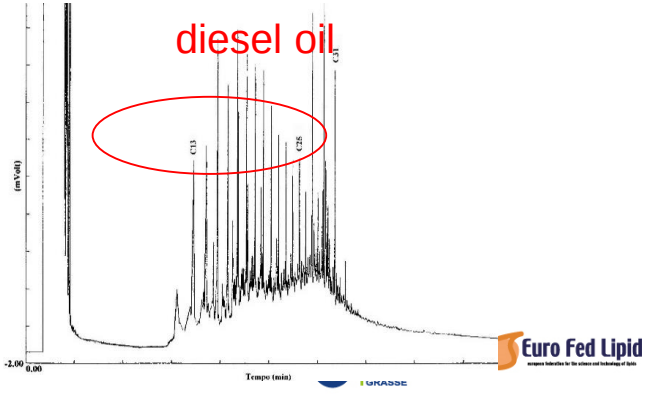


Grapeseed oil before and after refining

Refining removes about 30% of the contamination (more volatile compounds)

GC profiles may help to identify possible sources of contamination



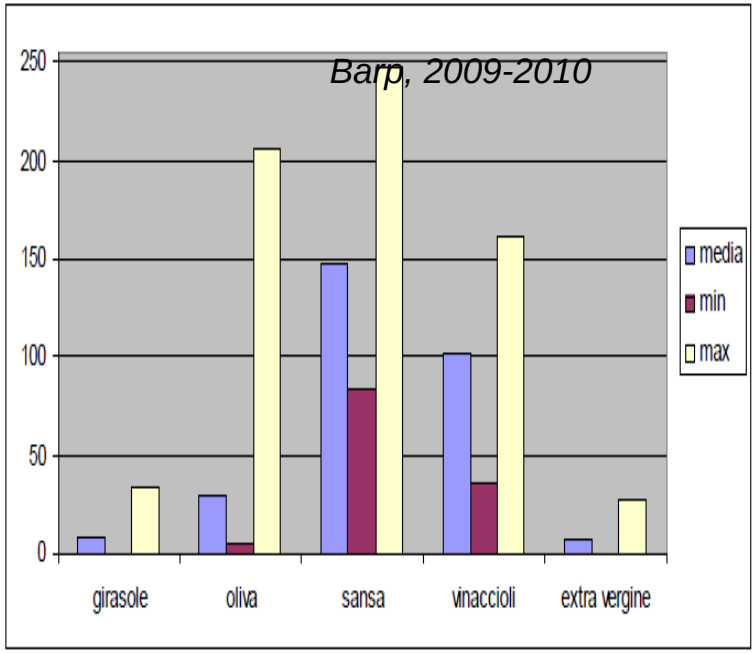


Vegetable oils from the market (2000/2005)

	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

Some occurrence data



Vegetable oil from the market (2009/2010)

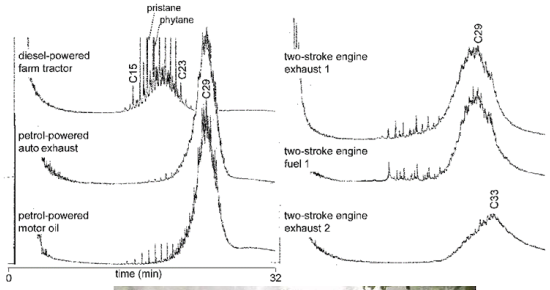
- Sunflower oil (13 samples)
- Grapeseed oil (5 samples)
- Olive oil (16 sample)
- Olive pomace oil (7 samples)
- Extra virgin olive oil (12 samples)

Olive oil from the market (2014-2015)

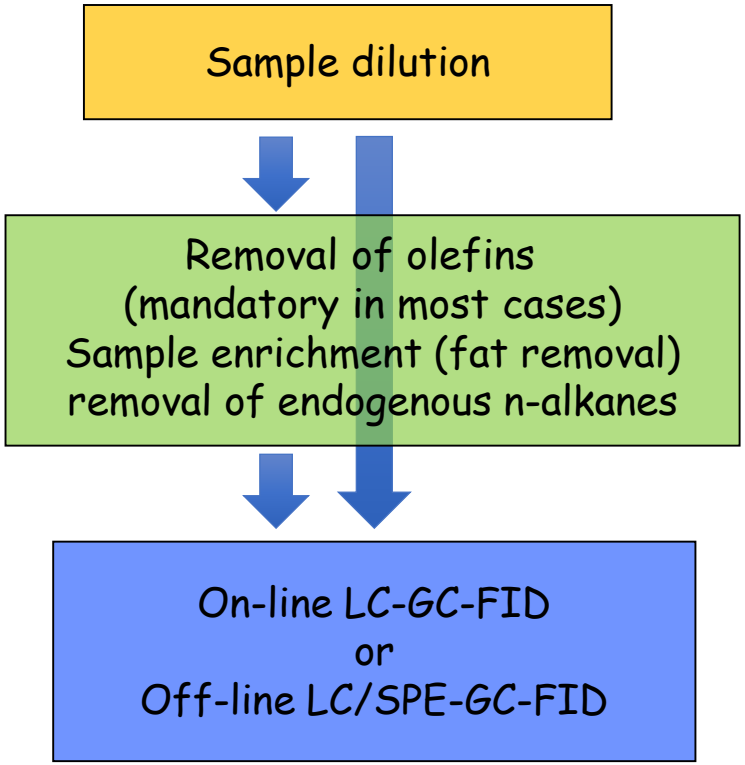
- Extra virgin olive oil (40 samples, mean MOSH 8 mg/kg,; only 2 sample with MOAH above LOQ 2 mg/kg))
- Olive oil (16 samples, mean MOSH 18 mg/kg))
- Olive pomace oil (11 samples, mean MOSH 174 mg/kg))

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)



Mineral oil determination in edible oils and fats



LC-GC procedure: 300-400 mg oil + internal std diluted to 1 mL → injection 50 uL

Sample enrichment (fat removal)

- Adsorption on fat retainers (silica gel, aluminum oxide, mixed beds)
- Saponification with strong alkali
- Use of a sulphuric acid-impregnated silica gel (not tested on MOAH)

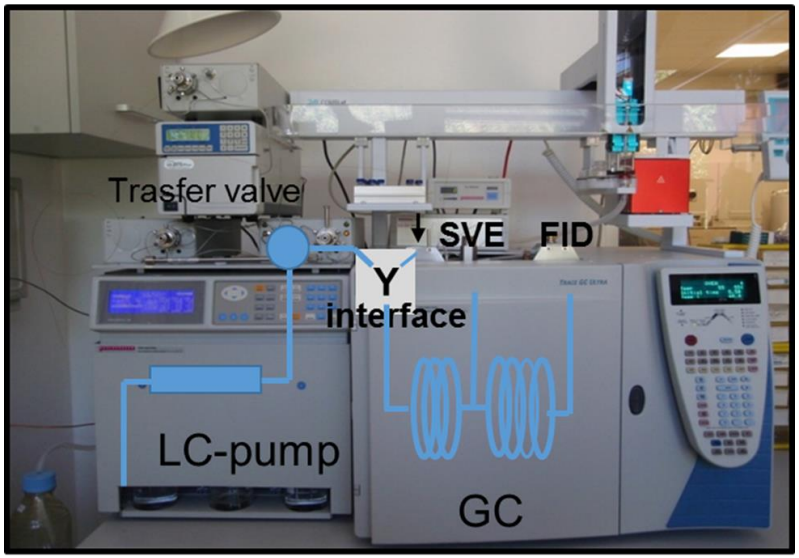
Sample purification

- Removal of olefins (epoxidation)
- Removal of n-alkanes (Alumina)

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)

EN method *BS EN 16995:2017* *"Vegetable oils and foodstuffs on basis of vegetable oils. Determination of mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH) with on-line HPLC-GC-FID analysis"*,

On-line methods



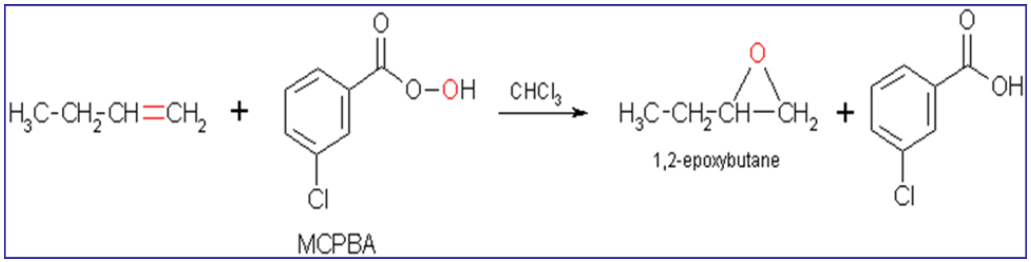
Transfer by the retention gap technique and partially concurrent eluent evaporation through the Y-interface

The LC column is a 25 cm x 0.3 mm i.d. (Lichrospher Si 60, 5 µm) able to retain up to 20 mg fat.

MOSH and MOAH separation is performed with a gradient starting with hexane (and reaching 30% dichloromethane after 2 min (300 µL min-1).

At the end of the MOAH fraction, the column is backflushed with CH2Cl2 and then reconditioned with hexane.

Removal of interfering olefins (mandatory for most oils) by epoxidation



MOAH analysis in vegetable oils is complicated by the presence of large amounts of olefins naturally present in the oil or formed during oil refining and, that, if injected into the HPLC column without adequate pre-treatment, co-elute with the MOAH overloading the GC column.
By proper derivatization, the polarity of the olefins can be enhanced such that these components are eluted after the MOAH.

Our investigation on epoxidation

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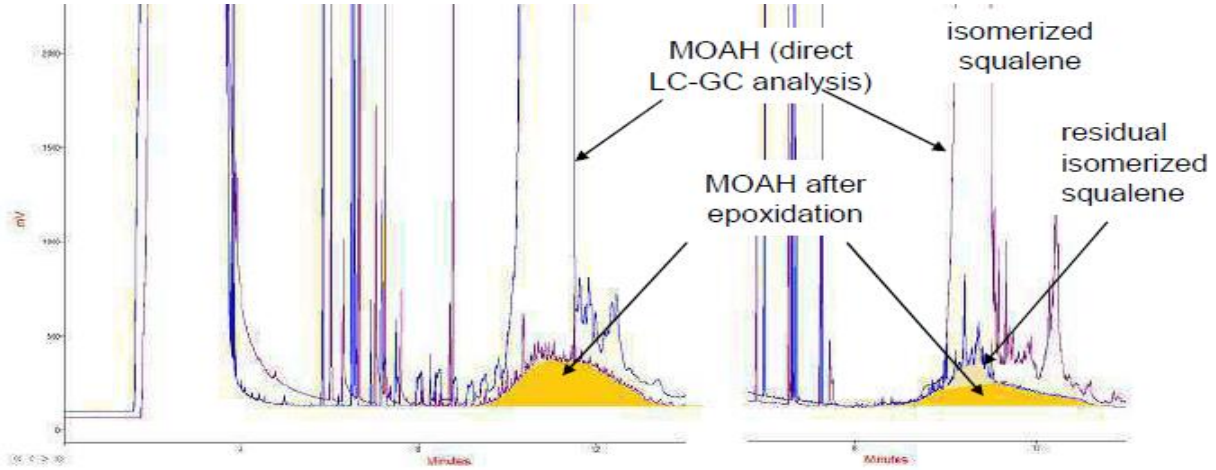
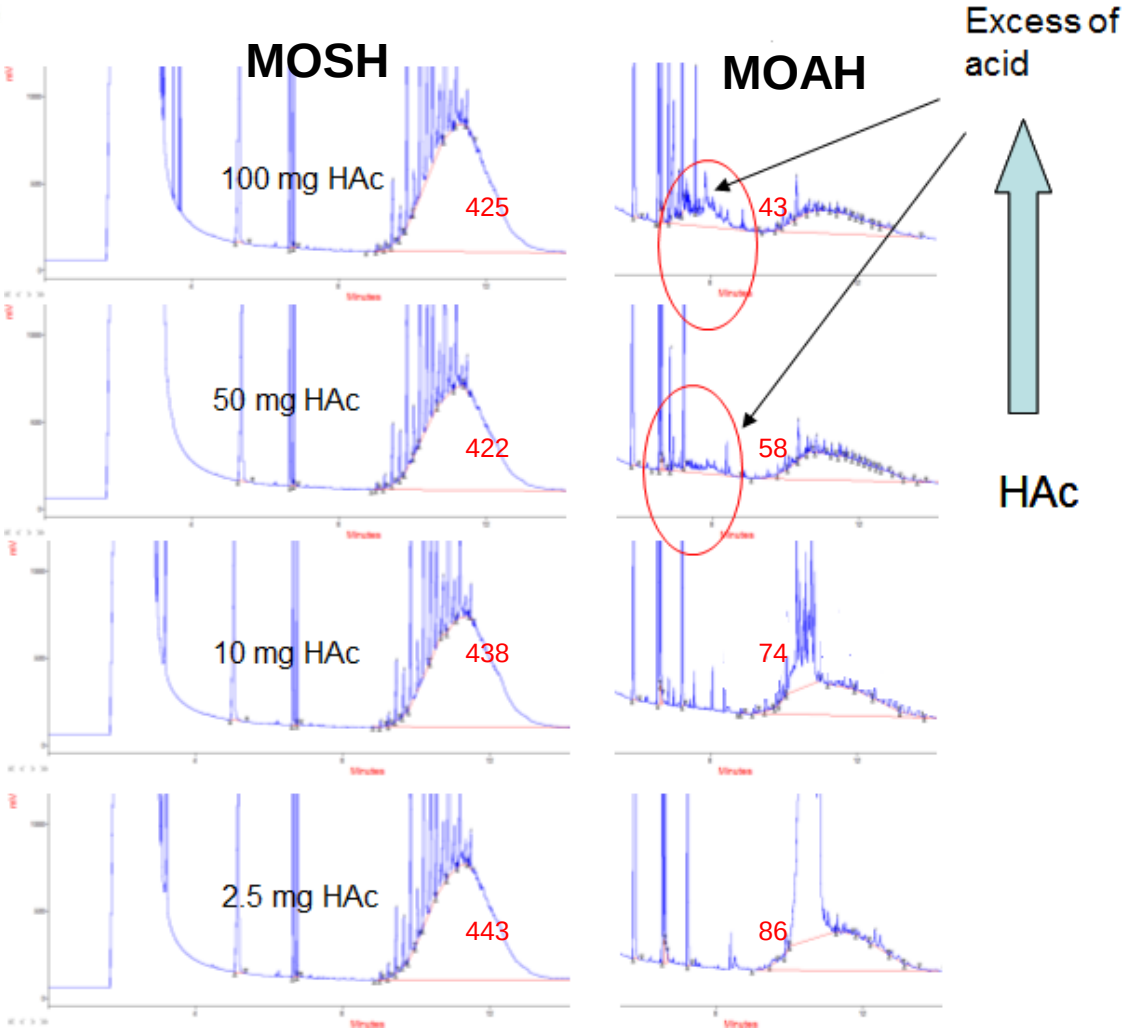


Sample dependent behaviour

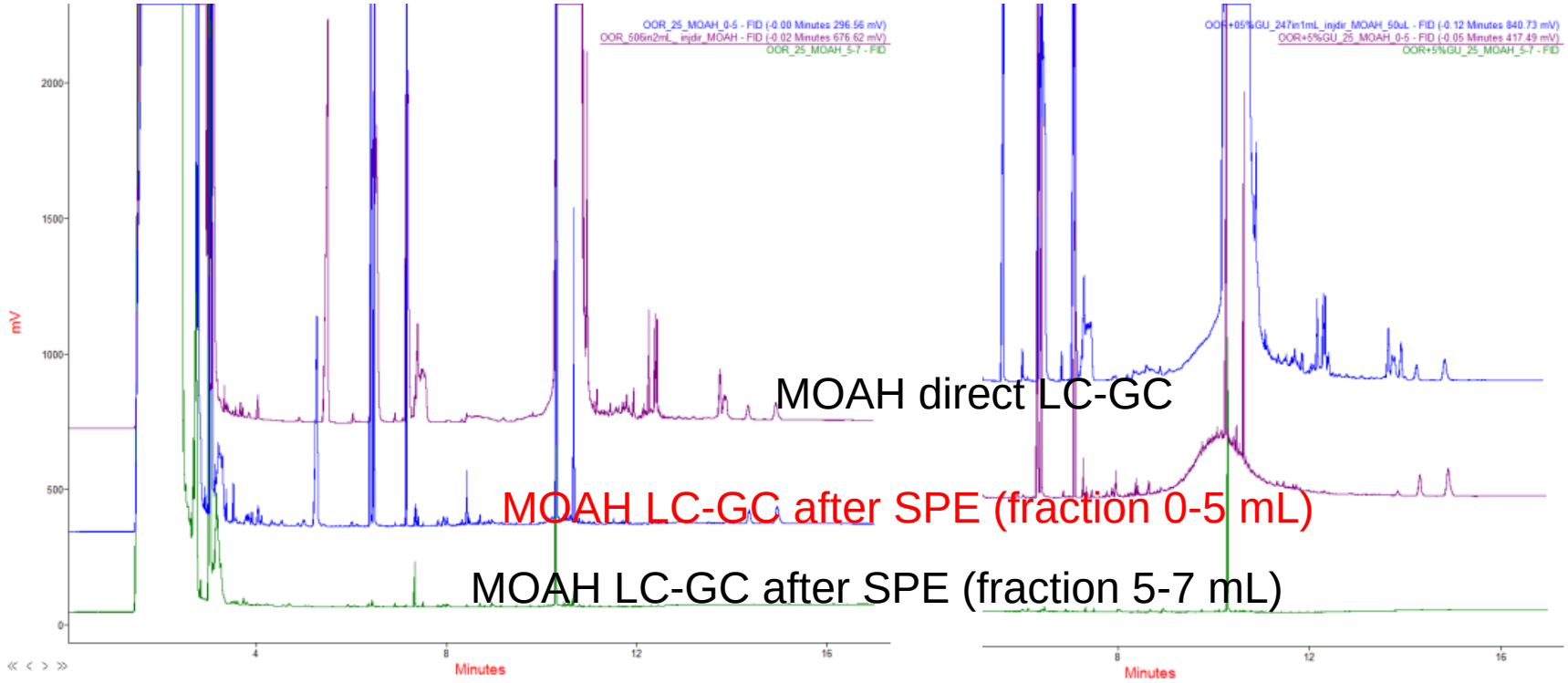
In conclusion:
epoxidation needs to be carried out under optimized conditions aimed at obtaining efficient removal of olefins, low aromatic losses and no artefact production.



MOSH and MOAH traces of a pomace olive oil after epoxidation with different amounts of 3-chloroperbenzoic acid.

Alternatives to epoxidation

SPE on 1 g silver silica (10%) was investigated as an alternative to epoxidation to reduce/ eliminate interference by olefins before on-line LC-GC.



Extra virgin olive oil

Extra virgin olive oil + 0.5% Ukrainian sunflower oil

Automated Solid Phase Extraction

SPE on 1g Si(Ag+) → LOQ around 2 ppm.

To reach higher sensitivity we explored the potential of automated SPE (5-10g of sorbent phase)

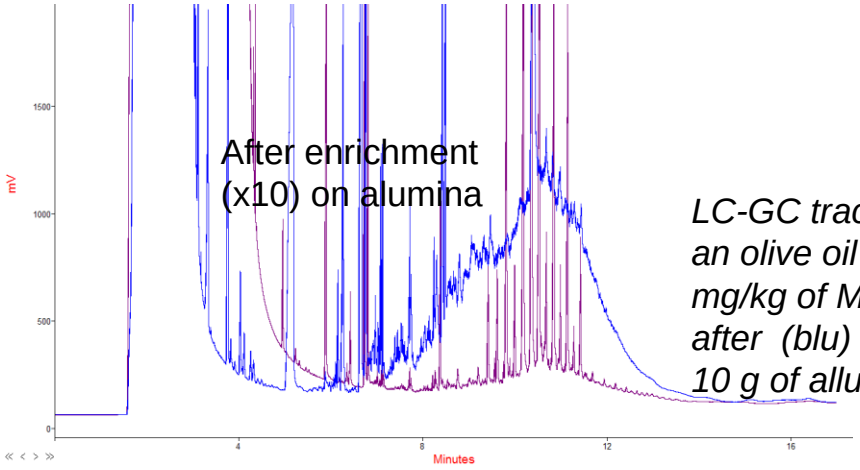
Optimal conditions were found for:

a) Fat removal (sample enrichment) to increase sensitivity and to remove endogenous *n*-alkanes

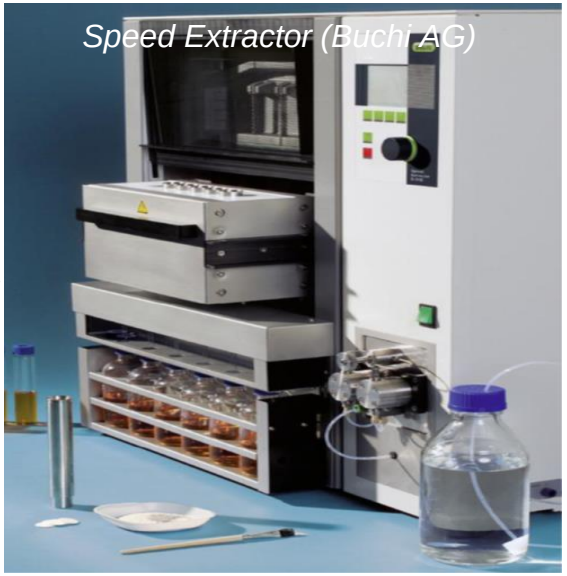
b) Sample enrichment, and purification of the MOAH from interfering olefins;

c) MOAH/MOAH fractionation prior to off-line GC-FID

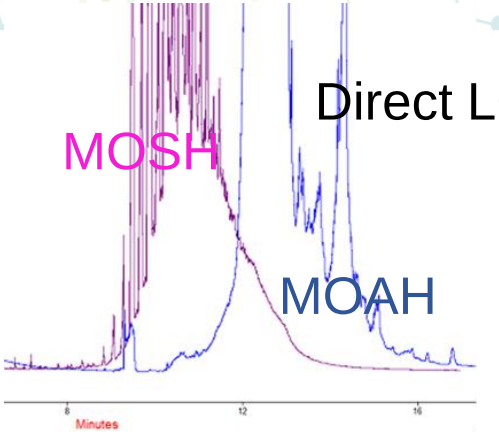
Example for (a)



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



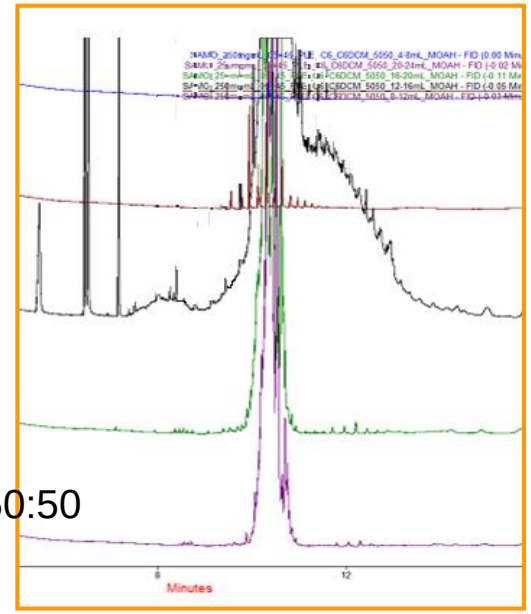
Sample: olive pomace oil (250 mg)

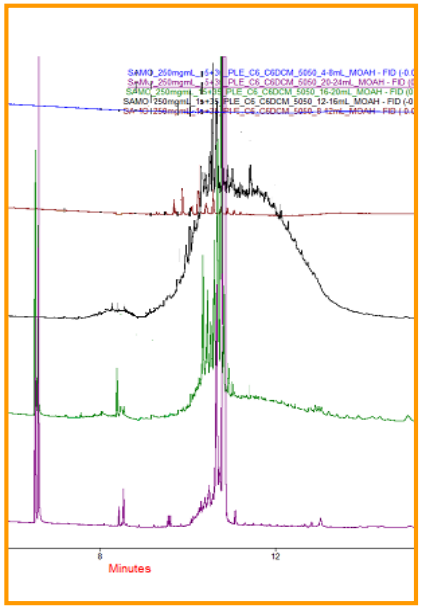


Direct LC-GC-FID

0.5 g Si-Ag + 4.5 g activated Si

1.5 g Si-Ag + 3.5 g activated Si





0-4 mL

4-8 mL

8-12 mL

12-16 mL

16-20 mL

Conditioning: Hex
Eluent: Hex/DCM 50:50

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

Microwave assisted saponification for MOSH & MOAH enrichment and purification

Microwave assisted extraction allows for rapid saponification of the sample and simultaneneous extraction of unsaponifiable matter. It has the advantage to eliminate the fat enabling to reach high enrichment factors and to avoid interference by wax esters.

Microvawe assisted saponification

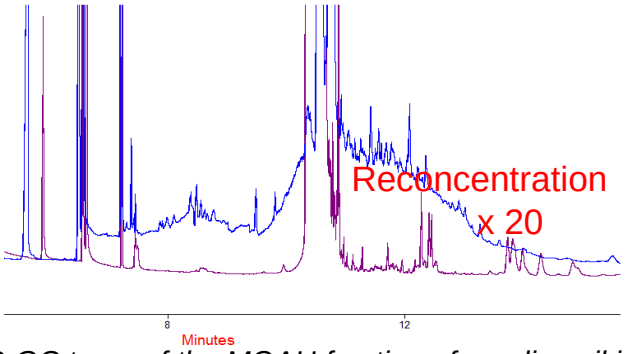
1-2 g sample + IS
+10 mL KOH in methanol
+ 10 mL hexane
MAS 120 ° C x 20 min

Transfer all the hexane extract
in a screw cup vial
wash with
CH3OH/H20 Vortex 1 min
Centrifugation

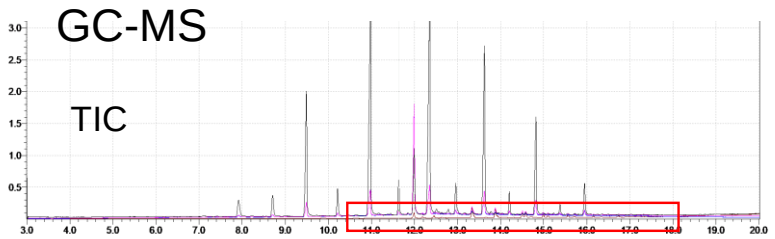
Epoxidation or passage on silver silica to eliminate interference of olefins

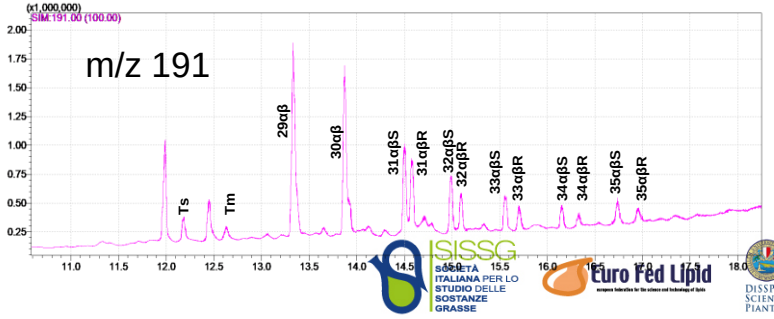
LC-GC analysis

Confirmation of the presence of mineral oil by GC-MS



LC-GC trace of the MOAH fraction of an olive oil injected directly or after MAS and SPE on silver silica





Microwave assisted saponification combined with automated solid phase extraction for off-line determination of MOSH and MOAH

1 g oil sample+ 20 µL IS +
10 mL KOH (1.5M) in methanol
+10 mL hexane

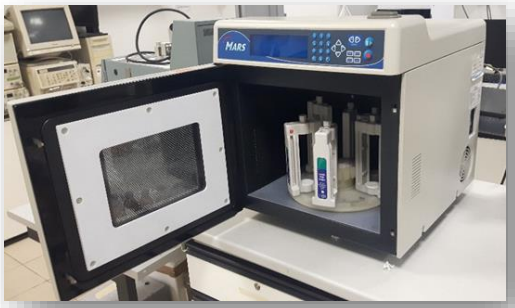
MAS 120 ° C per 20 min

Wash with CH3OH

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 50:50
MOSH (3-9 mL) MOAH (9-16 mL) →
concentration to 200 uL – 1 mL

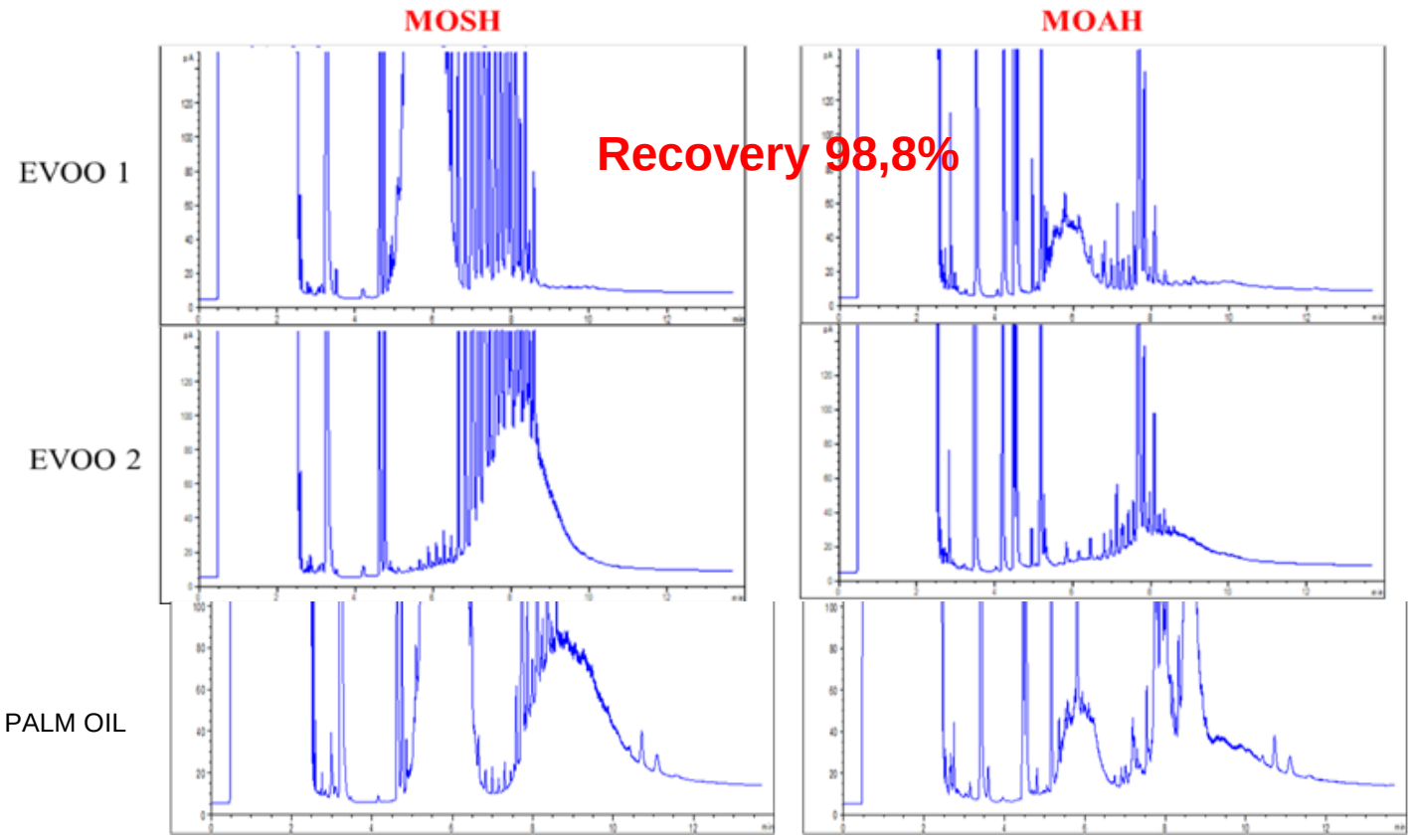
off-line GC-FID *(injection* 50 µL)



MARS, *Microwave Accelerated Reaction System*

SpeedExtractor (SPE automatizzata)

EVOO and PALM oil (spiked and not spiked with EXX-PRINT)



17.1.2017 EN Official Journal of the European Union L 12/95

RECOMMENDATIONS

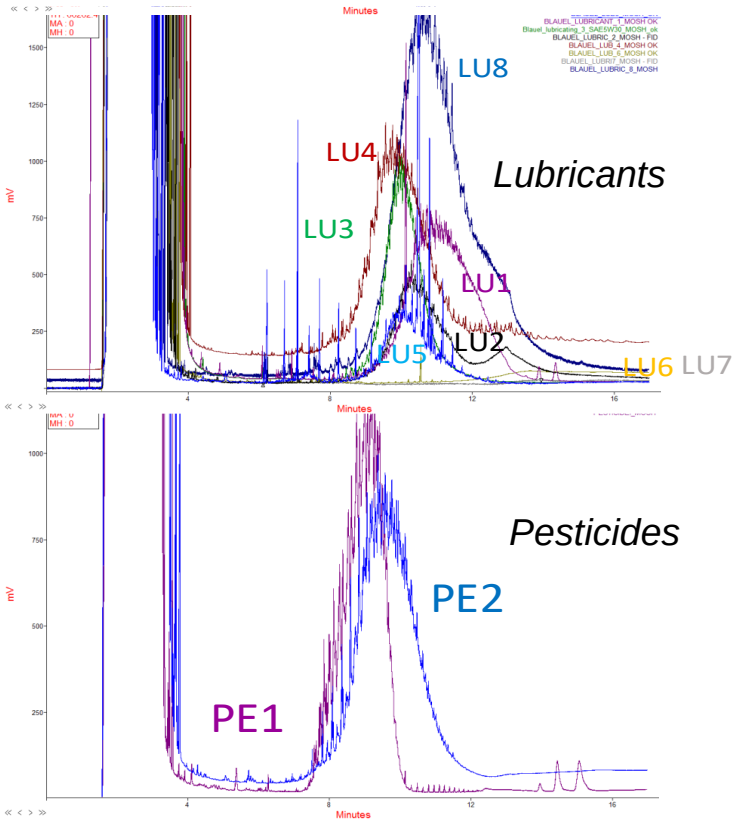
COMMISSION RECOMMENDATION (EU) 2017/84

of 16 January 2017

on the monitoring of mineral oil hydrocarbons in food and in materials and articles intended to come into contact with food

Including vegetable oils!

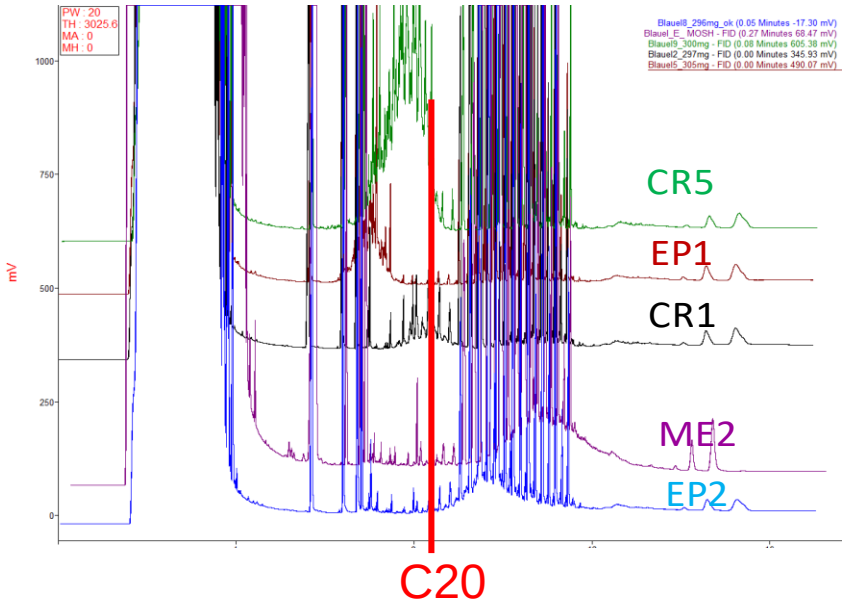
Preliminary results of a collaboration research between UniUD and Blauel & Co.



Olives from different sites in Greece:

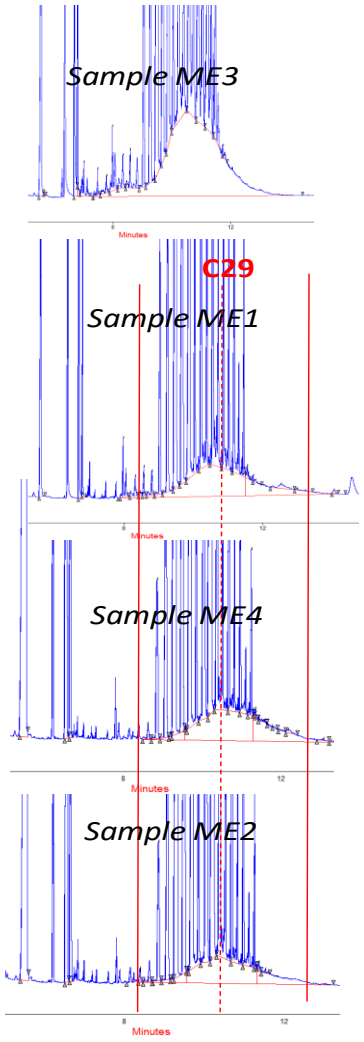
- ✓ *Crete (CR)*
- ✓ *Messinia (ME)*
- ✓ *Argolide (ANC)*
- ✓ *Epidauro (EP)*

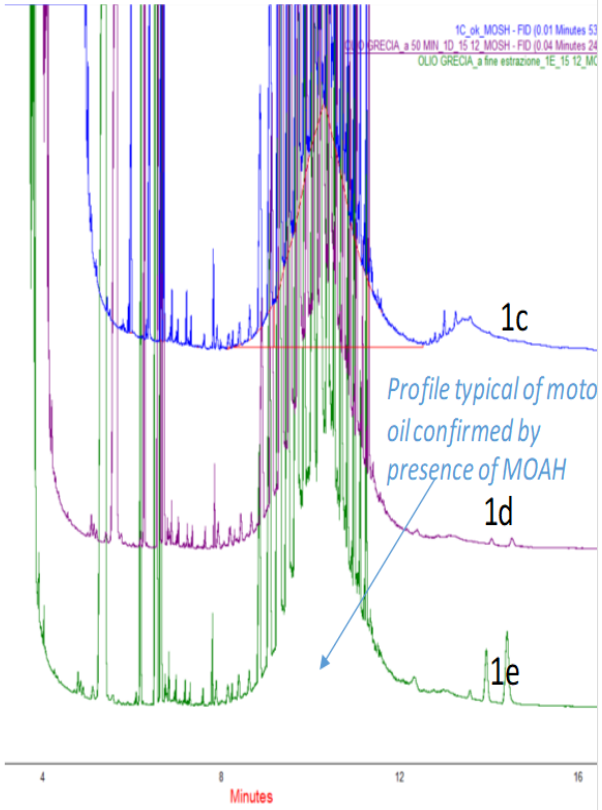
MOSH contamination focused on specific ranges of carbon chain length



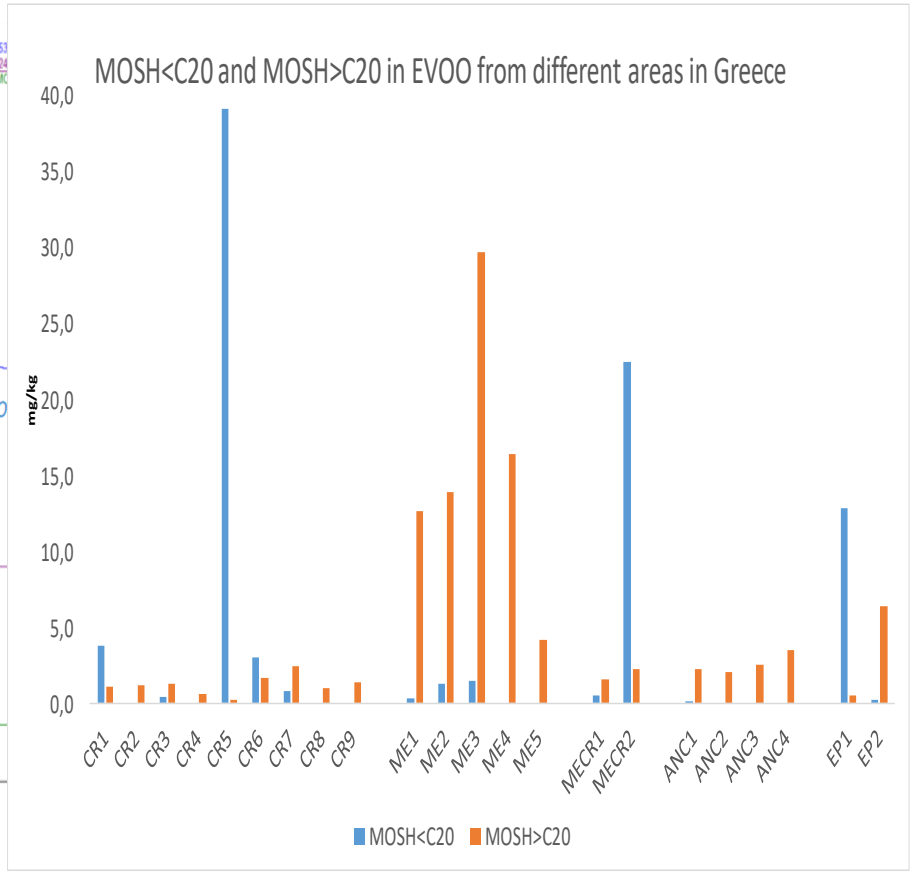
Contamination during harvesting

Messinia region



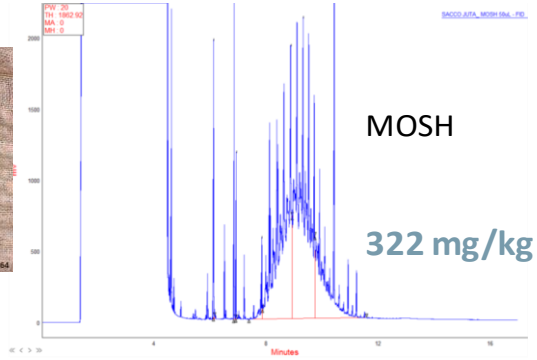


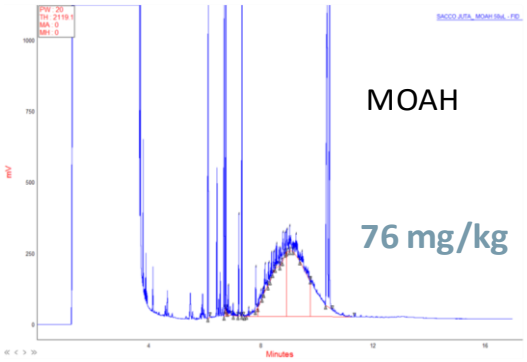
Chainsaw lubricated with motor oil
Producer 1



Contamination during transport in jute bags

In the jute bag



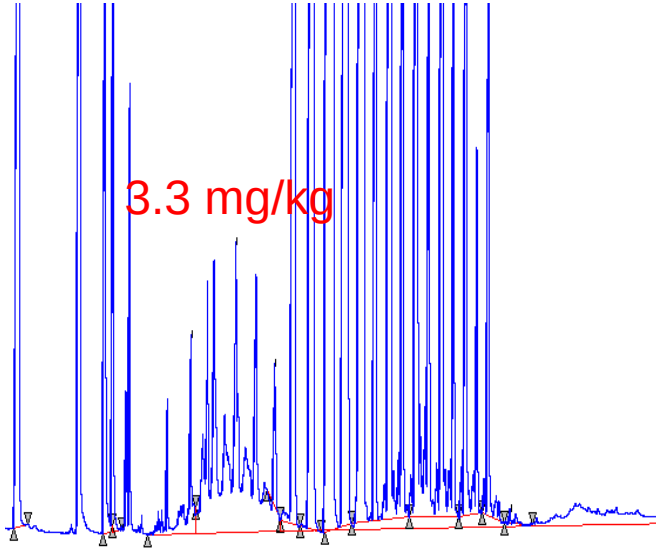


In the oil

(considering 100% transfer)

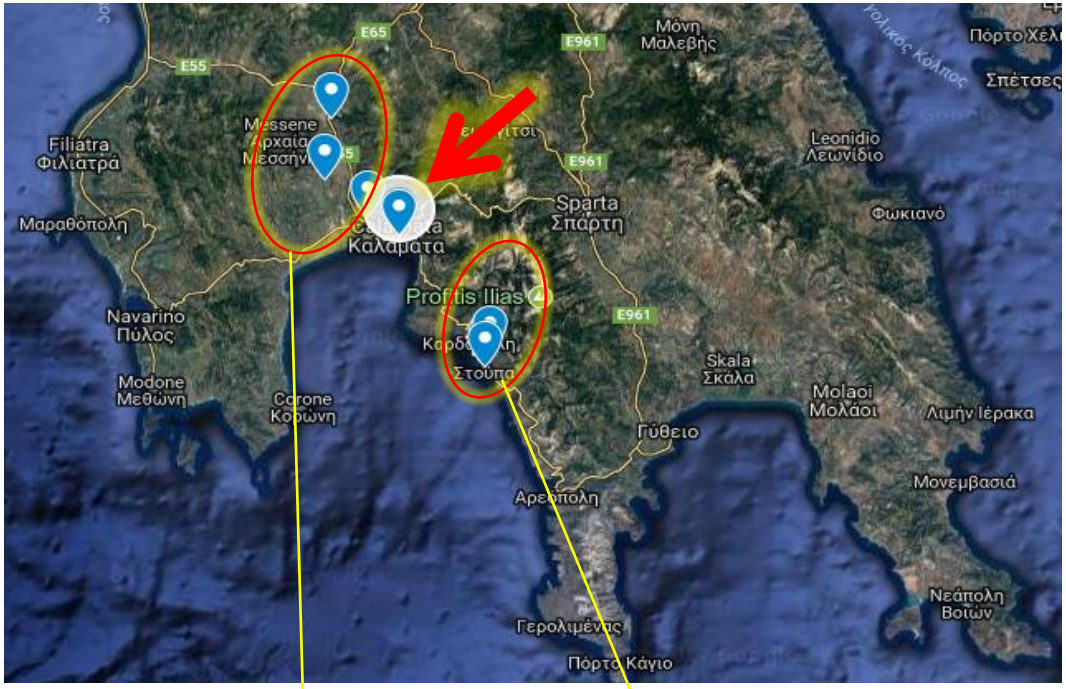
10 mg/kg

2 mg/kg

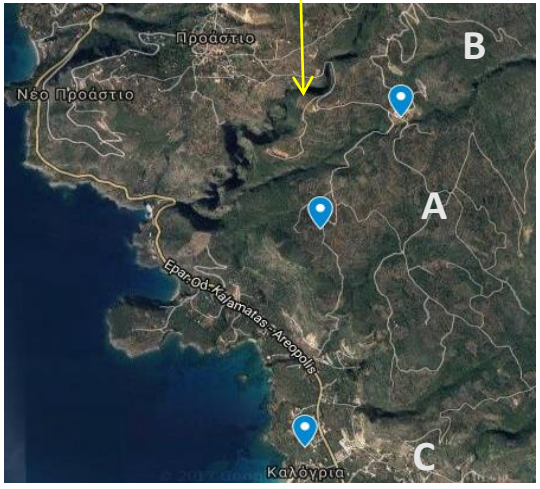


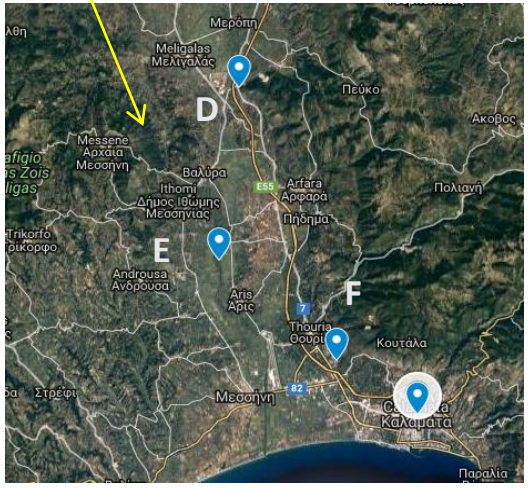
Monitoring environmental impact

Six areas differently exposed to environmental contamination in Greece



unpolluted areas





polluted areas

Environmental contamination: soil

mg/kg dry soil

	soil	isoalkanes	*n*-alkanes	*n*-alkanes carbon range	ratio *n*-alkanes/isoalkanes	total hydrocarbons
supposed "clean" areas	**A**	0,8	4,3	21-45	5,5	5,8
	B	1,9	6,4	23-36	3,3	9,0
	C	1,0	7,2	21-37	6,9	9,7
supposed "dirty" areas	**D**	3,7	6,1	21-37	1,6	11,0
	E	1,1	8,2	21-47	7,4	9,8
	F	1,1	1,6	23-35	1,5	3,4

Environmental contamination: olives

For each area two olive trees were identified to collect samples of olives in 2 periods

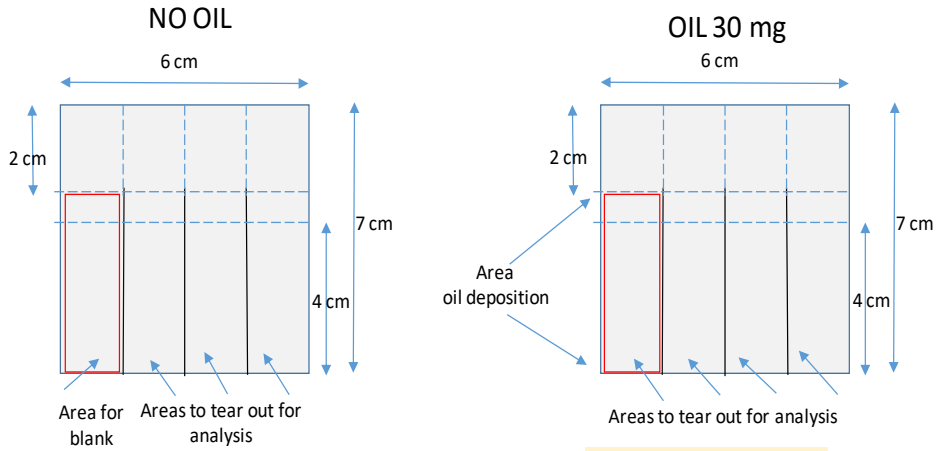
The olives were washed with water and after a LLE with hexane, the superficial contamination was determined by injecting the concentrated hexane extract into the LC-GC apparatus

Washed olives were ground and extracted in a microwave with hexane ethanol (1:1), the extracted fat was diluted and injected to determine total contamination

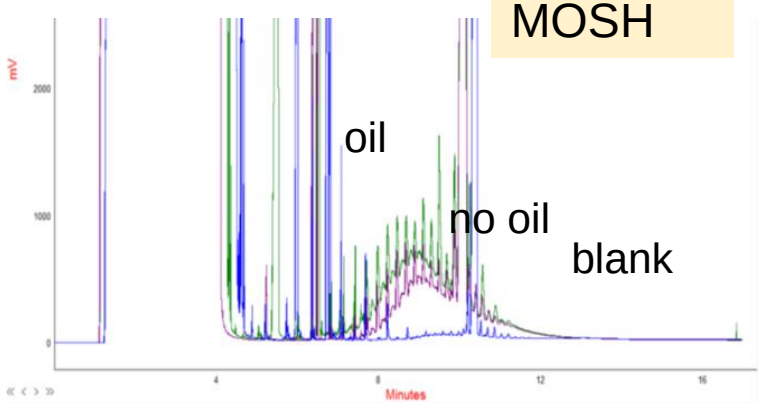
- Constant and very little contamination (on average around 2 mg/kg of oil was found in olives independently on the collection site
- Superficial contamination was about 1-2% referred to whole olive contamination

preliminary tests with passive traps

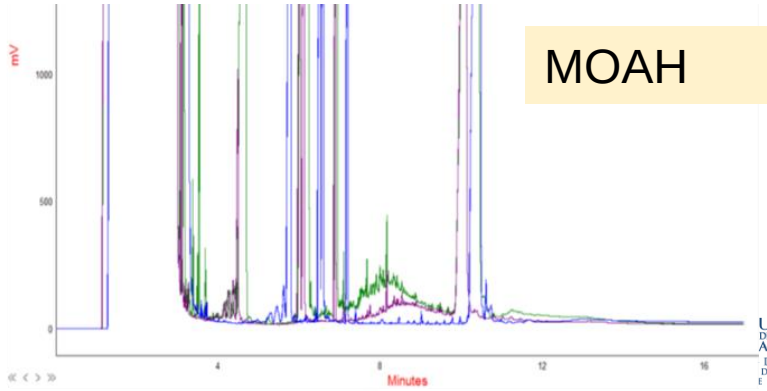
Passive traps made of paper filters (without oil and with 30 mg oil) exposed in highly trafficked road to determine the air contamination



MOSH

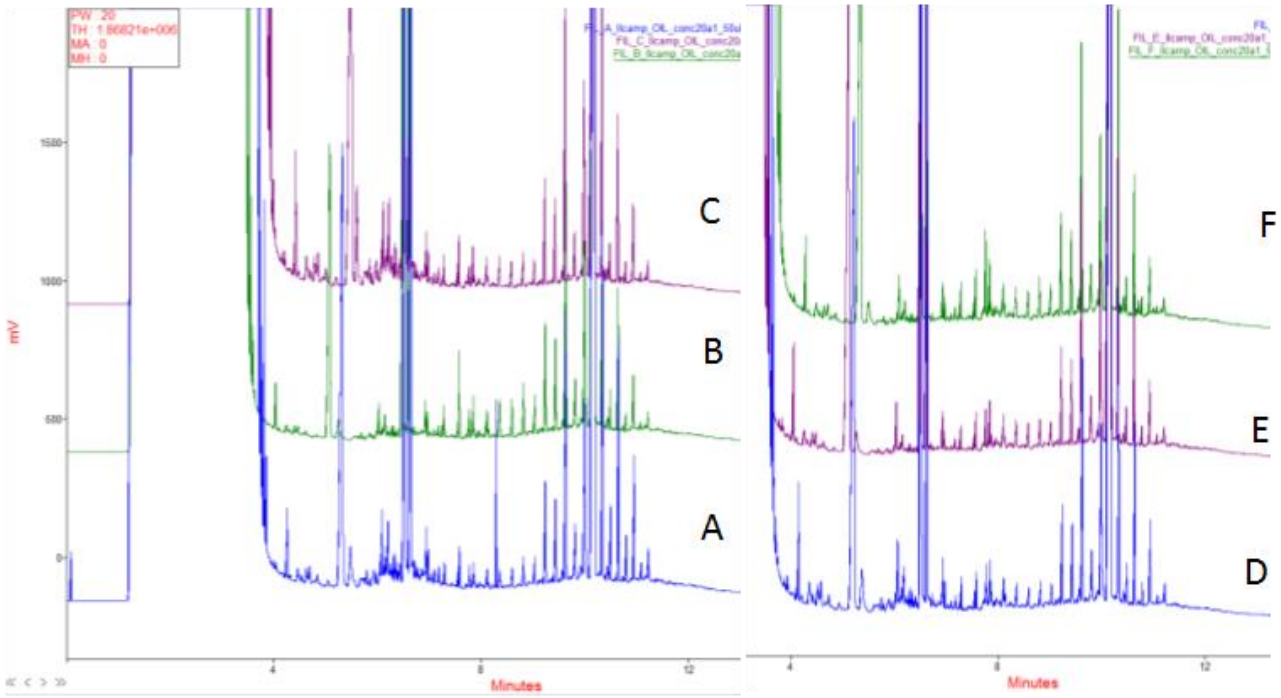


MOAH

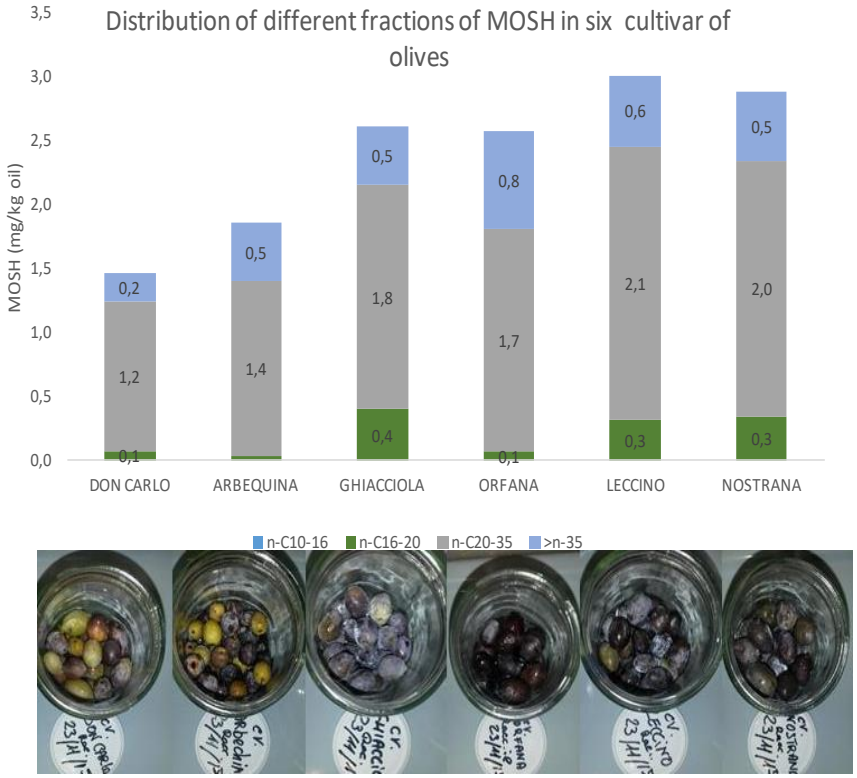


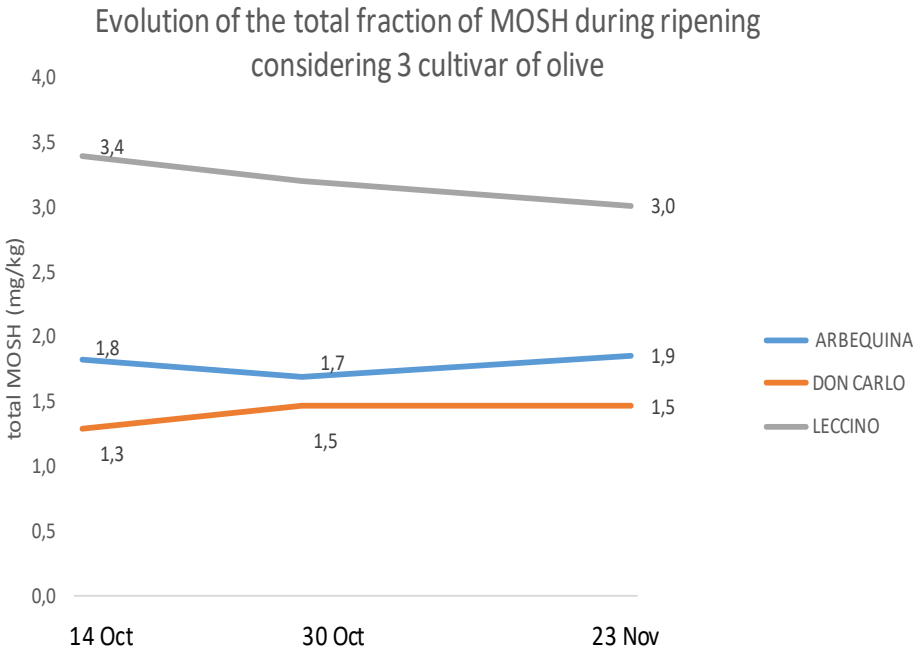
Passive traps

LC-GC traces of oil extracted from cardboard filters placed for 3 weeks in 6 different sites



MOSH content in olives from different cultivars (same olive grove) and evolution during ripening





Thank you for your kind attention

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