Standardization work for determination of mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH) in vegetable oils

Florence Lacoste

ITERG, French Institute for Fats and Oils

INNOVANT PAR NATURE

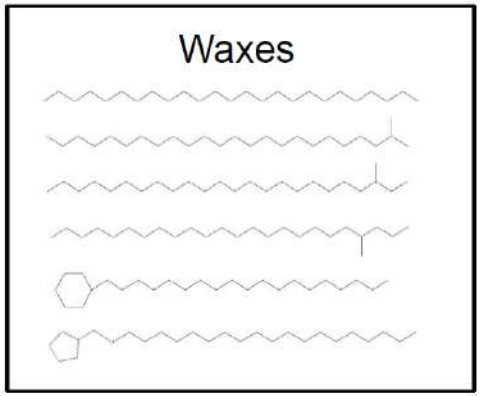
SISSG, Bologna, 12th December 2019

Agenda

- ❑ Chemical structures of mineral oil hydrocarbons according to petroleum experts
- ❑ Several cases of vegetable oil contamination with mineral oil hydrocarbons
- ❑ Critical points of the determination of mineral oil hydrocarbons
- ❑ ISO/TC34/SC11 - Standardization for MOSH determination in vegetable oils
- ❑ CEN/TC275/WG 13 - Standardization for MOSH & MOAH determination in Vegetable oils and foodstuff on basis of vegetable oils
- ❑ Current situation of the analysis of MOSH & MOAH in vegetable oils
- ❑ Possible improvements of the method (lower LOQs, markers)
- ❑ Conclusion

Mineral oil (MO) composition according to **Concawe**

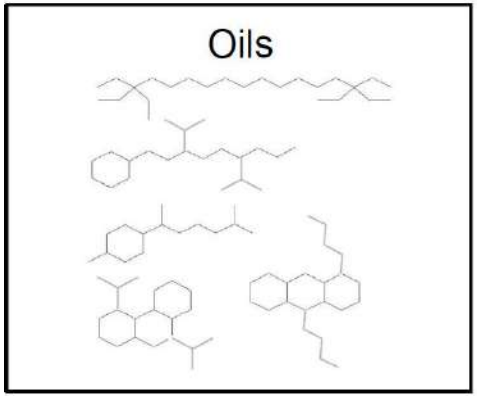
Concawe is a division of the European Petroleum Refiners Association



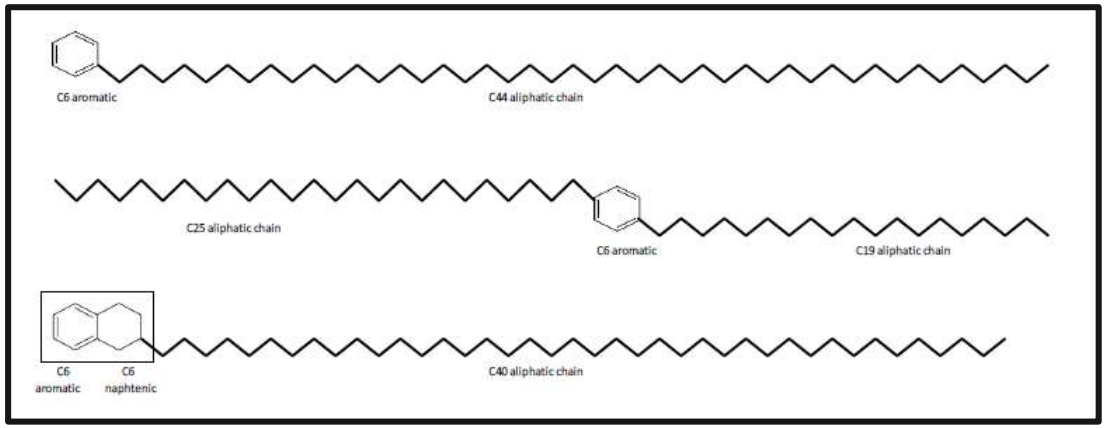
MOSH =

← n-alkanes mostly in waxes (>80%)

iso-alkanes + naphtenes in mineral oil →



MOAH = mono ou di-aromatics with very long alkyl chains



Bad MOAH = 3-7 PAC
(eliminated through refinement)

Harmless MOAH = highly alkylated aromatics
(what is left after 3-7 PAC elimination)

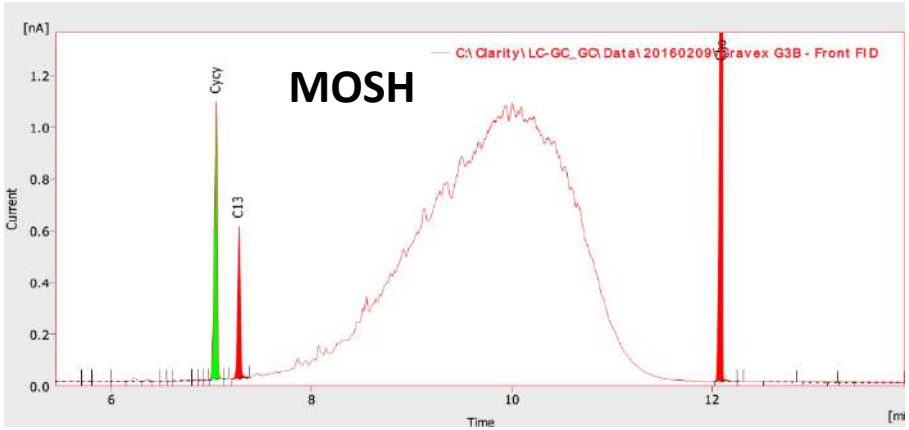
Dirk Danneels & Juan-Carlos Carrillo, Fresenius - nov 2017

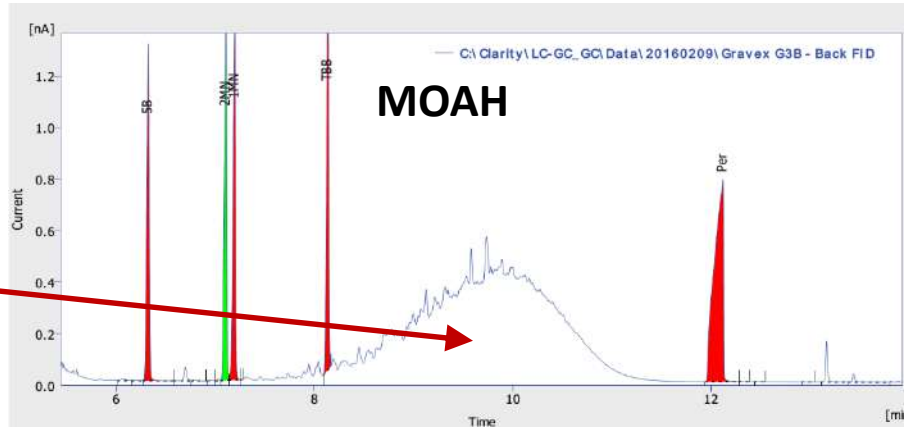
Naphtenic oil specification - Example

Naphthenic process oil suitable for carbonless copy paper

Typical Physical Characteristics

			Gravex 913
Colour (ASTM)		ISO 2049	L0.5
Density at 15 °C	kg/m3	ISO 12185	892
Refractive Index at 20 °C		ASTM D 1218	1.488
Flashpoint COC	°C	ISO 2592	150
Pour Point	°C	ISO 3016	< -60
Kinematic Viscosity		ISO 3104	
at 20 °C	mm2/s		19
at 40 °C	mm2/s		8.8
at 100 °C	mm2/s		2.1
Sulphur (X-Ray)	%m/m	ISO 14596	0.1
Carbon Type Distribution		DIN 51378 /	
C/A (S-corr.)	%	ASTM D 2140	11
C/N (S-corr.)	%	mod.	44
C/P (S-corr.)	%		45
Refractive Intercept (RI)		DIN 51378	1.0440
Viscosity Gravity Constant (VGC)		DIN 51378	0.865
Aniline Point	°C	ISO 2977	67
Clay Gel Analysis		ASTM D 2007	
polar components	%m/m		0.1
aromatic components	%m/m		30.6
saturated components	%m/m		69.3
Evaporation Loss (22h/107°C)	%m/m	ASTM D 972	28
Noack Volatility (1h/250°C)	%m/m	ASTM D 5800	
PCA-Content (DMSO method)	%m/m	IP 346	< 3.0



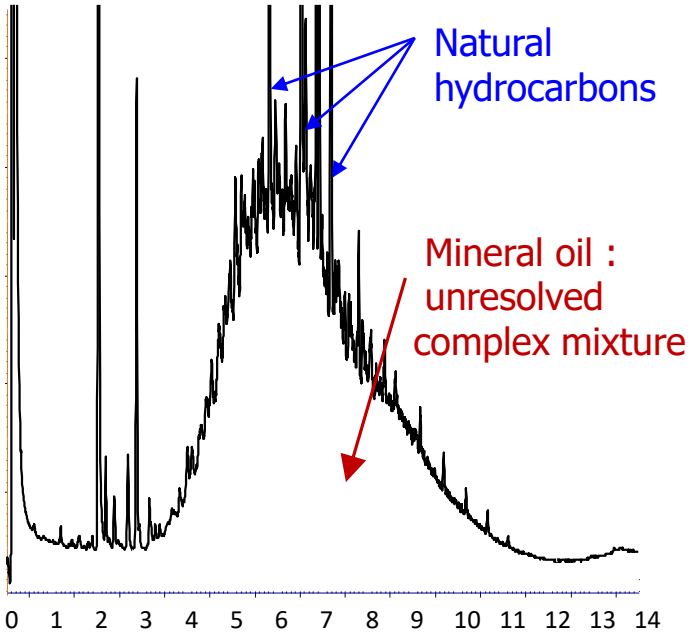


➢ Superposition of the MOSH & MOAH humps

ITERG
Expertise Corps Gras

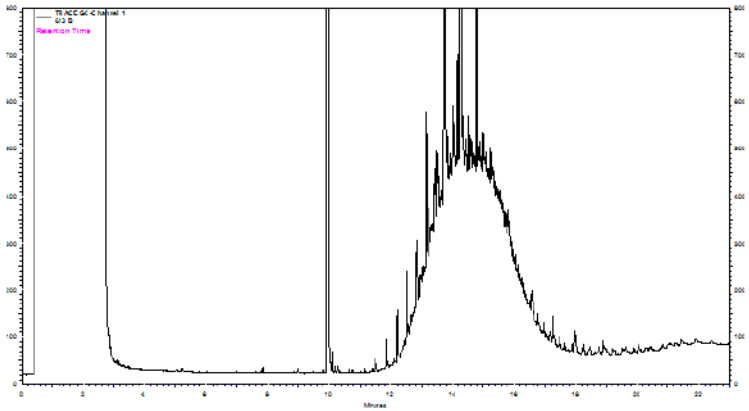
Mineral Oil detected in oils & fats

- 2008 → contamination of sunflower oil from Ukraine with a mineral oil from unknown origin
- 2009 → contamination of walnut oil with a food grade lubricant oil during refining process
- 2010 → identification of compounds eluted as mineral oil in grapeseed oils
- 2011 → contamination of milk fat with a food grade lubricant oil during production

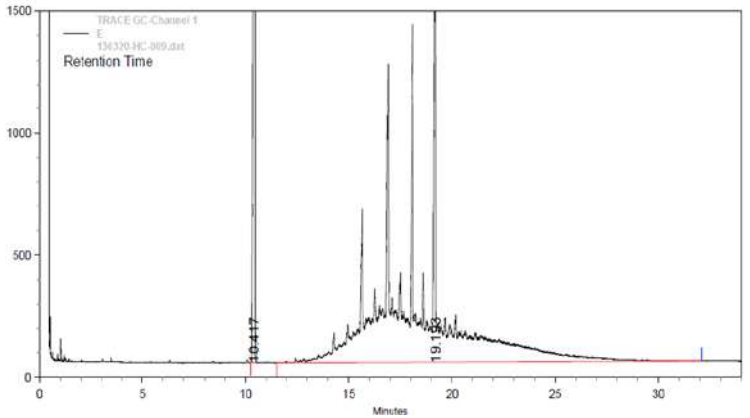


Contaminated Sunflower oil (MOSH)

Walnut oil - MOSH = 372 mg/kg



Grapeseed oil - MOSH = 235 mg/kg

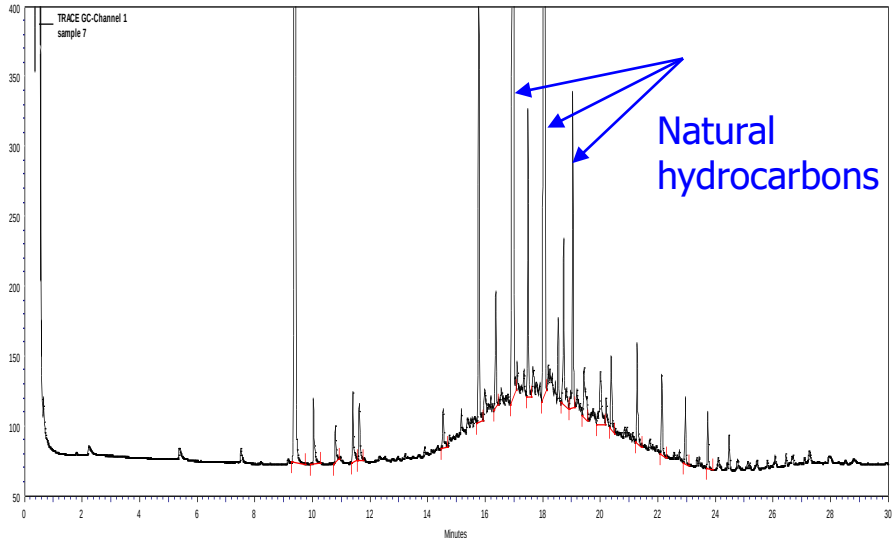


ITERG
Expertise Corps Gras

Mineral oil determination - Critical points

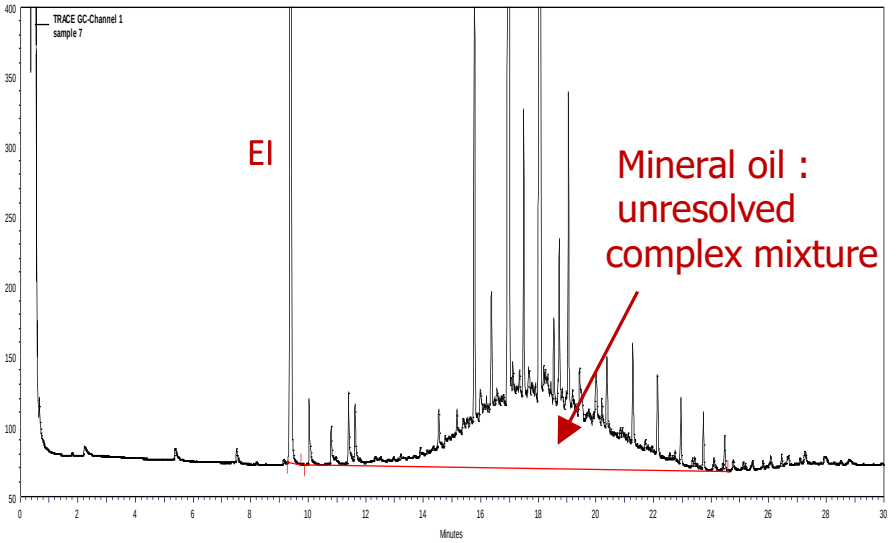
Principle of the methods

- First step : isolation of hydrocarbons by liquid chromatography or HPLC
- Second step (optional) : extra purification and concentration in order to increase the sensitivity
- Third step : gas chromatography analysis with FID detection



Critical points

- Integration of the hump & subtraction of the "natural hydrocarbons"
- Quantification of hydrocarbons without loosing the volatile ones
- Removal of interference compounds *(olefins in MOAH fraction)*
- Limit of quantification as low as possible
- Blank level to be under control



Standardization : work program

ISO/TC34/SC11

ISO work :

→ determination of saturated aliphatic hydrocarbons in vegetable oils (MOSH)

→ crude and refined vegetable oils

→ pre-trial in 2012

→ collaborative study in 2013 & 2014

ISO 17780: 2015 for MOSH

Limit of application : 50 mg/kg

CEN/TC275/WG13

EC requirement :

→ determination of mineral oil

→ method able to distinguish MOSH (Mineral Oil Saturated Hydrocarbons) and MOAH (Mineral Oil Aromatic Hydrocarbons)

→ crude and refined vegetable oils

→ foodstuffs on basis of vegetable oils

UNION EUROPEENNE
Fonds Européen de développement Régional

EN 16995 : 2017 for MOSH & MOAH

Limit of application : 10 mg/kg (without prior cleanup)

ITERG
Expertise Corps Gras

ISO 17780 - MOSH in oils with LC purification and GC-FID

ISO 17780 - Animal and vegetable fats and oils - Determination of aliphatic hydrocarbons in vegetable oils

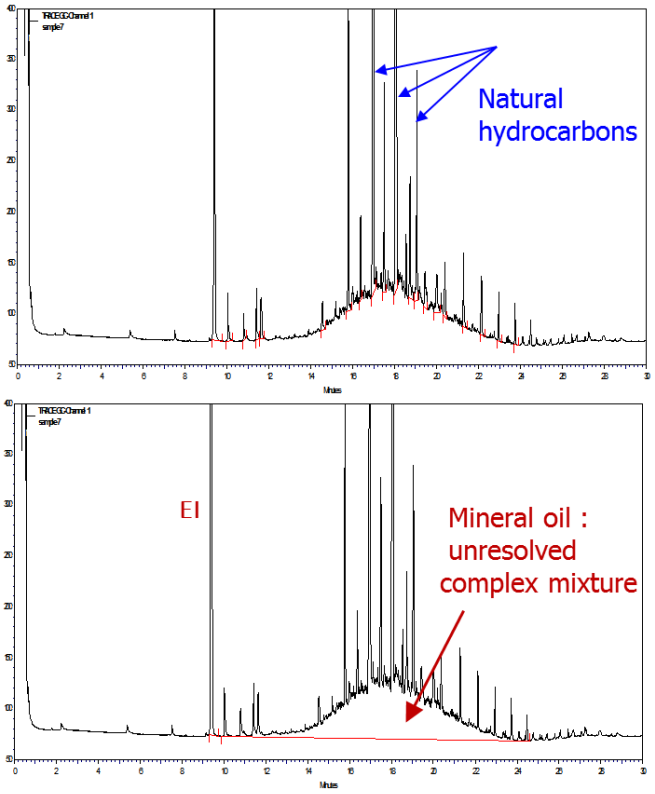
Scope of the method

- Method applicable for the determination of saturated aliphatic hydrocarbons from C10 to C56 of natural origin present in vegetable oils, and for detecting the presence of mineral oil and diesel oil.
- Method applicable to all types of crude and refined edible oils and fats, for concentrations of mineral oils from **50 mg/kg to 1 000 mg/kg**.

Principle of the method

- Fractionation of the sample by liquid chromatography on silica gel impregnated with AgNO3
- Quantification with C18 (IS)
- Injection of a reference standard blend (C10-C40) and a C48 standard
- GC condition on an apolar column (15 m)
- Double integration (subtraction of the peaks above the hump)

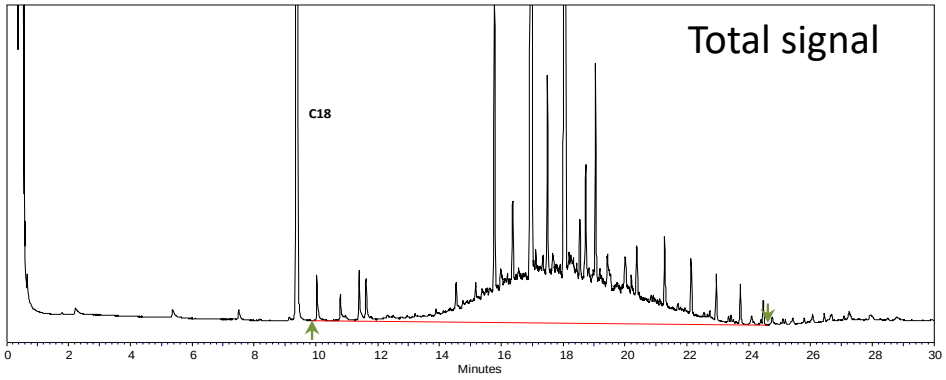
Silica gel with $AgNO_3$ → 18,5 g
Test portion → 1 g
Elution with hexane → 55 ml

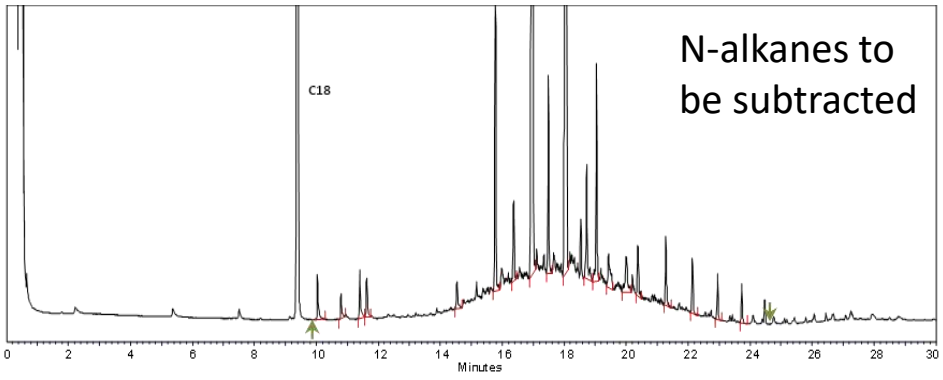


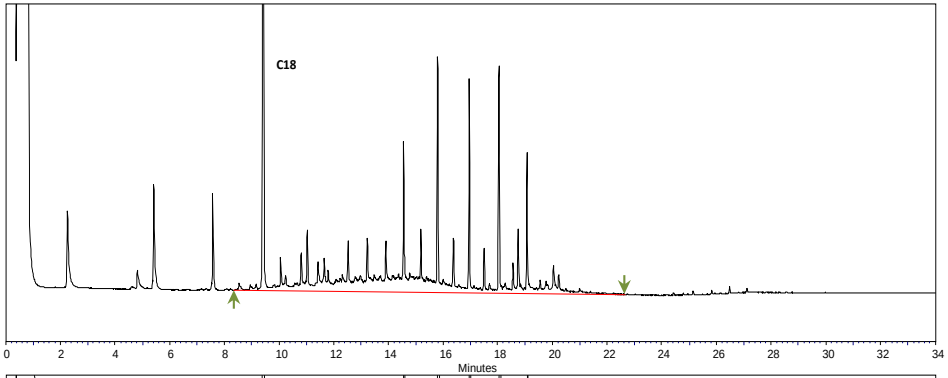
8

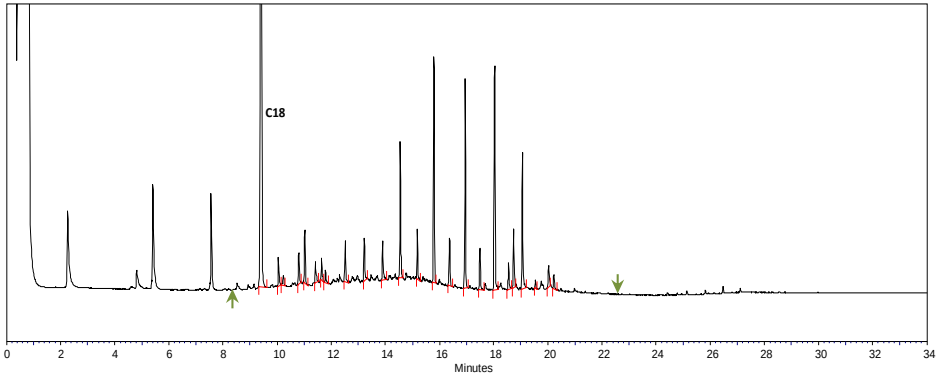
ITERG Expertise Corps Gras

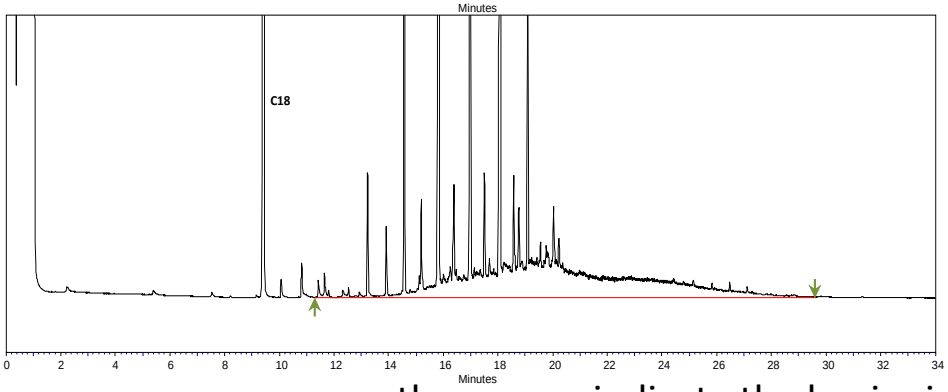
ISO 17780 - Integration of the chromatograms

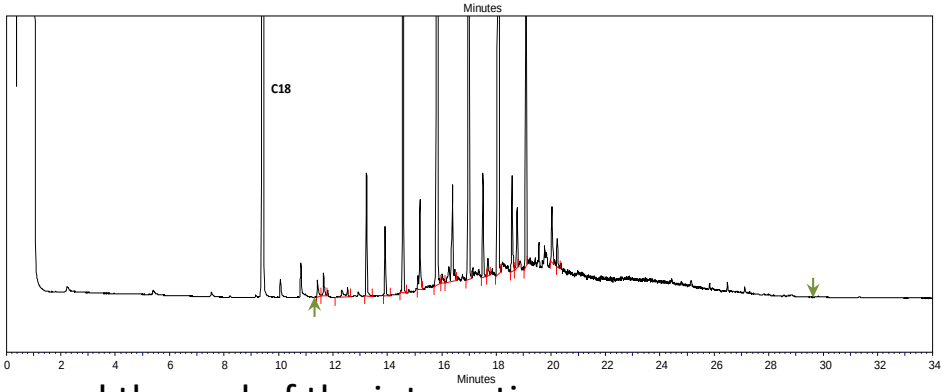






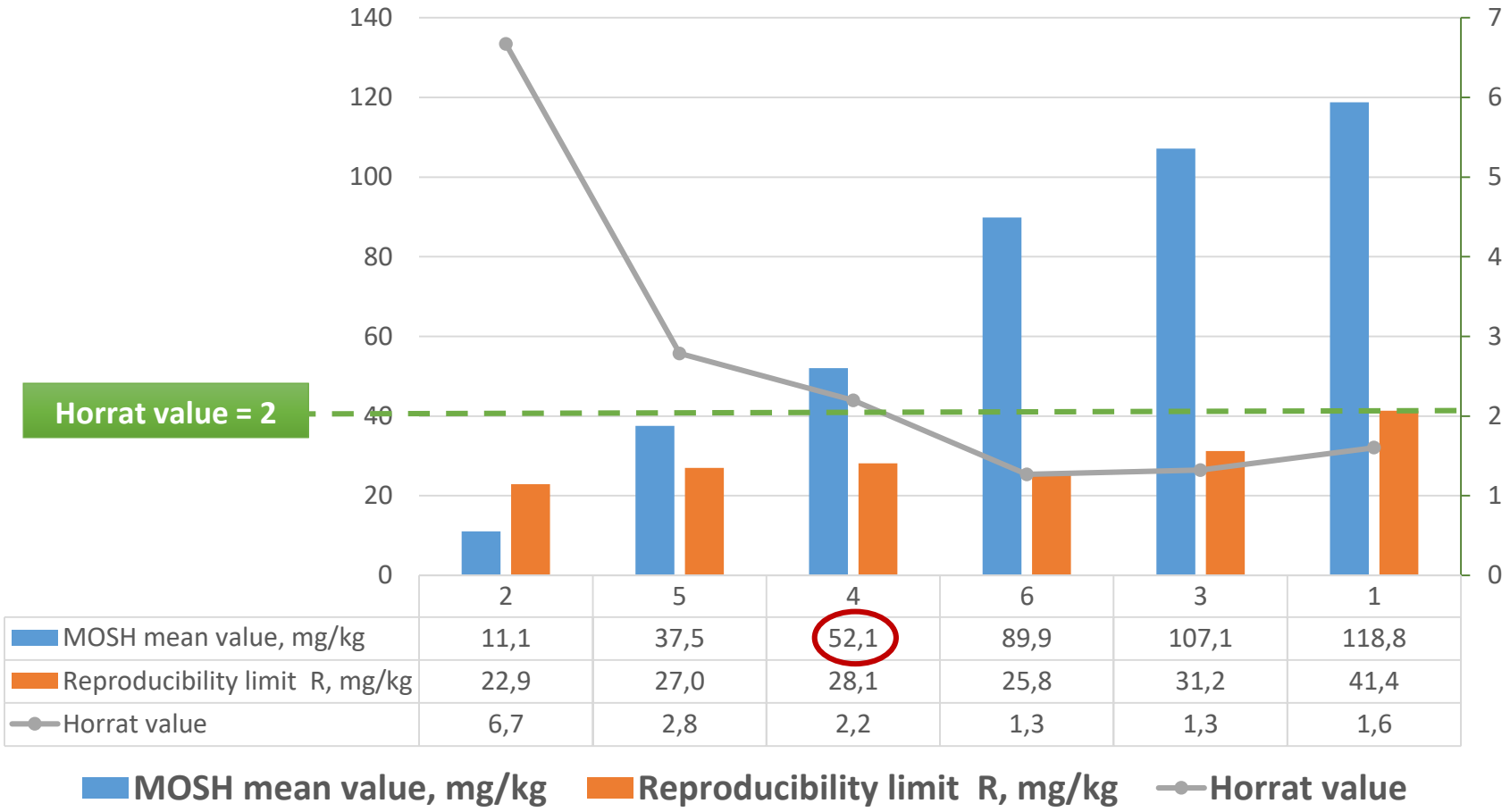






the arrows indicate the beginning and the end of the integration

ISO 17780 - 2014 CT - Reproducibility



	2	5	4	6	3	1
MOSH mean value, mg/kg	11,1	37,5	52,1	89,9	107,1	118,8
Reproducibility limit R, mg/kg	22,9	27,0	28,1	25,8	31,2	41,4
Horrat value	6,7	2,8	2,2	1,3	1,3	1,6

MOSH mean value, mg/kg **Reproducibility limit R, mg/kg** **Horrat value**

Reproducibility limit *R* → absolute difference between 2 single test results, obtained with this same method on identical test material in different laboratories

HORRAT > 2,0 → Method reproducibility is problematic

Limit of application : 50 mg/kg due to dispersion of the results

ITERG
Expertise Corps Gras

MOSH and MOAH : on-line-HPLC-GC-FID

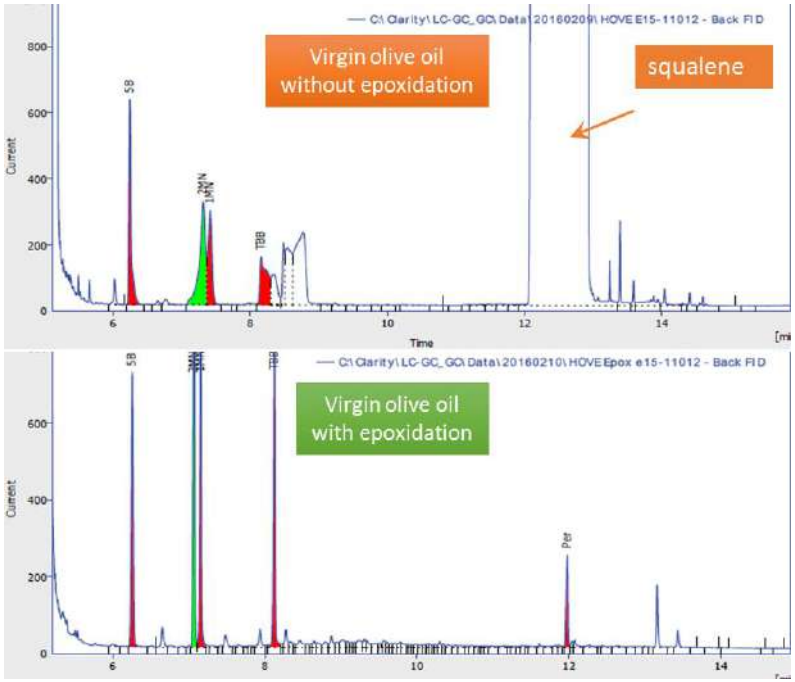
EN 16995 - Vegetable oils and foodstuff on basis of vegetable oils — Determination of mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH) with on-line HPLC-GC-FID analysis

Scope of the method

Determination of saturated and aromatic hydrocarbons (*from C10 to C50*) in vegetable fats and oils and foodstuff on basis of vegetable oils for which it has been interlaboratory validated, with online-HPLC-GC-FID. *This standard is not intended to be applied to other matrices.*

Principle of the method

- Fractionation of MOSH & MOAH with HPLC/UV
- On-line injection with large volume of each fraction followed by GC/FID analysis
- Quantification with bicyclohexyl (CyCy) for the MOSH fraction, 2-methylnaphthalene (2-MN) for the MOAH fraction
- *Optional purification by liquid chromatography on a Silica-ALOX column (to get rid of C23-C33 n-alkanes)*
- Clean-up by an epoxidation procedure (to avoid interferences with olefinic substances) = *mandatory*



SISSG, Bologna, 12th December 2019

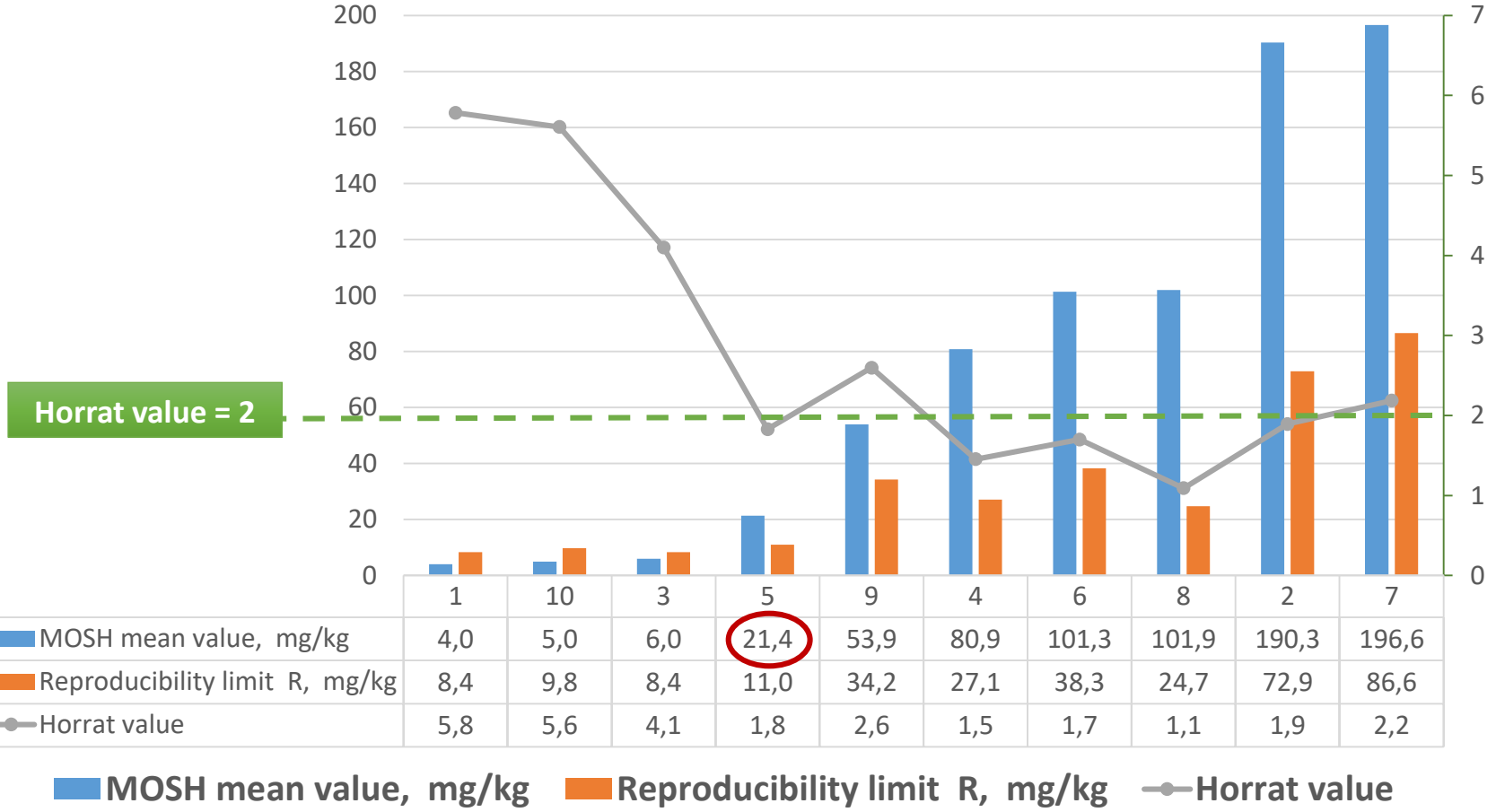
ITERG Expertise Corps Gras

MOSH – 2015 Collaborative study – Precision data

sample 1	refined sunflower oil
sample 2	crude soybean oil
sample 3	virgin olive oil

samples 4-5-6	refined sunflower oil
sample 7	refined olive pomace oil
sample 8	mayonnaise

sample 9	palm oil
sample 10	margarine



	1	10	3	5	9	4	6	8	2	7
MOSH mean value, mg/kg	4,0	5,0	6,0	21,4	53,9	80,9	101,3	101,9	190,3	196,6
Reproducibility limit R, mg/kg	8,4	9,8	8,4	11,0	34,2	27,1	38,3	24,7	72,9	86,6
Horrat value	5,8	5,6	4,1	1,8	2,6	1,5	1,7	1,1	1,9	2,2

According to the results of the collaborative study, the method has been proven suitable for MOSH mass concentrations above 10 mg/kg.

SISSG, Bologna, 12th December 2019

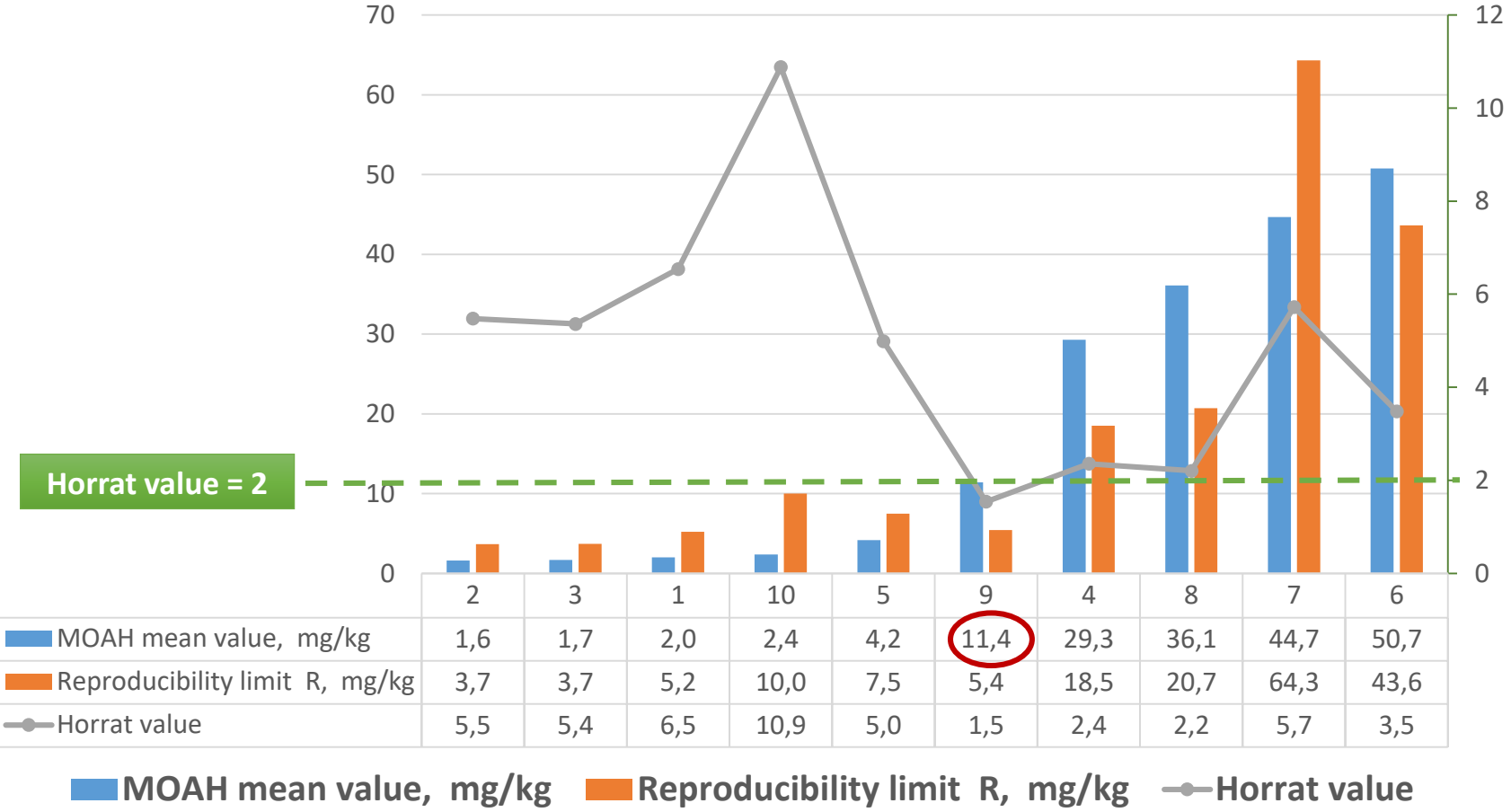
ITERG
Expertise Corps Gras

MOAH – 2015 Collaborative study – Precision data

sample 1	refined sunflower oil
sample 2	crude soybean oil
sample 3	virgin olive oil

samples 4-5-6	refined sunflower oil
sample 7	refined olive pomace oil
sample 8	mayonnaise

sample 9	palm oil
sample 10	margarine



	2	3	1	10	5	9	4	8	7	6
MOAH mean value, mg/kg	1,6	1,7	2,0	2,4	4,2	11,4	29,3	36,1	44,7	50,7
Reproducibility limit R, mg/kg	3,7	3,7	5,2	10,0	7,5	5,4	18,5	20,7	64,3	43,6
Horrat value	5,5	5,4	6,5	10,9	5,0	1,5	2,4	2,2	5,7	3,5

MOAH mean value, mg/kg **Reproducibility limit R, mg/kg** **Horrat value**

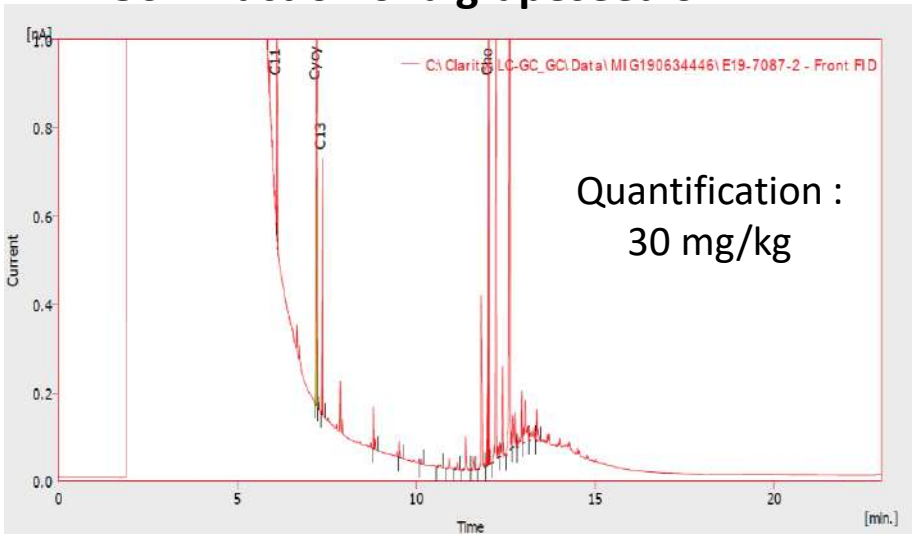
According to the results of the collaborative study, the method has been proven suitable for MOAH mass concentrations above 10 mg/kg.

SISSG, Bologna, 12th December 2019

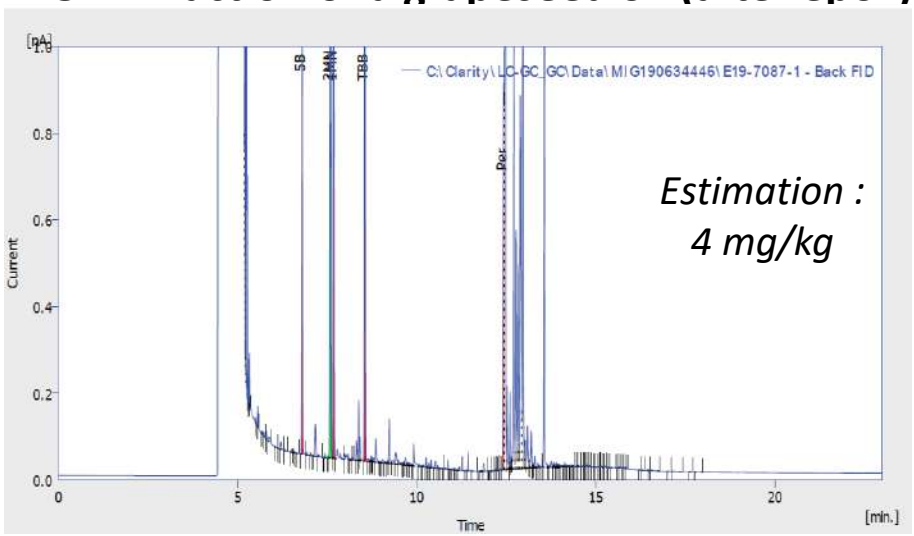
ITERG
Expertise Corps Gras

MOSH & MOAH - Chromatograms without prior cleanup

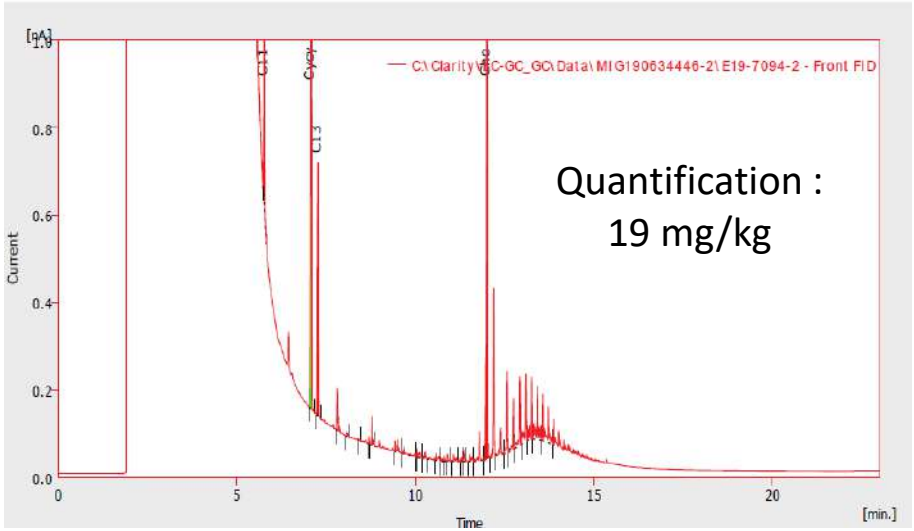
MOSH fraction of a grapeseed oil



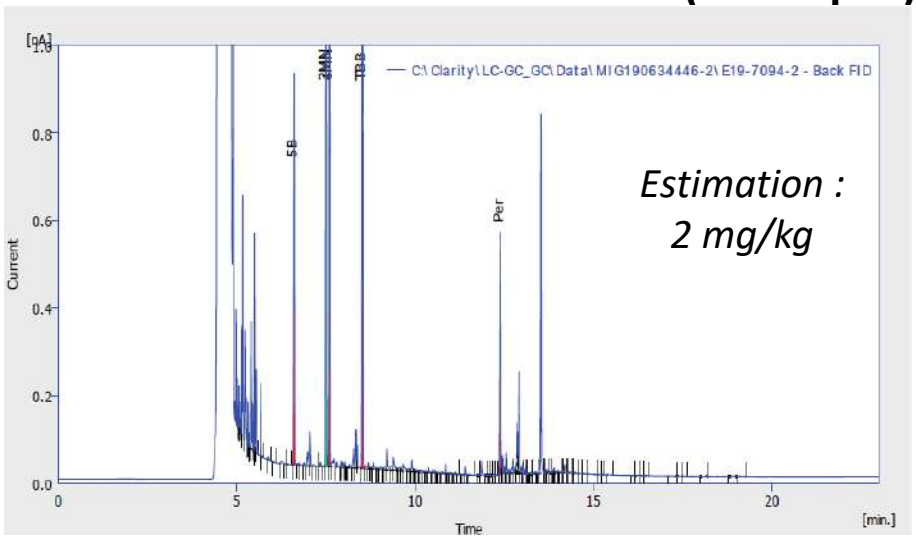
MOAH fraction of a grapeseed oil (after epox)



MOSH fraction of a hazelnut oil



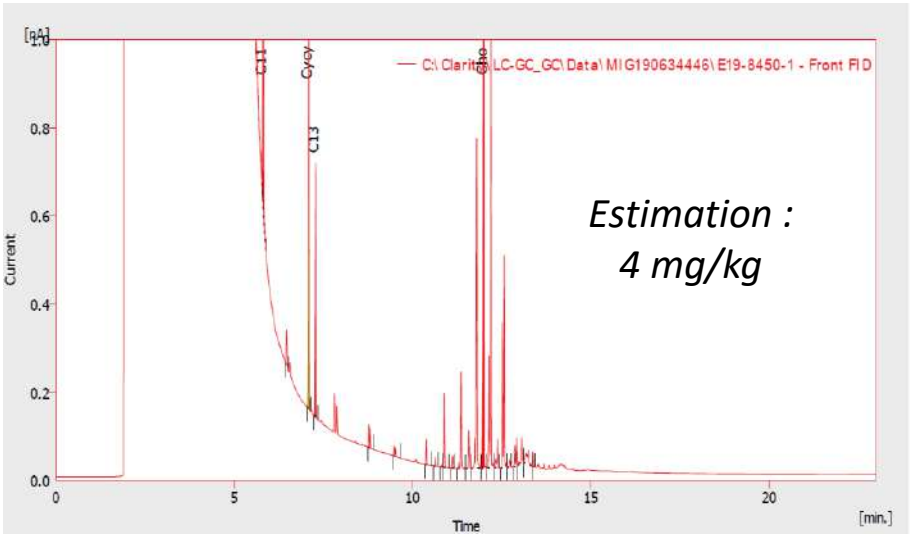
MOAH fraction of a hazelnut oil (after epox)



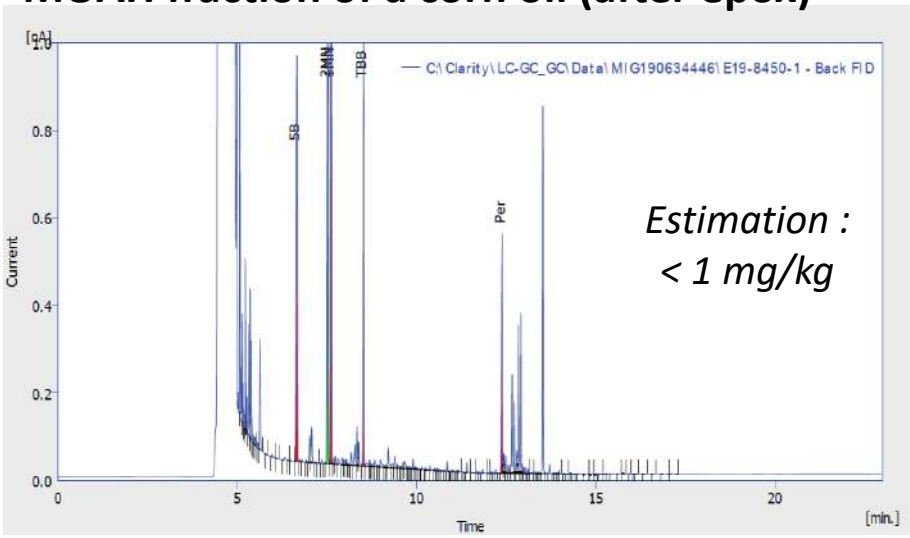
ITERG
Expertise Corps Gras

MOSH & MOAH - Chromatograms without prior cleanup

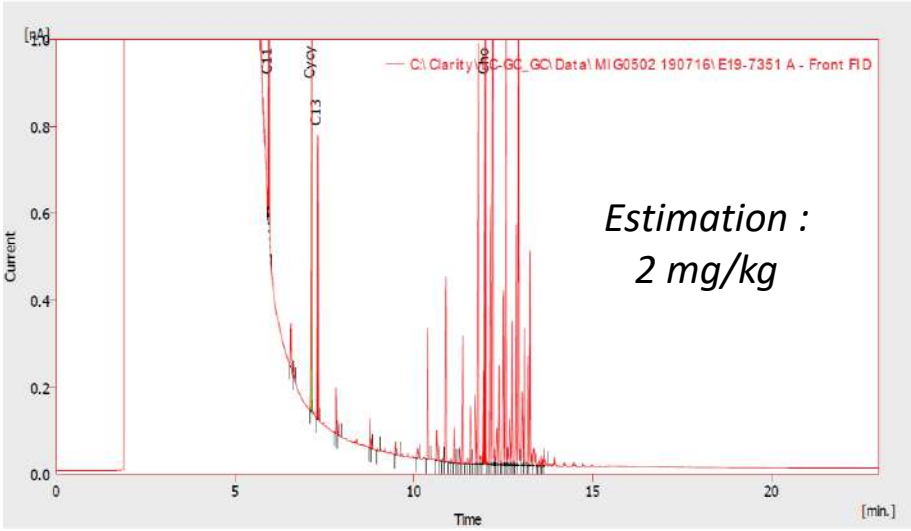
MOSH fraction of a corn oil



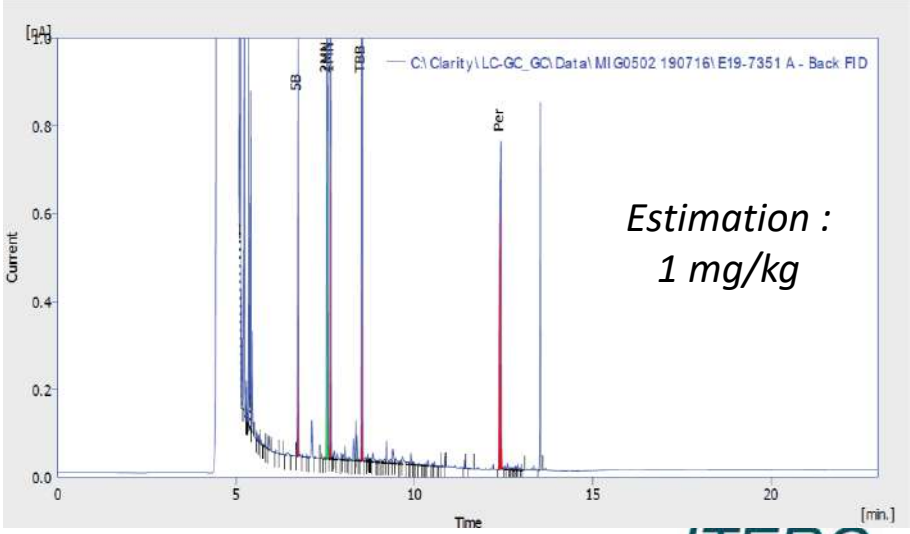
MOAH fraction of a corn oil (after epox)



MOSH fraction of a sesame oil



MOAH fraction of a sesame oil (after epox)



ITERG
Expertise Corps Gras

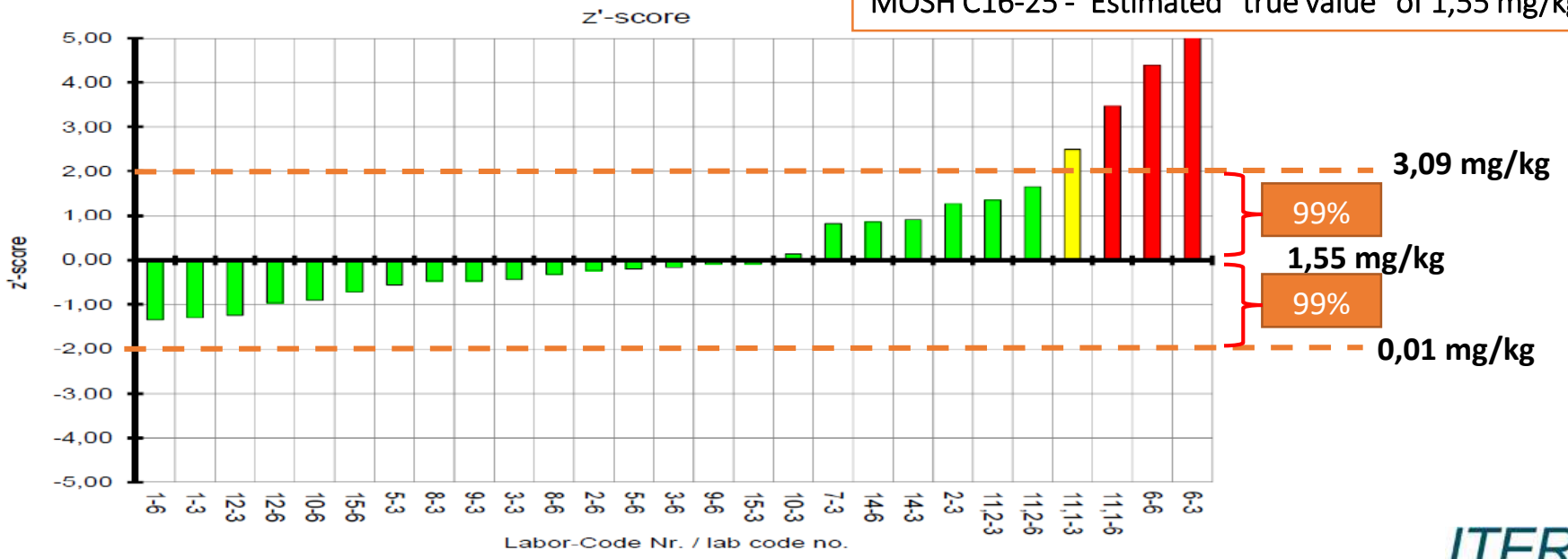
Current situation of the analysis of MOSH & MOAH

Mineral oil hydrocarbons in foods: is the data reliable ? *S. Koster, J. Varela, R. H. Stadler, J. Moulin, C. Cruz-Hernandez, J.Hielscher, C. Lesueur, J. Roïz, H. Simian. Food Additives & Contaminants: Part A (2019)*

DRRR-Proficiency Testing - RVEP 180635 - MO in edible oil (10/2018)

- The acceptable deviation from the estimated "true value" is quite high below 10 mg/kg for both MOSH and MOAH (from76% to 99%)
- Above 10 mg/kg the deviations are below 54%, which is usual for contaminant analysis
- Amounts of MOAH below 2 mg/kg are so difficult to quantify that statistical analysis cannot be performed

Samples 3 & 6 (non-spiked edible oil)
MOSH C16-25 - Estimated "true value" of 1,55 mg/kg



ITERG
Expertise Corps Gras

2019/2020 - Improvement of EN 16995 method

19 laboratories are willing to participate to this work :

- 1 lab from Belgium
- 1 lab from France
- 8 labs from Germany
- 3 labs from Italy
- 1 lab from Spain
- 3 labs from Switzerland
- 2 labs from The Netherlands

Improvement strategies proposed by the participants :

- MOSH: Clean up above ALOX column if needed
- MOAH: Clean up with SILICA column + epoxidation step
- Saponification (hot, KOH) + epoxidation step
- Fractionation of MOSH & MOAH on SILICA/ALOX column
- Epoxidation step (mandatory)
- HPLC on-line clean-up with ALOX after SILICA separation
- Characterization of MOSH or MOAH fraction by GC x GC FID/MS (if needed)
- Confirmation by MS (mandatory)

ITERG
Expertise Corps Gras

Possible improvements of the EN 16995 method

Cleanup for MOSH fraction with aluminium oxide *(already in EN 16995)*

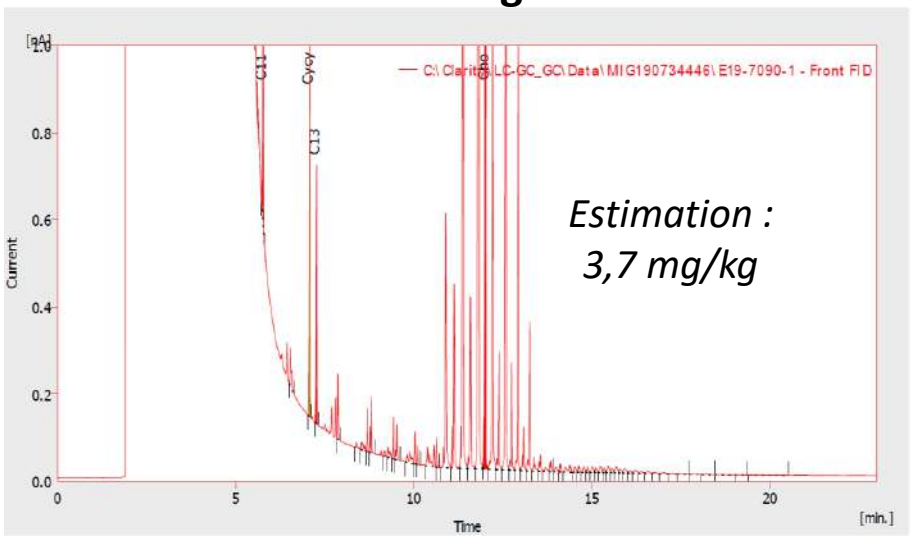
- 1 g of edible oil + internal standard + 2 ml of hexane
- Transfer the mixture to a 10 g of ALOX + 3 g of silica gel column
- Elute the aliphatic hydrocarbon fraction with 25 ml of hexane
- Evaporate the solvent under reduced pressure
- Analyze the sample solution by online-HPLC-GC-FID

Enrichment for MOAH prior to epoxidation (*already in EN 16995*)

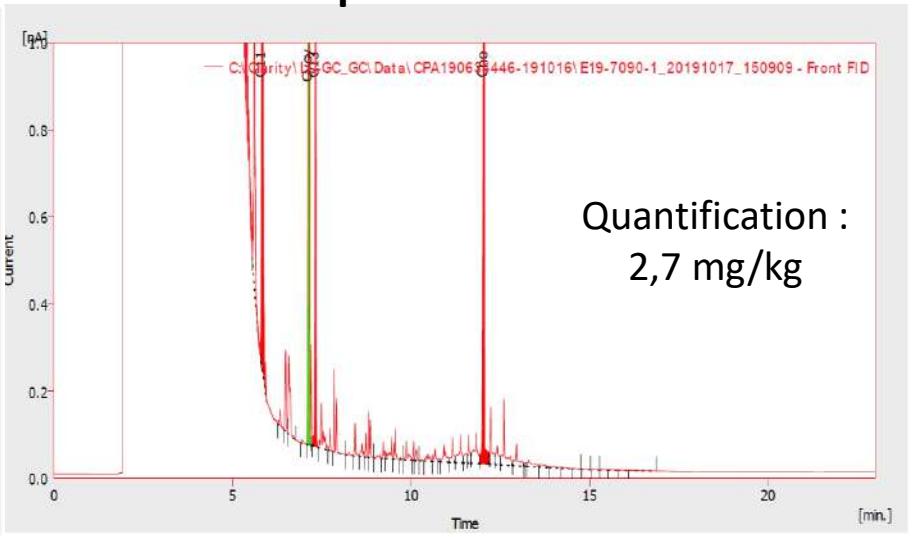
- 1 g of edible oil + internal standard + 2 ml of hexane
- Transfer the mixture to a 12 g silica gel column
- Elute the hydrocarbons with 45 ml of a 20 % mixture of dichloromethane and n-hexane
- Discard the first 10 ml of eluate, collect the subsequent 35 ml in a round flask containing 40 mg of clean sunflower oil
- Evaporate the solvent under reduced pressure
- Proceed to the epoxidation step
- Analyze the sample solution by online-HPLC-GC-FID

MOSH & MOAH : clean-up steps

MOSH fraction of a virgin olive oil - Before and after clean-up of the MOSH fraction

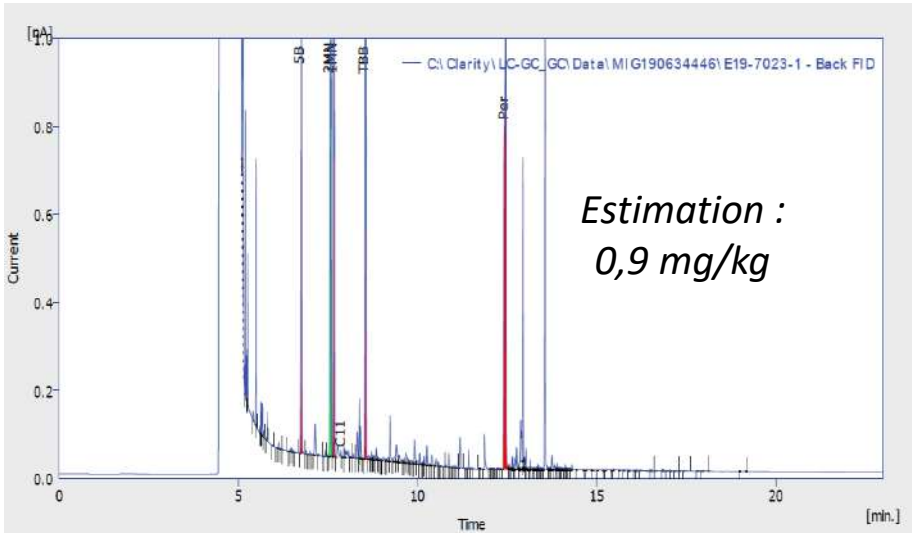


Estimation :
3,7 mg/kg

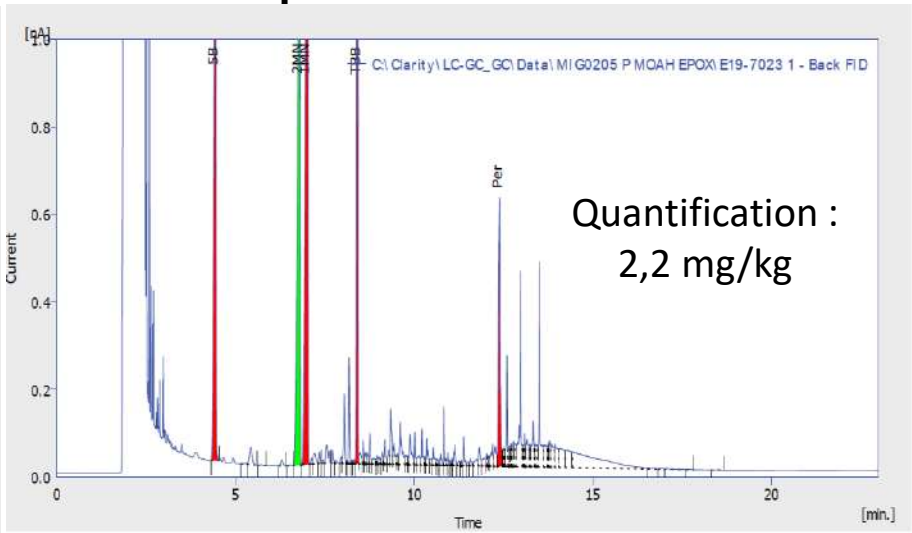


Quantification :
2,7 mg/kg

MOAH fraction of a walnut oil - Before and after clean-up of the MOAH fraction



Estimation :
0,9 mg/kg

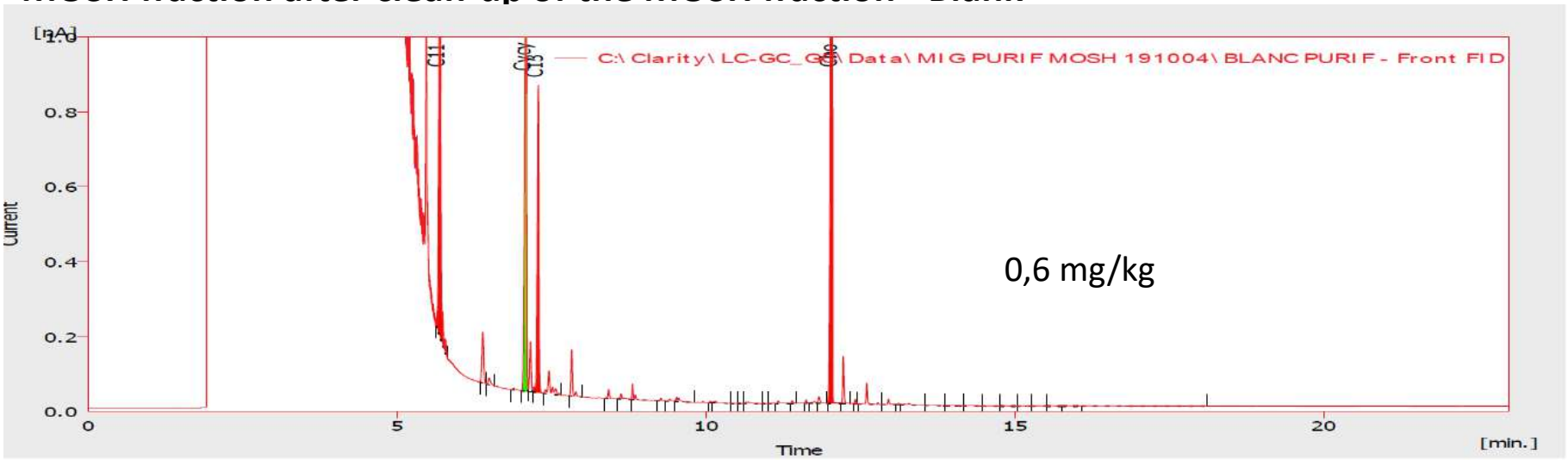


Quantification :
2,2 mg/kg

ITERG
Expertise Corps Gras

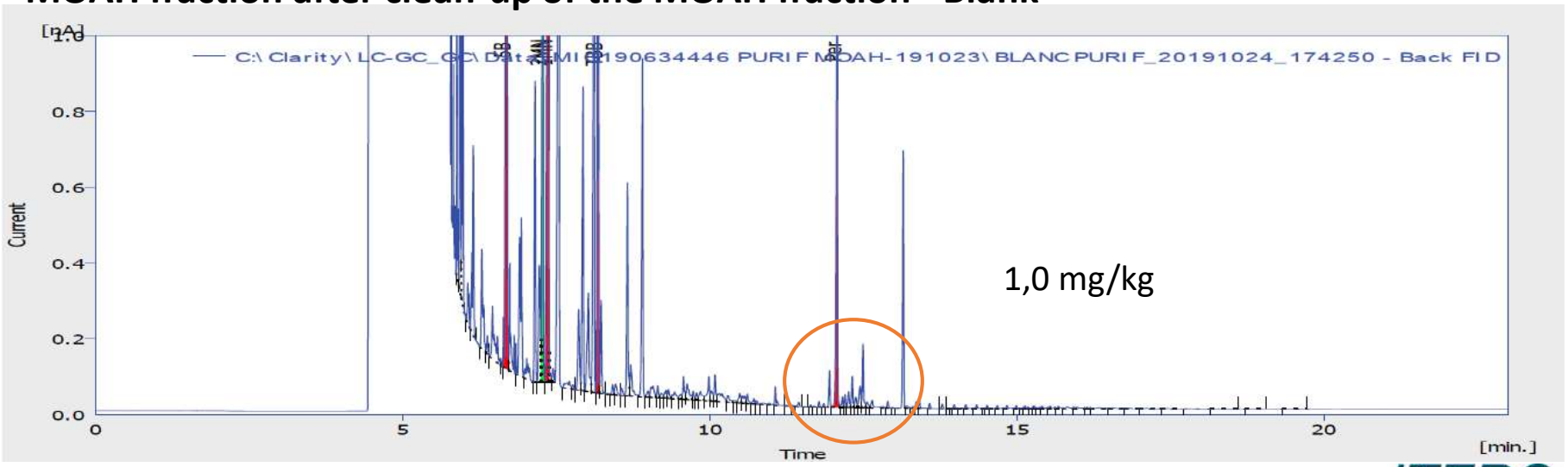
Clean-up steps - Blank levels

MOSH fraction after clean-up of the MOSH fraction - Blank



0,6 mg/kg

MOAH fraction after clean-up of the MOAH fraction - Blank

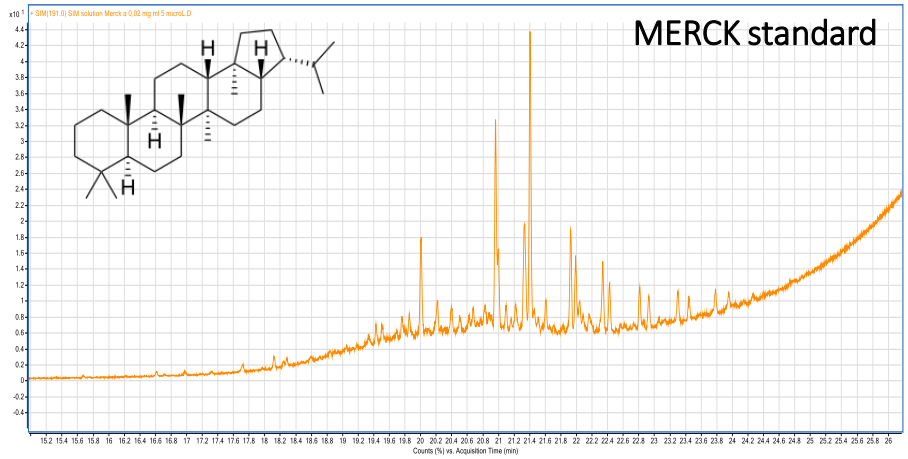


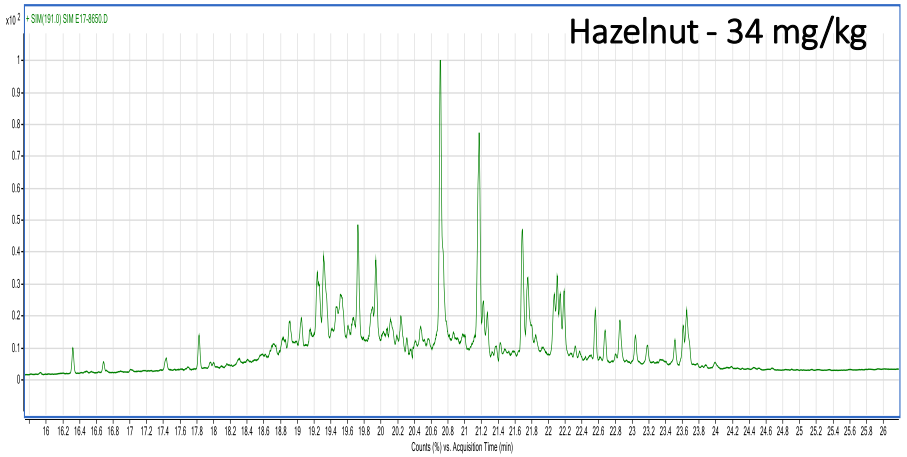
1,0 mg/kg

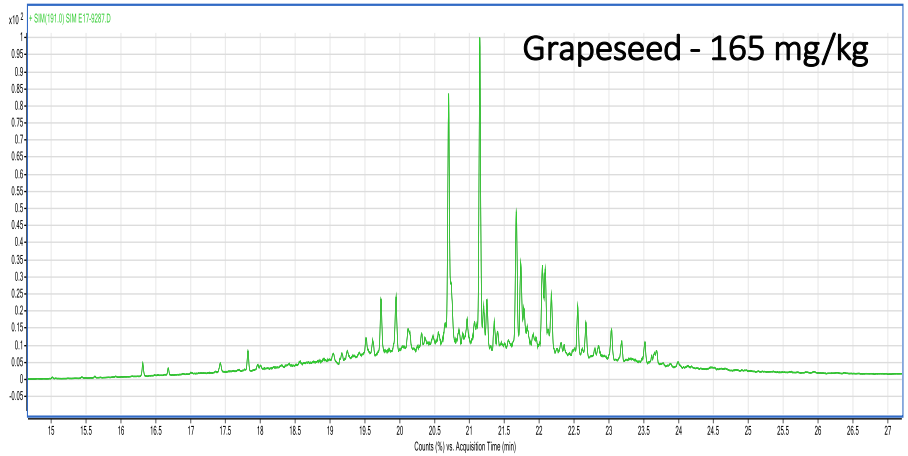
ITERG
Expertise Corps Gras

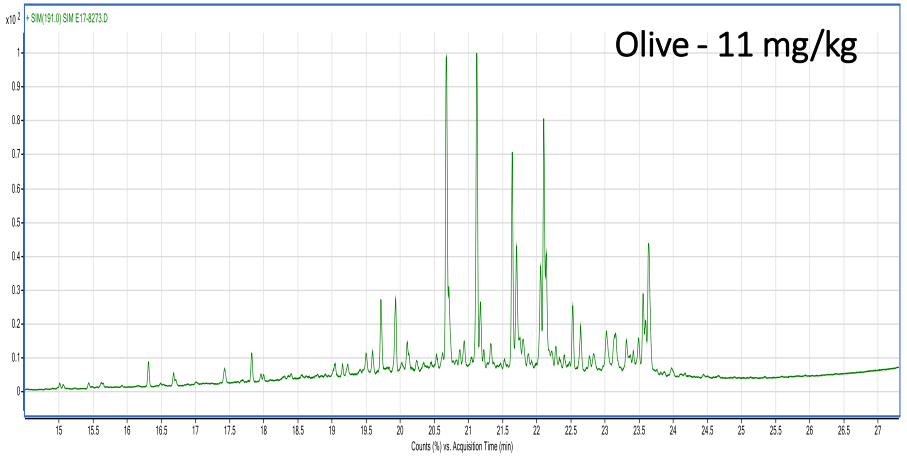
MOSH : Hopanes qualitative analysis in vegetable oils

- Purification of 1 g oil on silica-$AgNO_3$ following ISO 17780 & GC/MS-SIM (m/z 191)









Relative hopane content confirming the mineral origin of hydrocarbons contaminating foods and human milk
T. Populin, M. Biedermann, K. Grob, S. Moret, L. Conte. Food Additives and Contaminants. 2014

ITERG
Expertise Corps Gras

Conclusion

- Mineral oil hydrocarbons are present at different levels in nearly all foods, including in vegetable oils (EFSA, 2012)
- Mineral oil may be introduced at different stages of the vegetable oil and fat production (FEDIOL code of practice for the management of mineral oil hydrocarbons)
- no EU legislation regulating the limits of MOH in vegetable oils (till now) but a recommendation (EU) 2017/84 on the monitoring of mineral oil hydrocarbons in food
- Big pressure from Consumers Association to ban foodstuffs with MOAH content higher than 0,5 mg/kg
- Standardized method MOSH & MOAH published in 2017 with a limit of application at 10 mg/kg for both MOSH & MOAH
- Limit of application might be improved by sample enrichment prior to the online LC-GC/FID analysis

ITERG
Expertise Corps Gras

Many thanks for your attention !

Recherche | Qualité | Innovation | Production

ITERG
11 rue Monge
33610 CANEJAN

Tél : 05.56.36.00.44
iterg@iterg.com
www.iterg.com

The author would like to thank the following collaborators for their participation:

- *Hugues Griffon, ITERG, France.*
- *Ludger Brühl, Max Rubner-Institut (MRI), Germany*
- *Monika Mainka, SGS Institut Fresenius GmbH, Germany*
- *Eileen Schulz, Institut Kirchhoff Berlin GmbH, Germany*
- *Lilli Reinhold, LAVES LVI BS/H, Germany*
- *Franziska Janusch, Eurofins WEJ Contaminants GmbH, Germany*
- *Thomas Funke, Chemisches und Veterinäruntersuchungsamt Münsterland-Emscher-Lippe, Germany*
- *Silke Horst, LUA Dresden, Germany*
- *Sabrina Moret, University of Udine, Italy*
- *Emiliano Castellano, Silliker Italia S.p.A, Italy*
- *Martin Rijkee, Handelslaboratorium Dr. A. Verwey, the Netherlands*
- *Maurus Biedermann, Kantonales Labor Zürich, Switzerland*
- *Thomas Baumgartner, COOP, Switzerland*

UNION EUROPEENNE
Fonds Européen de développement Régional