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Mitigation of mineral oil compounds in edible oils and fats

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Thematic background

- Mineral oil hydrocarbons (MOH) enter the food chain. While MOSH accumulate in the body, some MOAH are suspected to show carcinogenic effects.
- Edible oil may contain especially high amounts of MOH compared to other food.
- Producers want to minimize contamination and examine many samples from their raw materials up to their products.
- Analysis results of different laboratories often showed poor comparability and therefor a reliable basis for important and fast decisions for minimization was missing.

Characterization of sources and pathways

- Step by step monitoring of the whole production chain
- Assessment of the sources
- Model experiments upon the fate of MOH during processing and refining

Development of procedures for elimination of MOH during refining

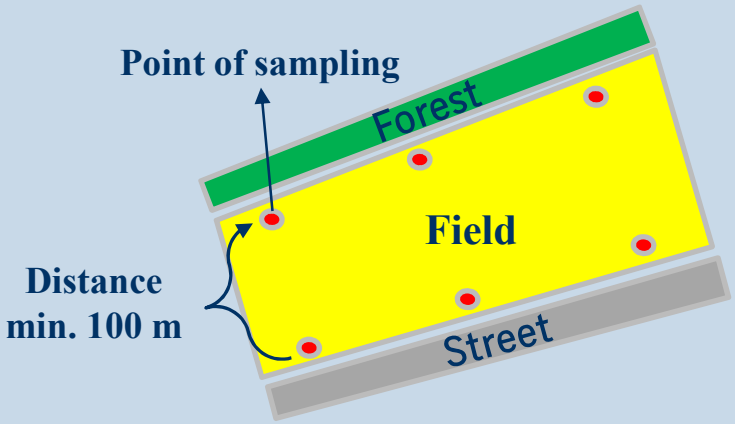
- Adsorbents
- Winterization
- Deodorization
- Control of the combination and scale-up in industry

Improvement of the actual LC-GC method

- Concentration procedure
- Separation of interfering substances
- Validation of the analysis method

- **Supporting information about minimization of MOH**
- **Publication, lectures and seminars**

Assessment of environmental effects on rapeseed contamination (hand picked seeds)

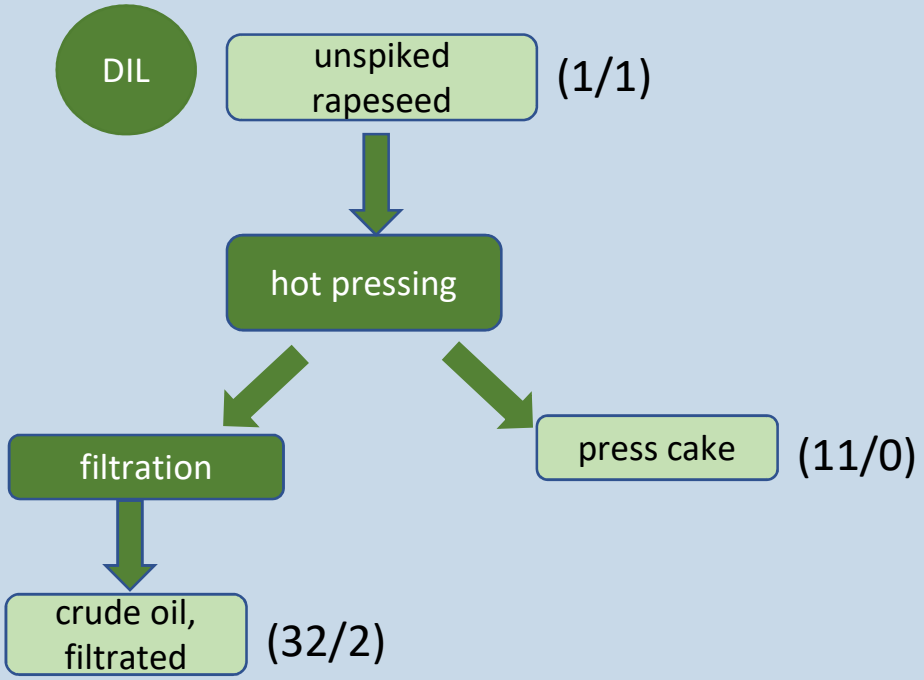


Limit of quantification: 0,5 mg/kg						
Place	Street			Forest		
	No	MOSH (mg/kg)	MOAH (mg/kg)	No	MOSH (mg/kg)	MOAH (mg/kg)
L615	1	< 0,5	< 0,5	1	< 0,5	< 0,5
	2	< 0,5	< 0,5	2	< 0,5	< 0,5
	3	< 0,5	< 0,5	3	< 0,5	< 0,5
L638	1	< 0,5	< 0,5	1	< 0,5	< 0,5
	2	< 0,5	< 0,5	2	< 0,5	< 0,5
Halle	1	< 0,5	< 0,5	1	**1,5**	< 0,5
	2	**0,8**	< 0,5	2	**0,8**	< 0,5
A44	1	< 0,5	< 0,5	1	< 0,5	< 0,5
	2	< 0,5	< 0,5	2	< 0,5	< 0,5
B80	1	< 0,5	< 0,5	1	**1,0**	< 0,5
	2	< 0,5	< 0,5			

Sources of contamination

	Samples (n)	MOH [mg/kg]
Environment (hand picked)	88	<0,1 – 4,1
Cultivation (incl. MOH containing plant protection products)	12	<0,5
Harvest	25	1,6 – 2,6
Transport (truck and ship)	14	1,1 – 3,8
Storage	4	<1 – 2,25
Import	57	<1 – 21
Rapeseed pods (surface)	8	5,7 – 61,3

Hot pressing of clean rapeseeds by a laboratory press



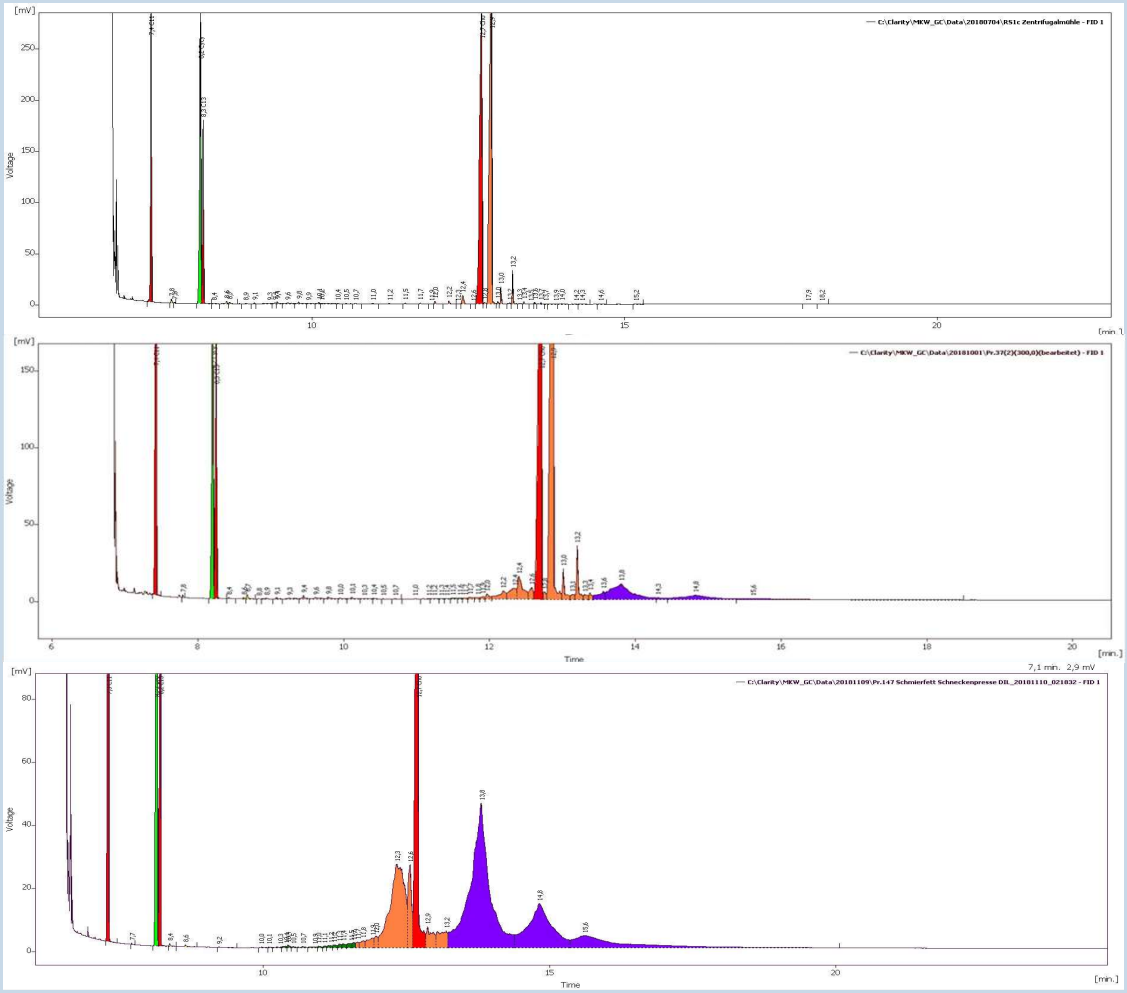
(MOSH/MOAH) in mg/kg

LC-GC-FID Chromatograms of MOSH of extracted seeds and the pressed oil

MOSH, clean seeds
1 mg/kg

MOSH, crude oil
after pressing
32 mg/kg

MOSH, lubricating oil
from screw press



Analysis of lubricating oils by GC-FID, LC-GC-FID, and GCxGC-MS:

- **Collecting of more than 70 lubricating oils from the whole field of the commodity chain of edible oils**

- Some examples:

  -gear oils

  -compressor oils

  -engine oils (used and unused)

  -fluid coupling oils

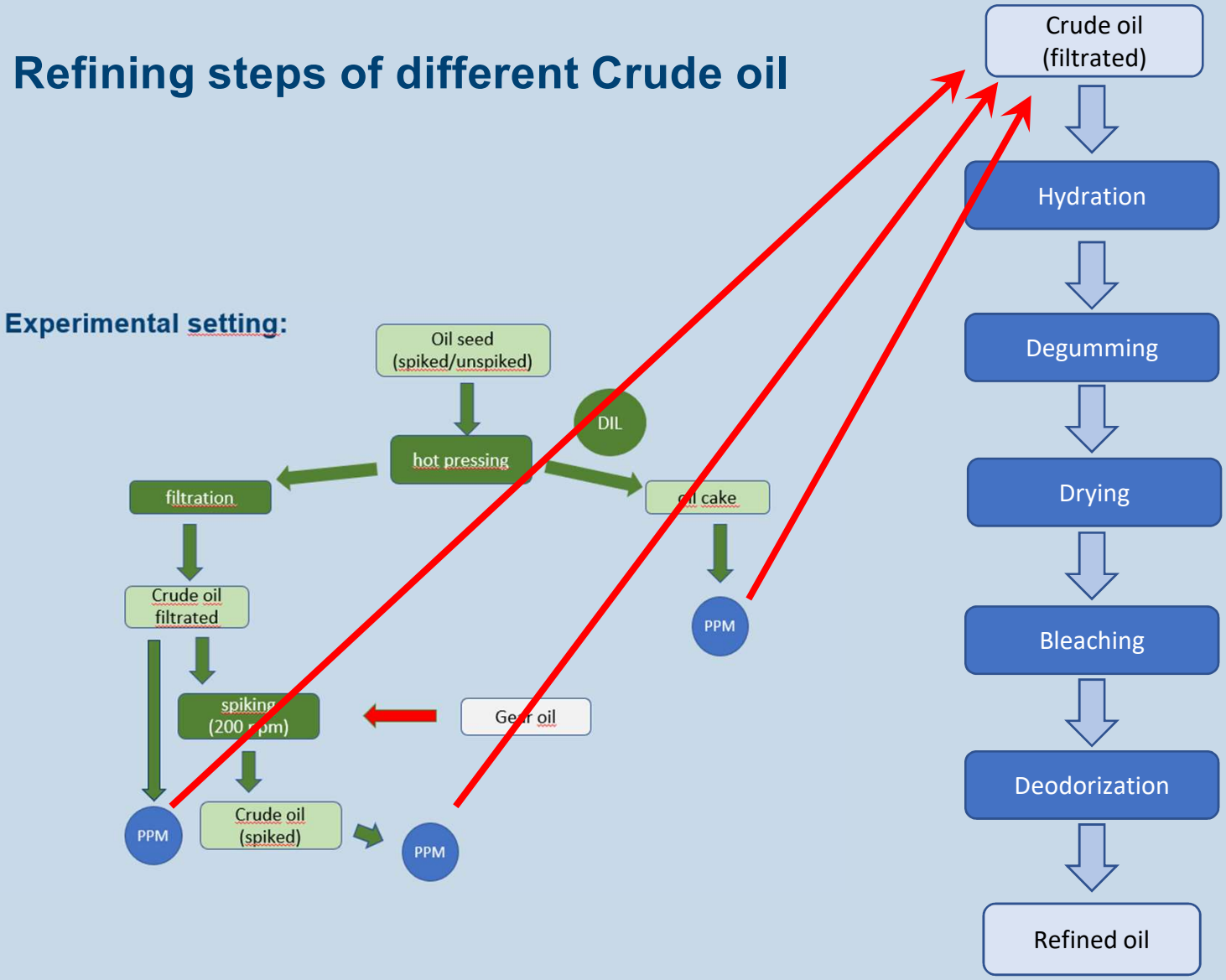
  -servosteering oils

  -bearing food grade lubrication oils

  -synthetic lubrication oils for use in

  -food and pharma industry…

Refining steps of different Crude oil



Parameters of refinig steps

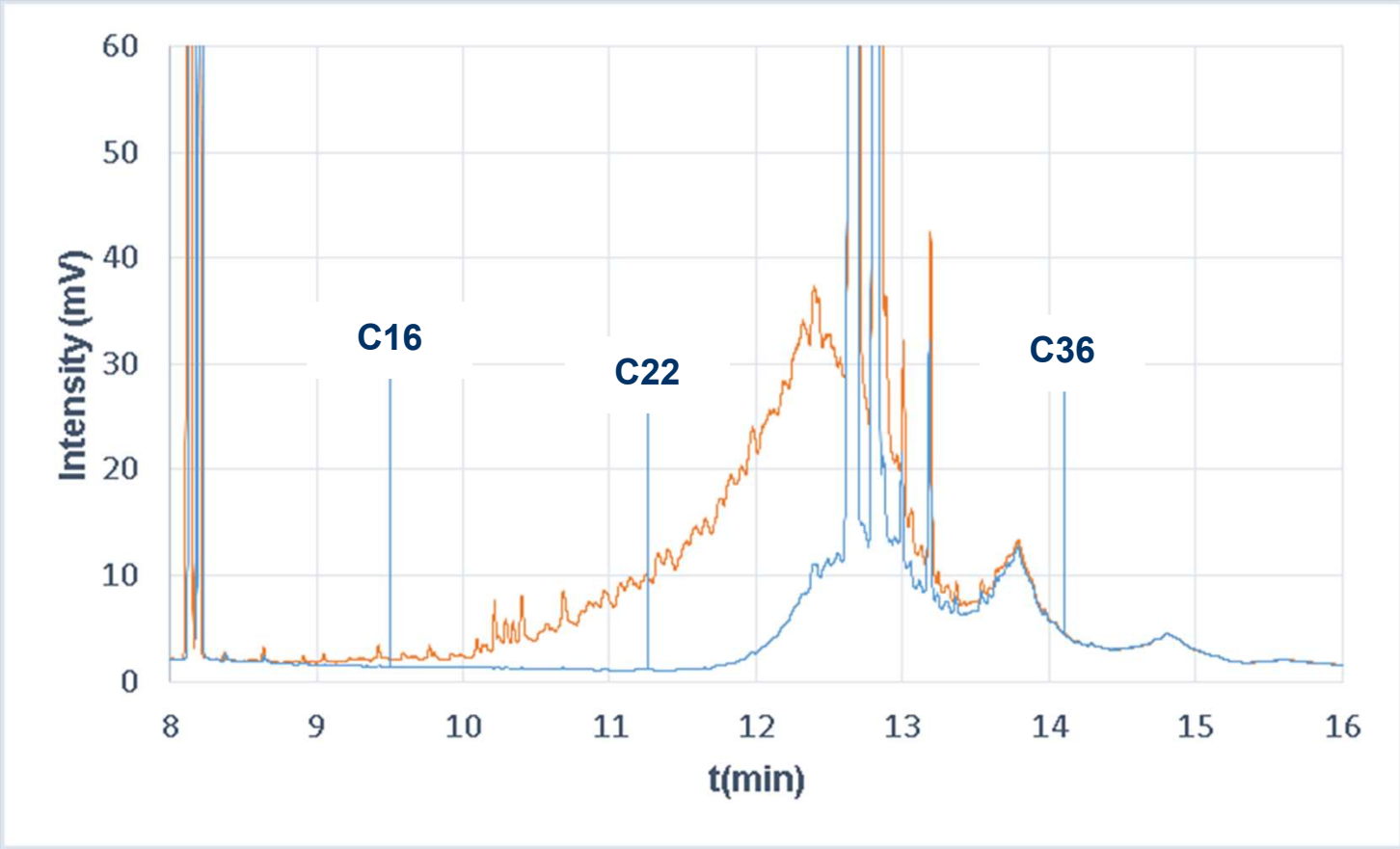
	Hydration	Degumming	Washing	Bleaching
Medium	Deionized water	H_3PO_4(20%)	Deionized water	Tonsil Optimum 210FF
Amount	10%	0,6%	10%	1,0%
Temperature	85°C	85°C	85°C	95°C
Time	45min	30min	20min	20min

	Drying	Deodorization
Medium	-	Steam
Amount	-	ca. 1%/h
Temperature	95°C	240°C
Pressure	Max. 30 mbar	2-3mbar
Time	-	60min

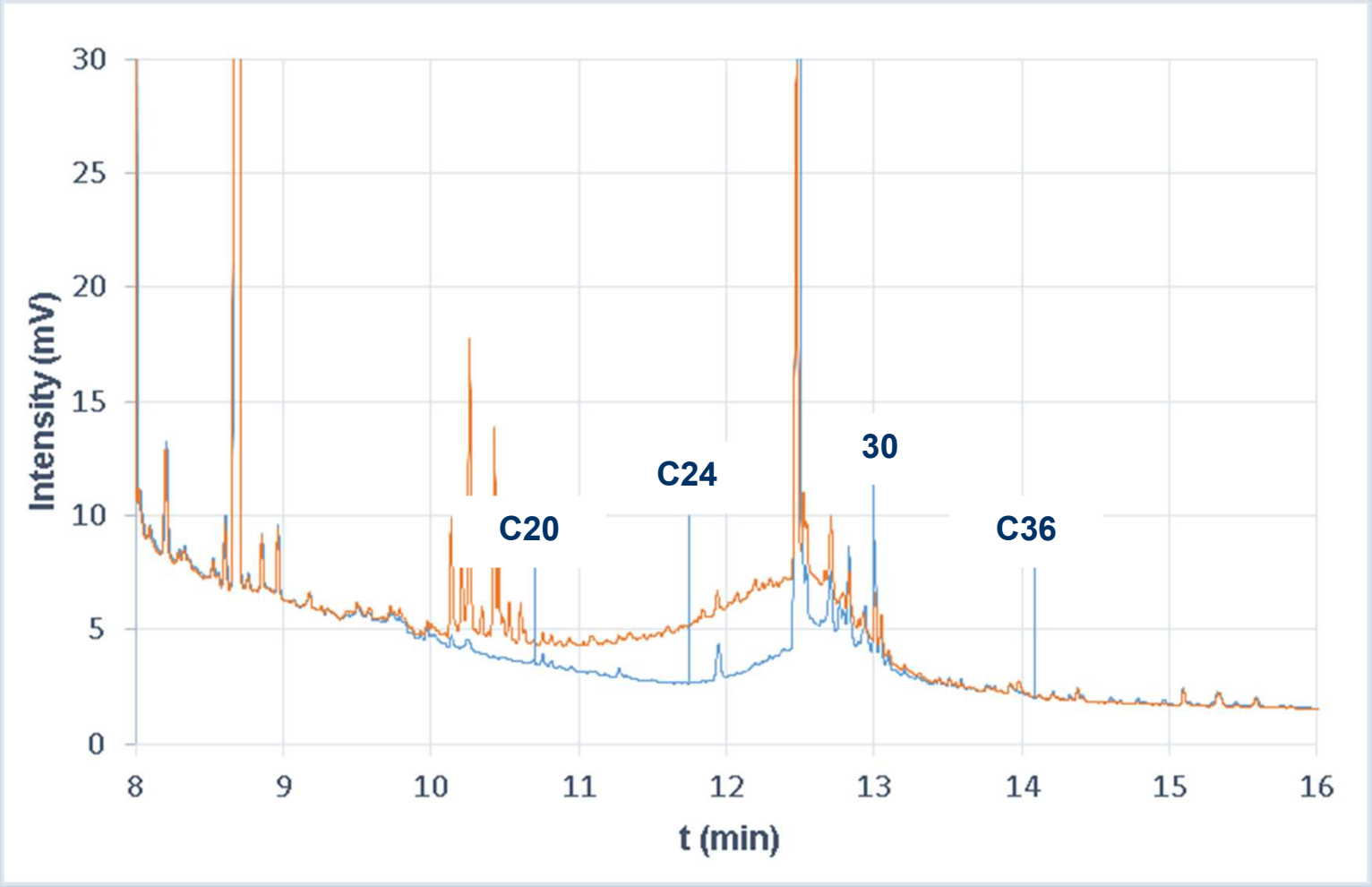
Results for sunflower seed and sunflower oil (after removal of natural n-alkanes by ALOX-treatment and removal of olefins by epoxidation)

Sample	Crude oil (from unspiked seed)		Crude oil (from spiked seed)		Spiked Crude oil	
Treatment	MOSH/PAO [mg/kg]	MOAH [mg/kg]	MOSH/PAO [mg/kg]	MOAH [mg/kg]	MOSH/PAO [mg/kg]	MOAH [mg/kg]
Filtration	11	<1	103	15	336	49
Hydration	11	<1	99	14	344	51
Degumming	11	<1	103	15	347	50
Drying	-	-	103	15	347	49
Bleaching	11	<1	100	18	340	56
Deodorization	9	<1	40	12	166	37

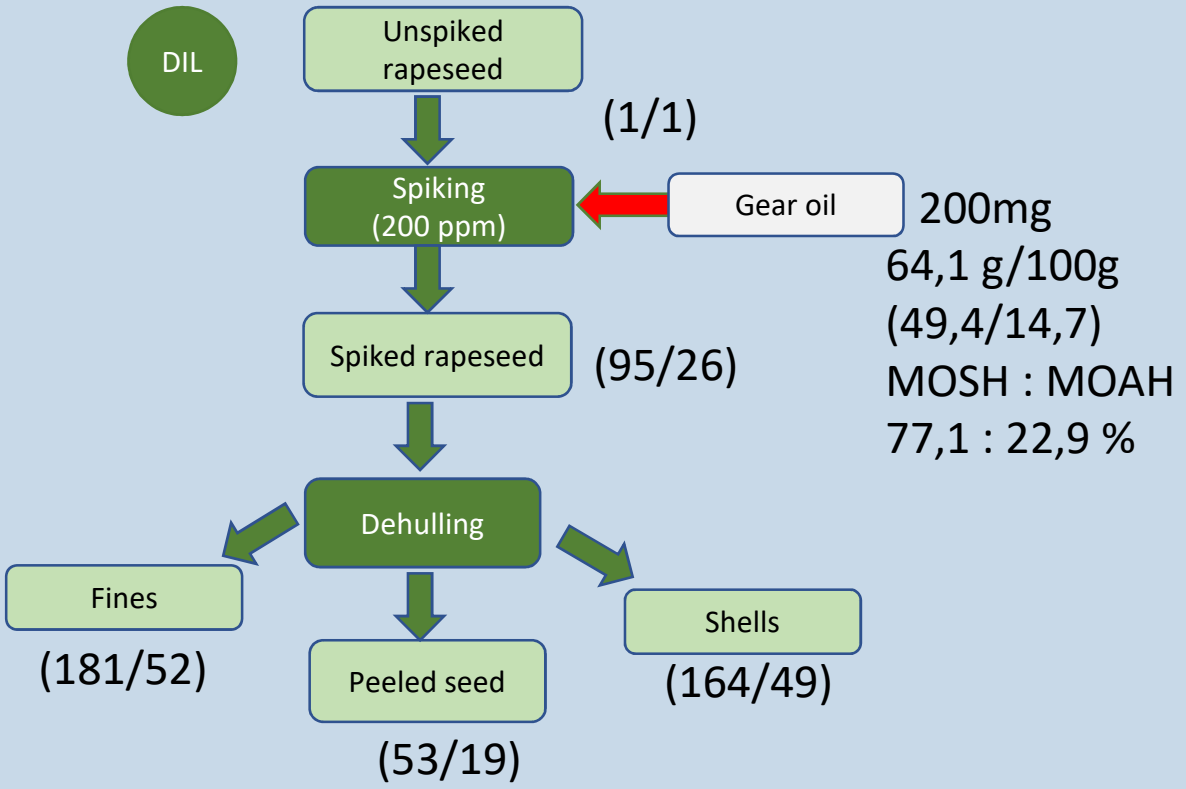
Removal of MOSH during standardized deodorisation



Removal of MOAH during standarized deodorisation

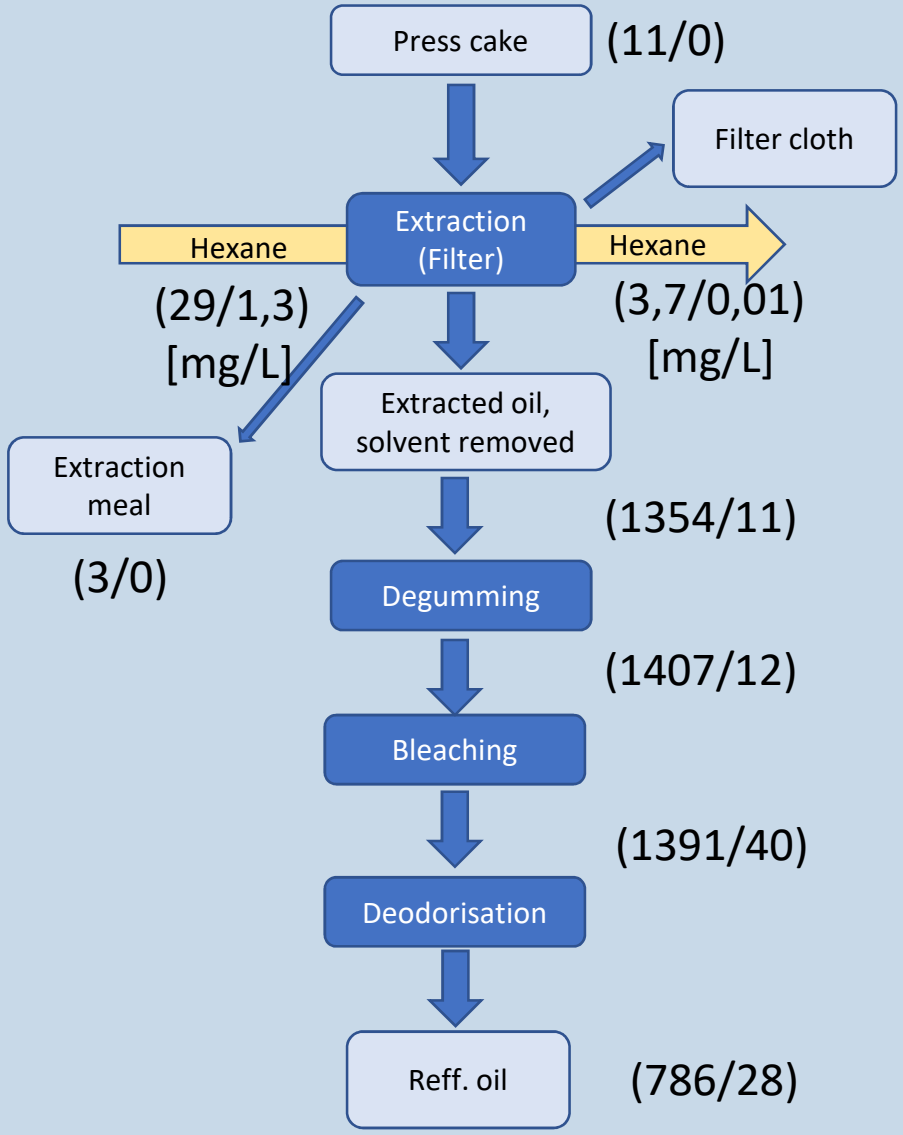


Dehulling and pressing of spiked rapeseed oil

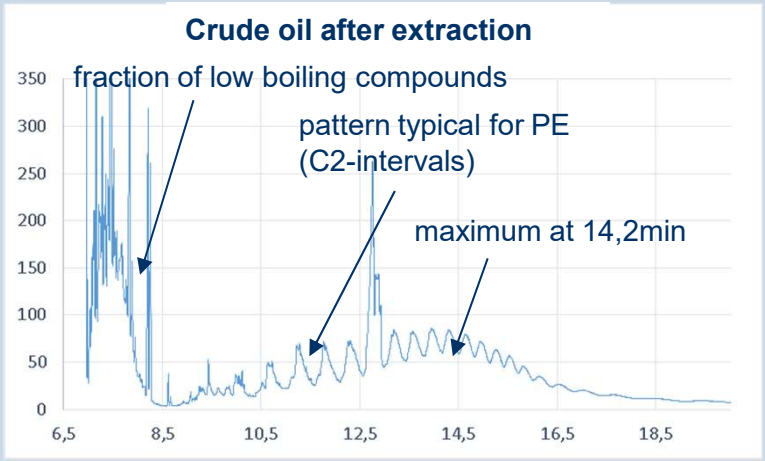


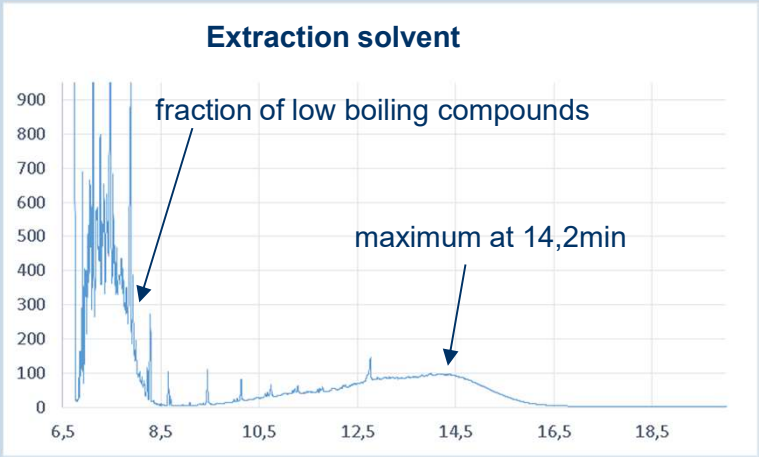
(MOSH/MOAH) in mg/kg

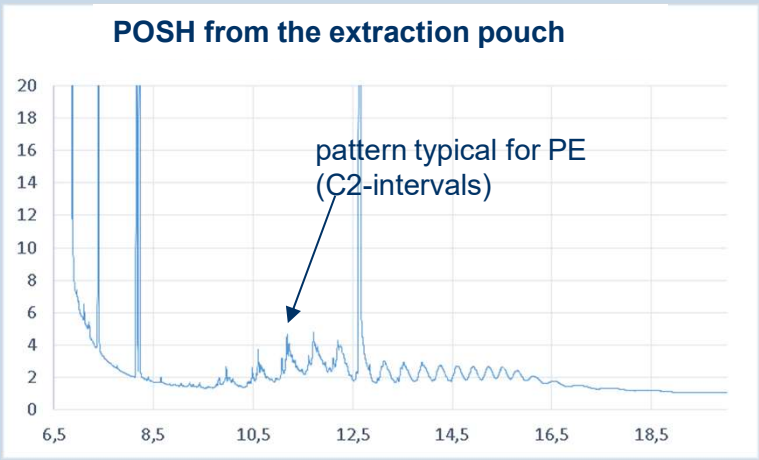
Extraction of press cake from rapeseed and refining of the crude oil



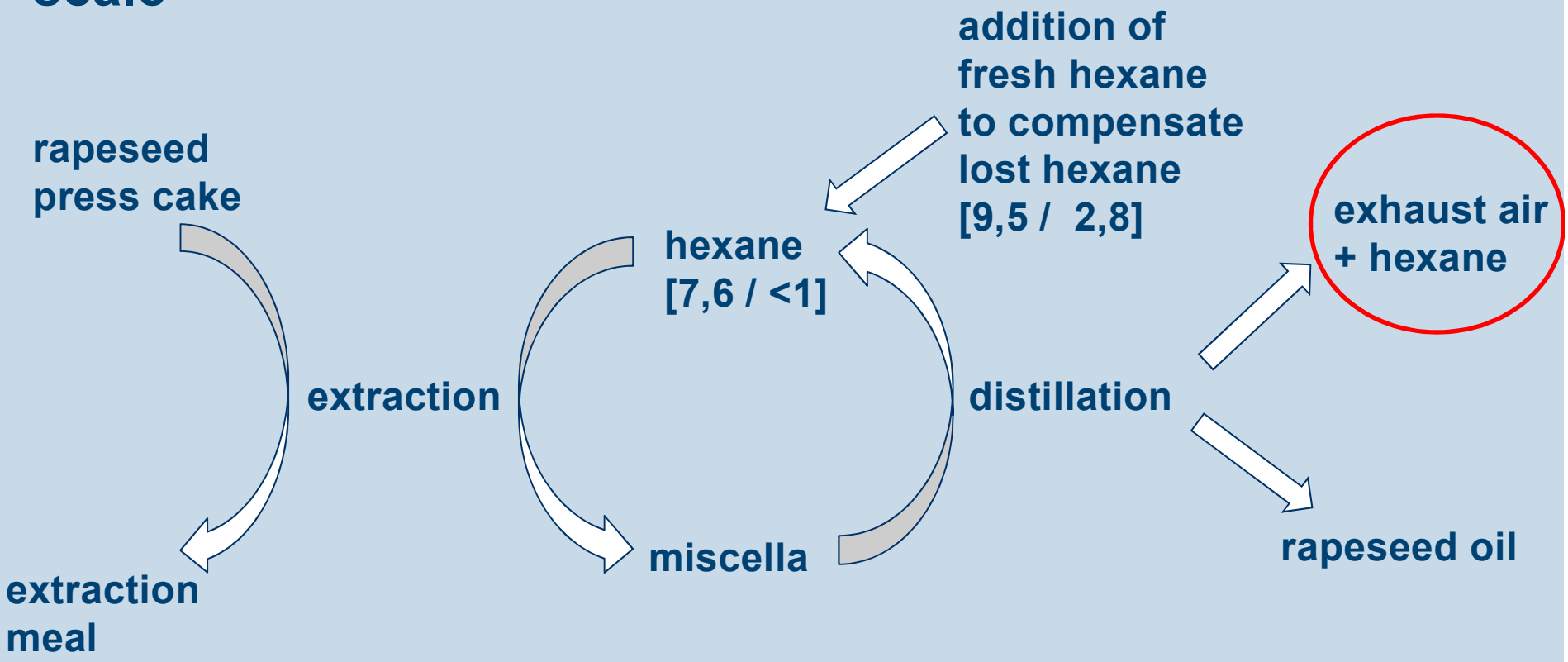
Identification of sources of MOH





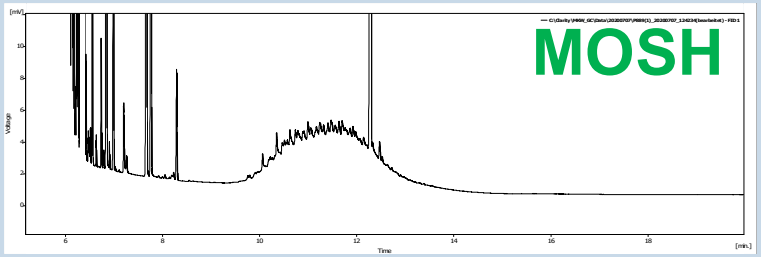


Hexane extraction of rapeseed press cake at industrial scale



[MOSH / MOAH]
[mg/kg / mg/kg]

Rcovery of hexane from exhaust air



clean
exhaust air

white MOH

Counter current
packed column

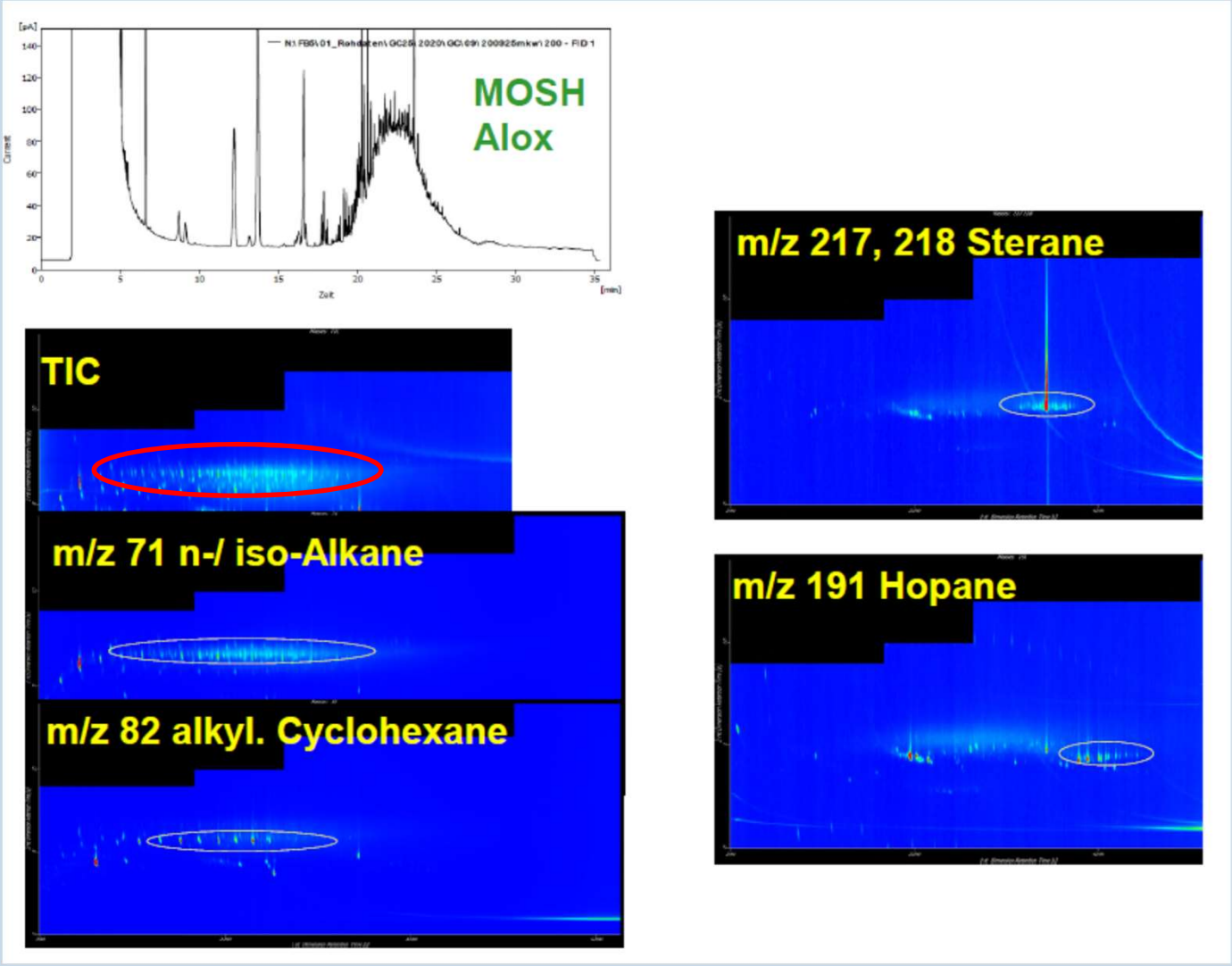
distillation

oil extraction

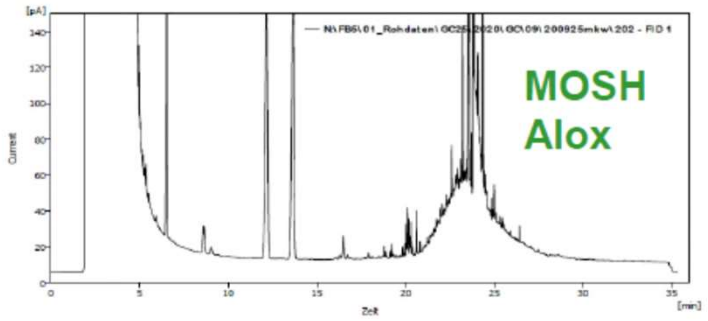
exhaust air
+ hexane

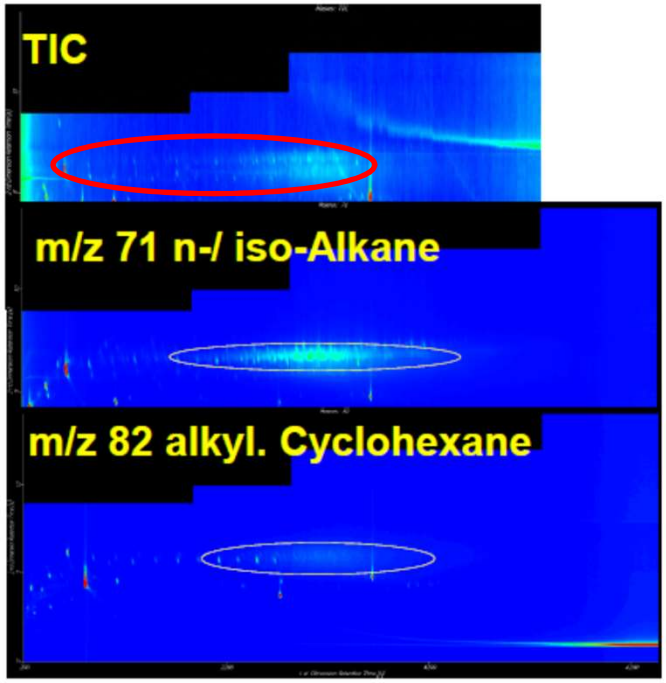
white MOH
+ hexane

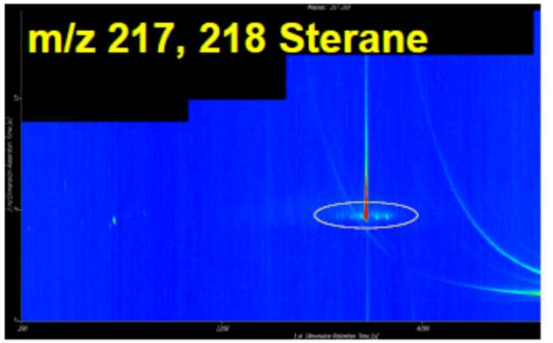
Rapeseed oil before deodorisation, MOSH, with Alox clean-up (no MOAH)

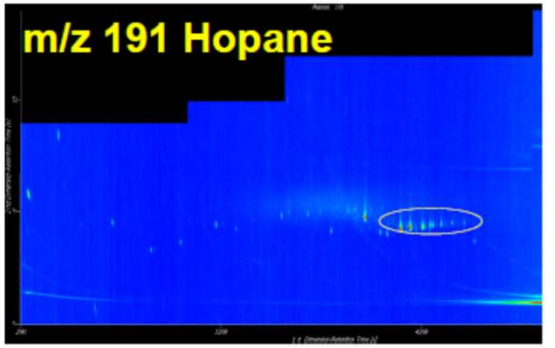


Rapeseed oil after deodorisation, MOSH, with Alox clean-up (no MOAH)









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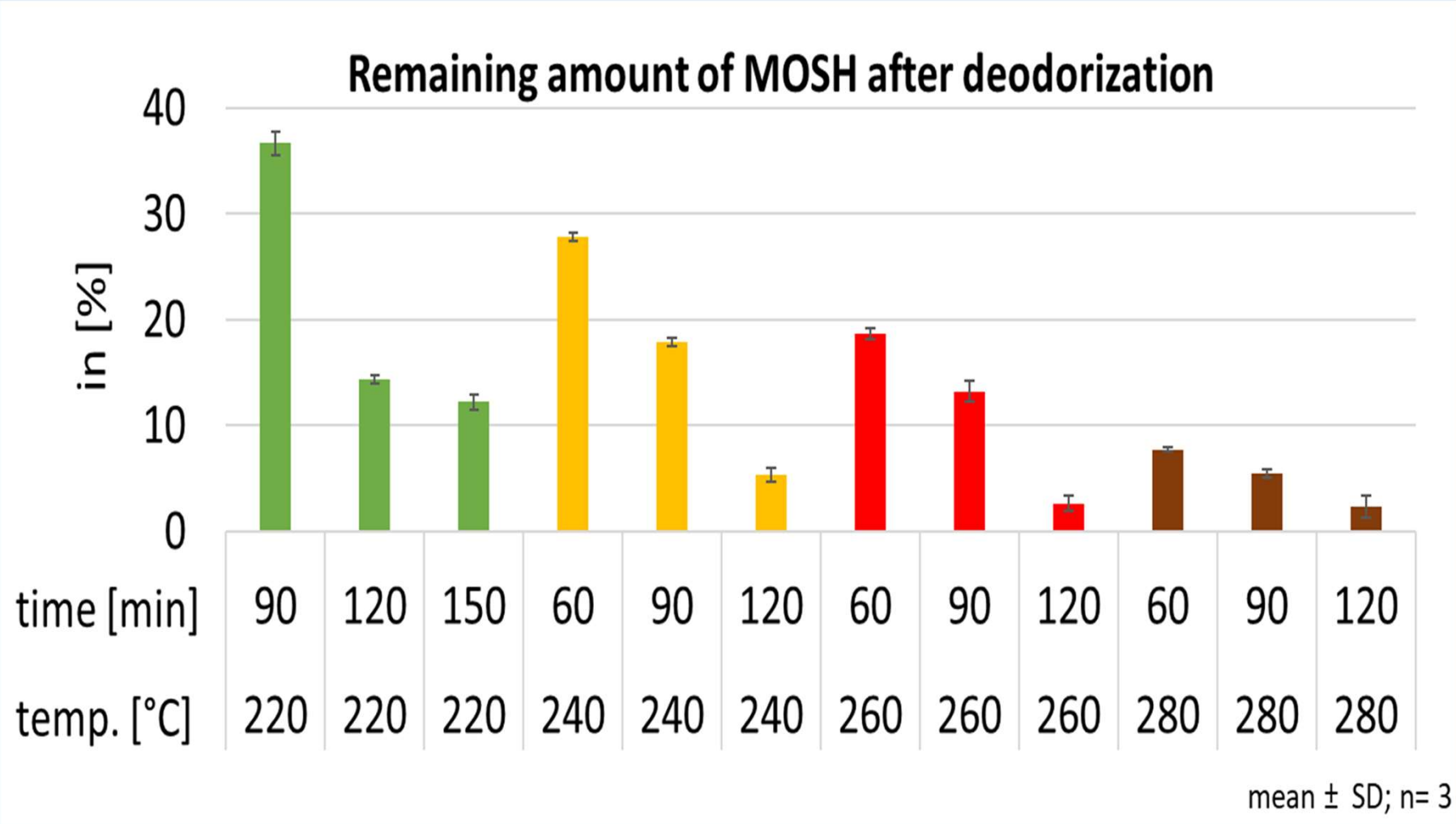
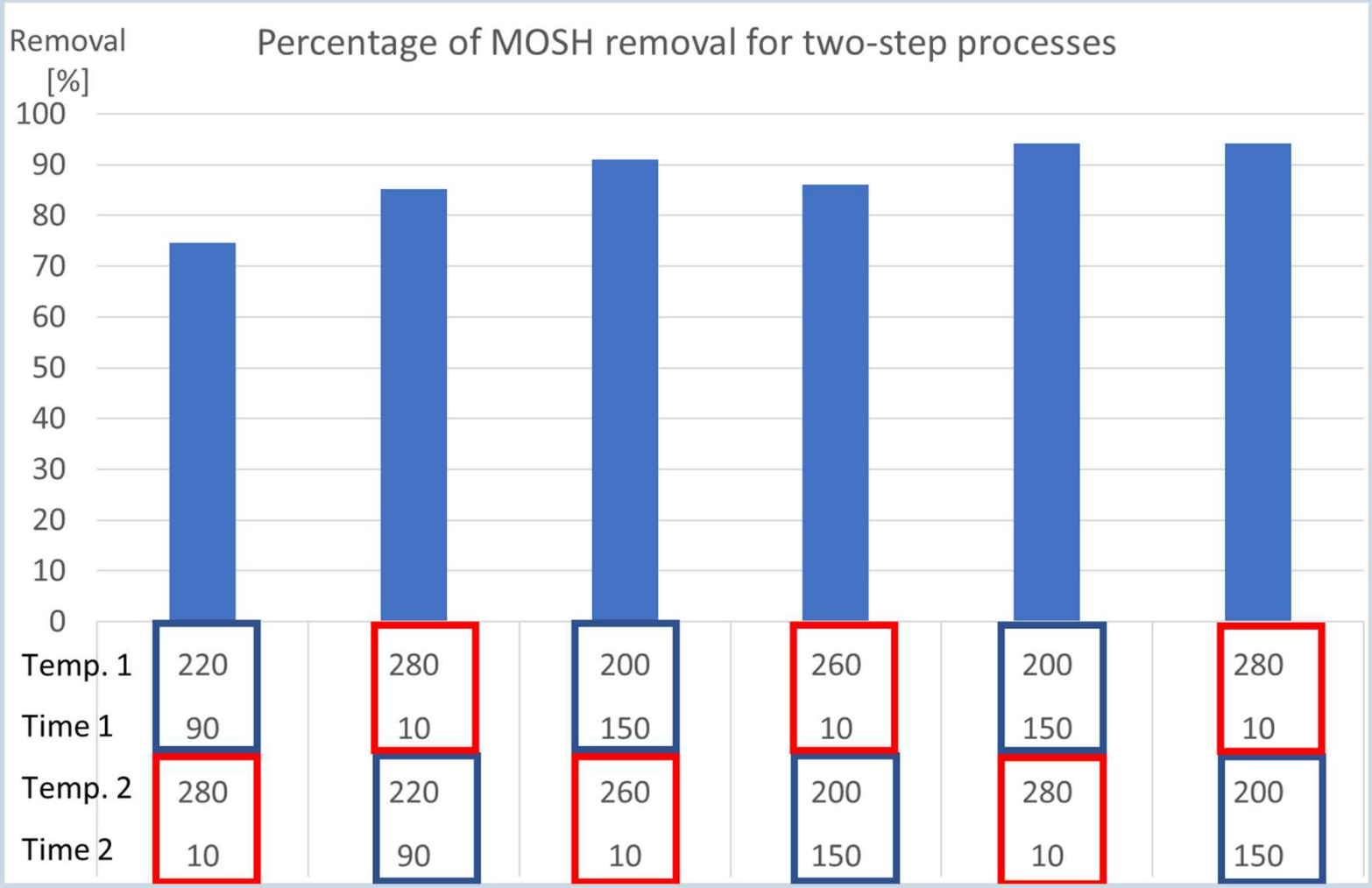
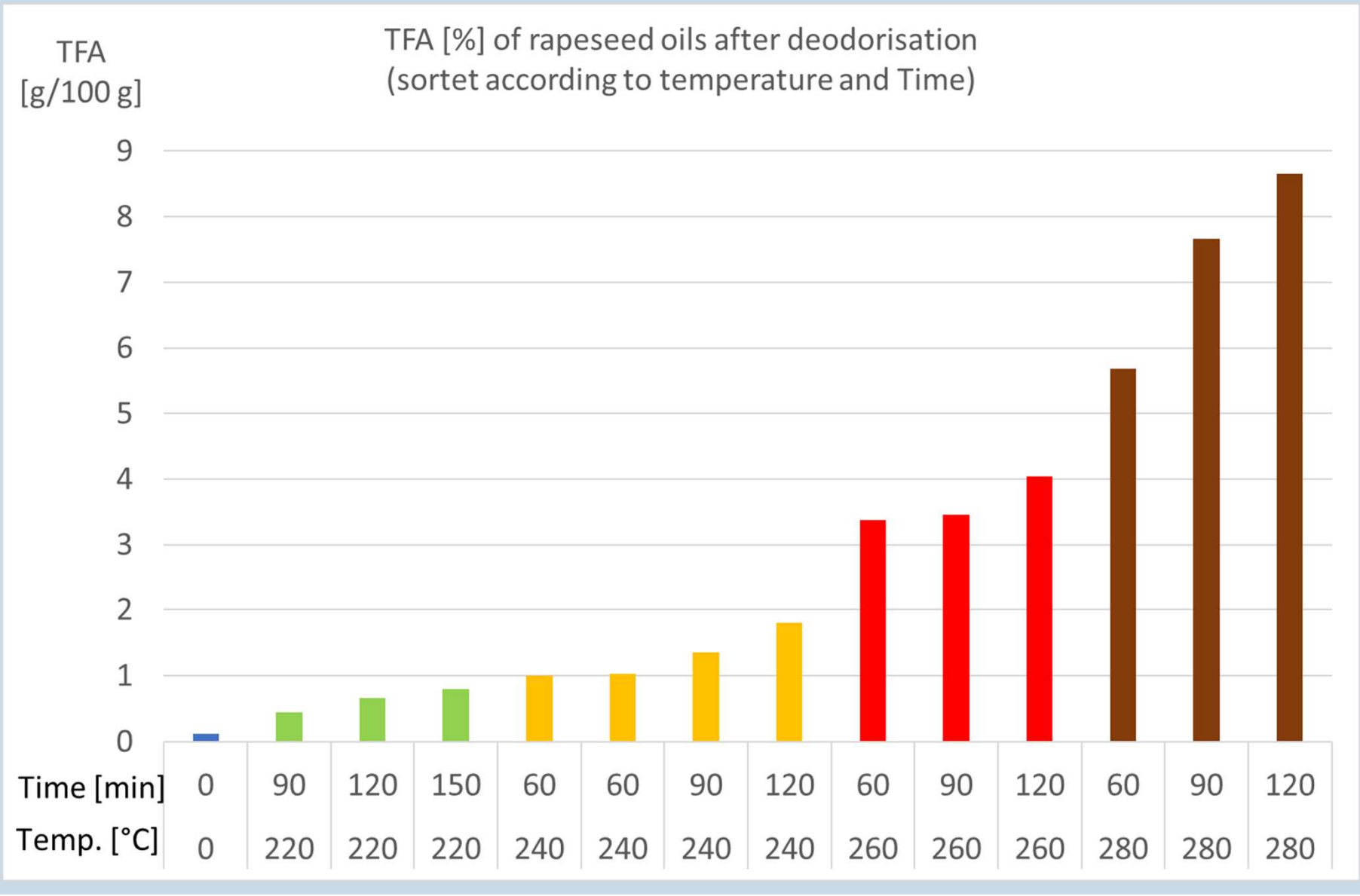
Removal of MOH by deodorisation

Removal of MOSH using different deodorisation parameters

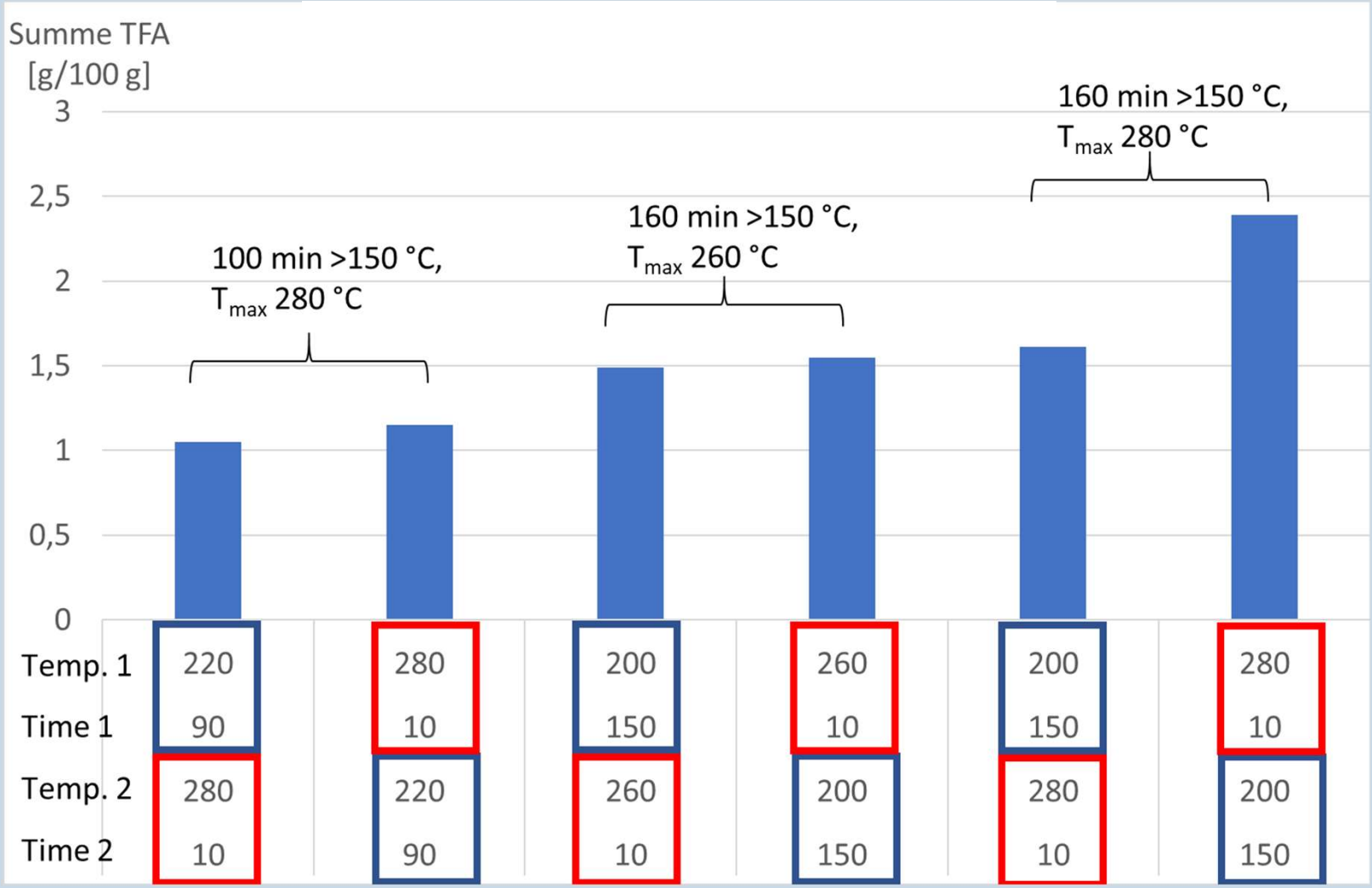


Removal of MOSH using two step deodorisation

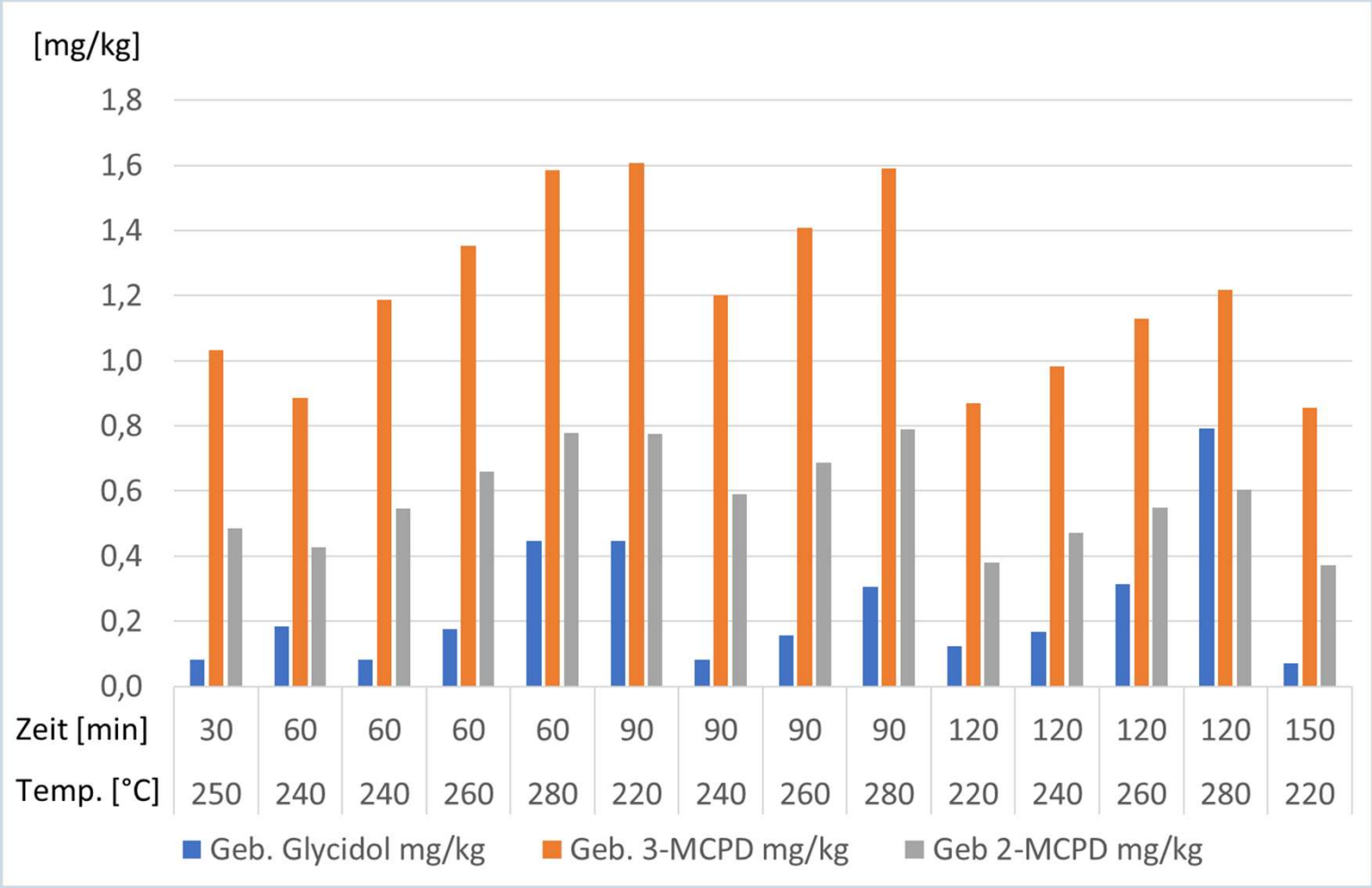



TFA [%] of rapeseed oils after deodorisation
(sortet according to temperature and Time)
TFA
[g/100 g]
9
8
7
6
5
4
3
2
1
0
Time [min] 0 90 120 150 60 60 90 120 60 90 120 60 90 120
Temp. [°C] 0 220 220 220 240 240 240 240 260 260 260 280 280 280

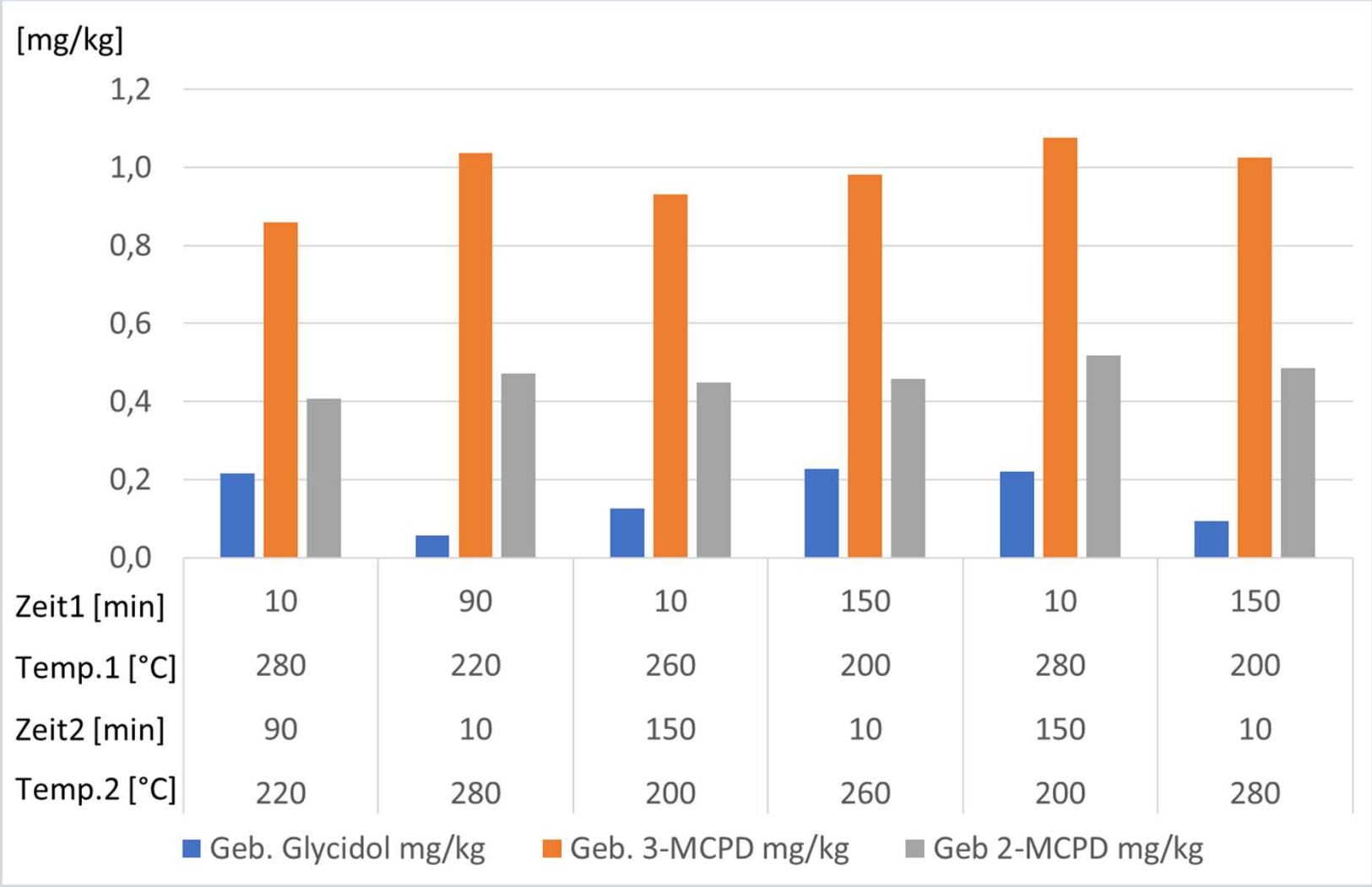
TFA [%] formation during 2-step deodorisation



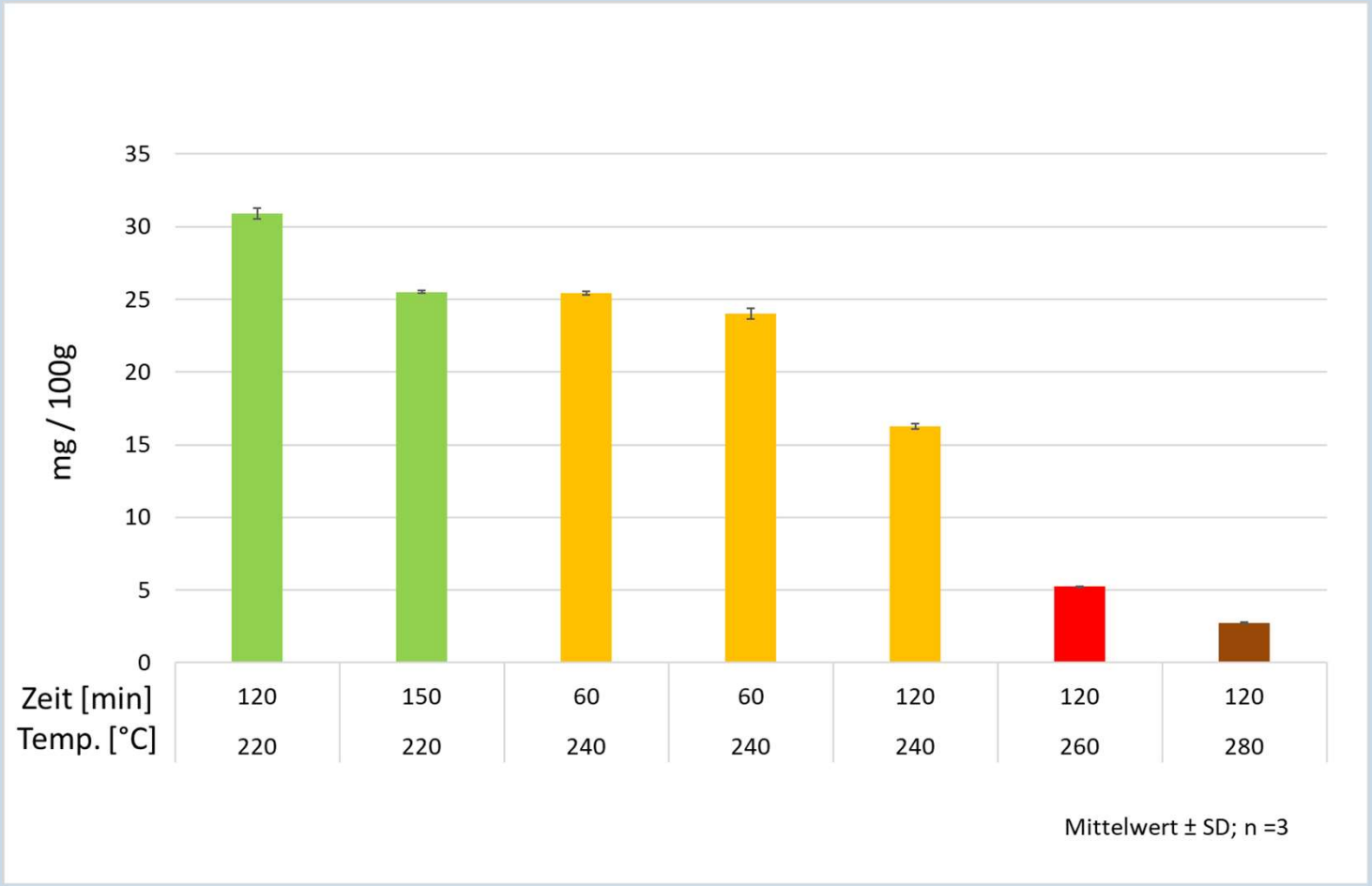
Determination of esterified Glycidol, 3- und 2-MCPD [mg/kg] after one-step deodorisation of rapeseed oil



Glycidol, 3- und 2-MCPD [mg/kg] after 2-step deodorisation



Degradation and removal of tocopherols by one-step deodorisation



Comparison of best one-step and two-step processes

Parameter10.7		Residual MOSH [mg/kg]	MOSH removal [%]	TFA [g/100g]	3-MCPDE [mg/kg]	2-MCPDE [mg/kg]	GLYCE [mg/kg]	Toc. [mg/kg]
1-Step	280°C 90min	7	95	5,6	1.6	0.8	0.3	11.7
1-Step	260°C 120min	3	97	4,0	1.1	0.5	0.3	4.3
1-Step	240°C 120min	7	95	1,8	1.0	0.5	0.2	13.6
2-Step	200°C 150min 280°C 10min	7	94	2,5	1.1	0.5	0.2	10.7
2-Step	200°C 150min 260°C 10min	11	91	1,5	0.9	0.4	0.1	15.8

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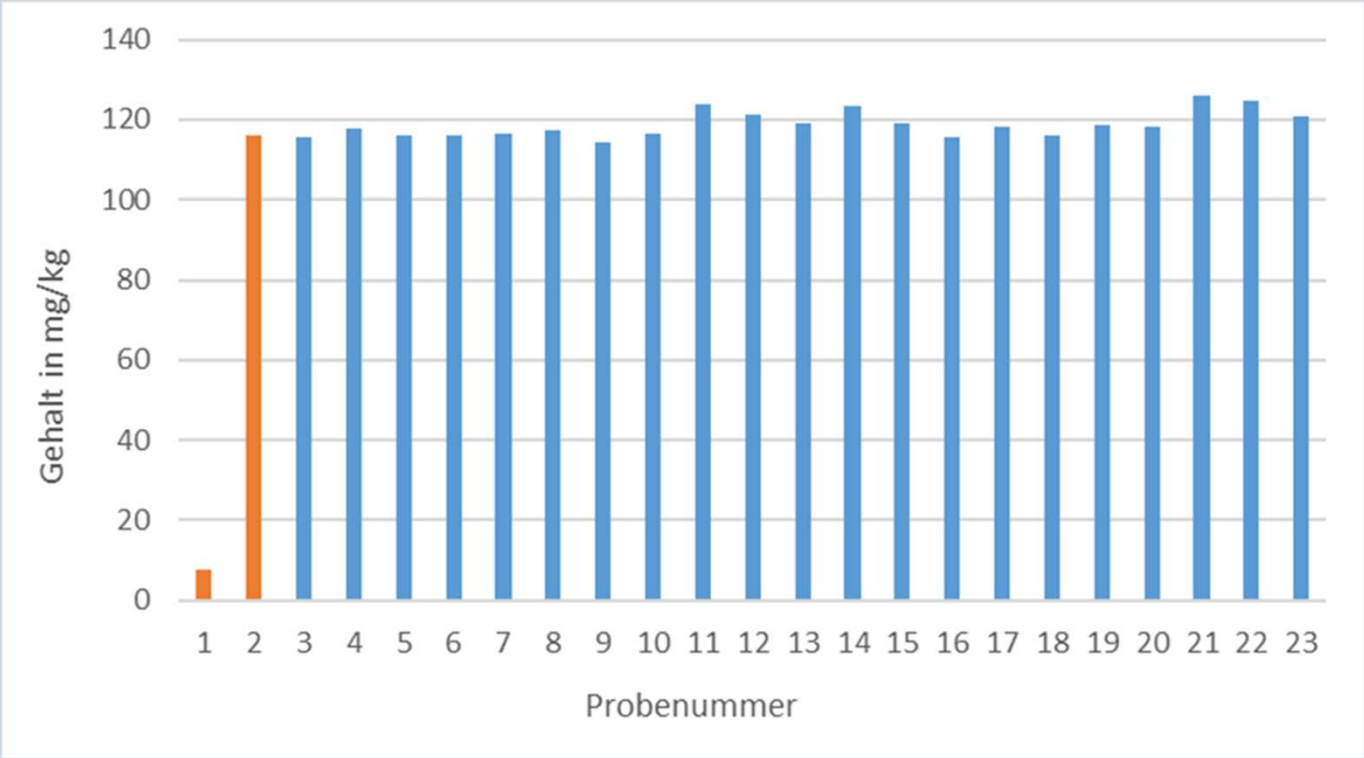
Removal of MOH by adsorption materials

Applied adsorptive materials

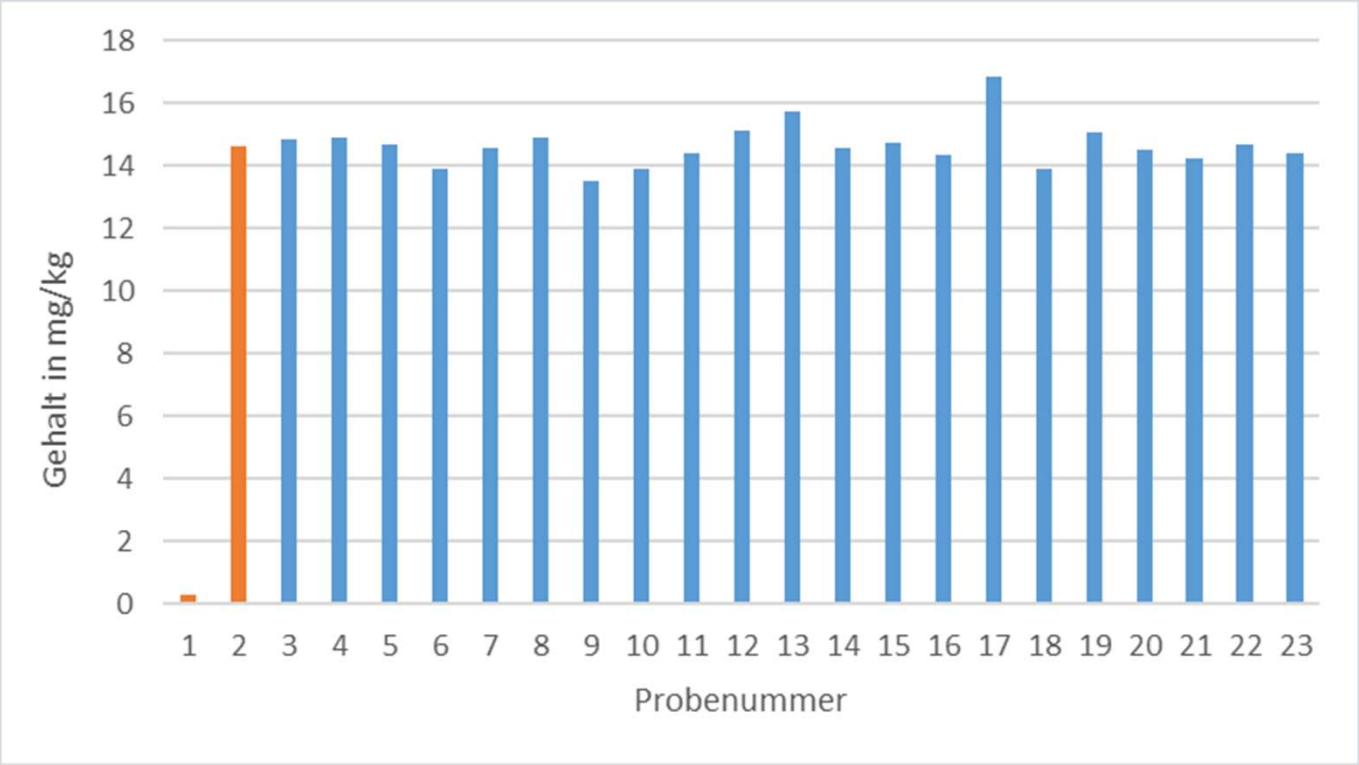
Probenbezeichnung	Lieferant / Hersteller	Spezifikationen
D60	Dallas Group	Synth. Magnesium-Silikat
1000R	Dallas Group	Synth. Magnesium-Silikat
1500R	Dallas Group	Synth. Magnesium-Silikat
2000R	Dallas Group	Synth. Magnesium-Silikat
Aluminium oxide 60	Merck KGaA	Aluminiumoxid, basisch
ColorSorb-XFP21_PAC	Jacobi Carbons GmbH	Aktivkohle
ColorSorb-CP1_PAC-S	Jacobi Carbons GmbH	Aktivkohle
ColorSorb-5000-PAC-S	Jacobi Carbons GmbH	Aktivkohle
ColorSorbXFP PAC-S	Jacobi Carbons GmbH	Aktivkohle
Köstrolith, 13X P-TR	Chemiewerk Bad Köstritz	Zeolith, getrocknet
Köstrolith, 13X P-calc.	Chemiewerk Bad Köstritz	Zeolith, calciniert
TriSyl	GRACE GmbH, Worms	Siliziumoxid (99,9%)
Celite, Silasorb	Lehmann & Voss & Co	Calcium-Silikat
Celite, Celkate T-21	Lehmann & Voss & Co	Magnesium-Silikat
MINCLEAR NQ 50	TOLSA S.A.	Sepiolith
MINCLEAR N 180	TOLSA S.A.	Sepiolith
MINCLEAR N 280	TOLSA S.A.	Sepiolith
MINCLEAR S 210	TOLSA S.A.	Sepiolith
MINCLEAR NC160	TOLSA S.A.	Sepiolith

Contents of MOSH in mg/kg detected in spiked oils after adsorption

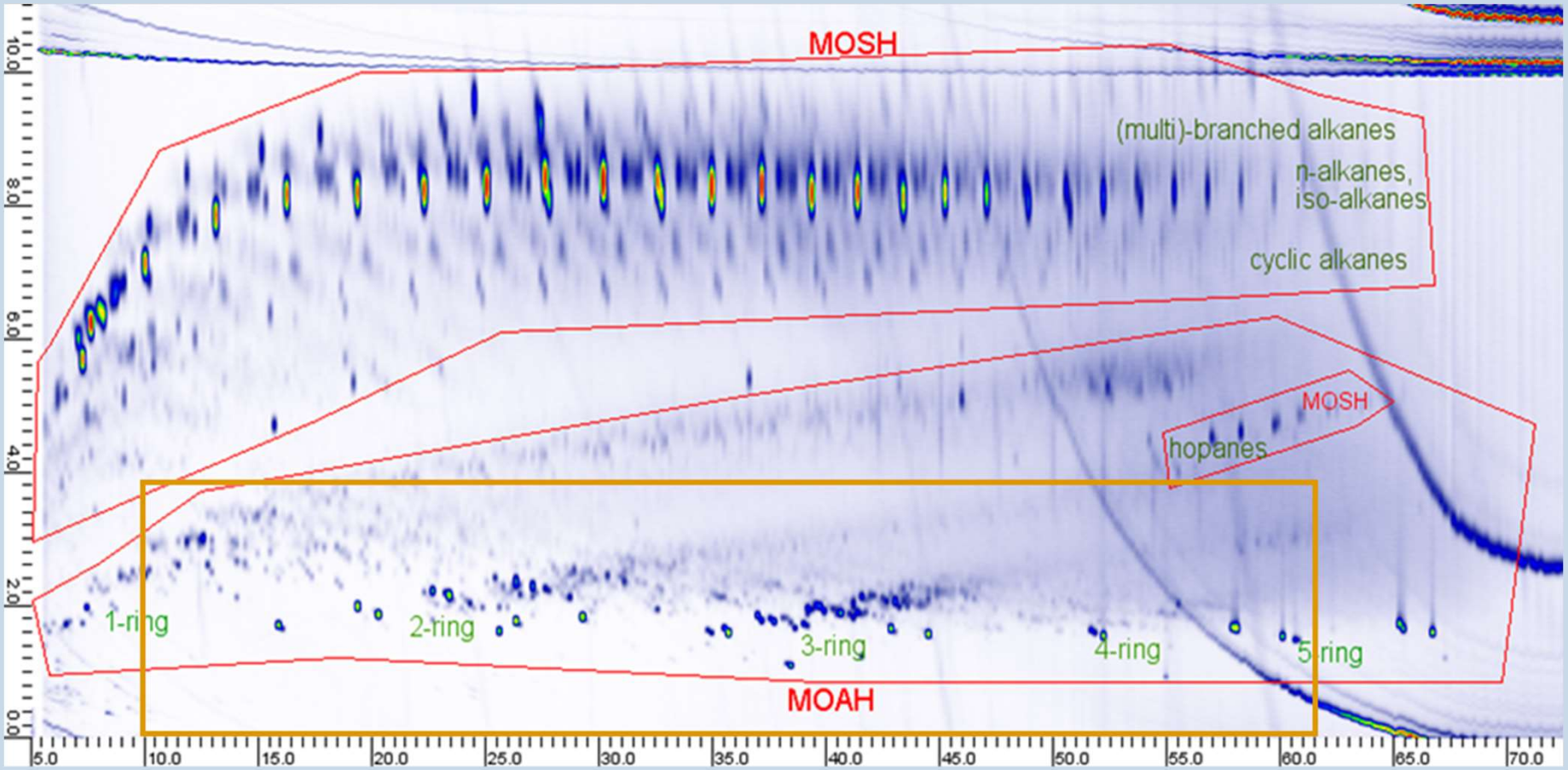
Spiking level at 120 mg/kg



Contents of MOAH in mg/kg detected in spiked oils after adsorption, spiking level at 14 mg/kg

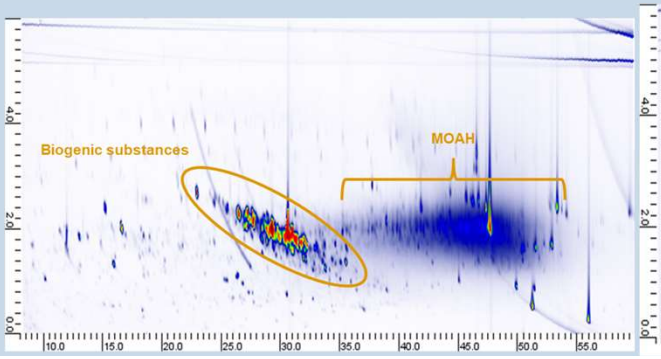


GC x GC-MS of a mixture of mineral oil products

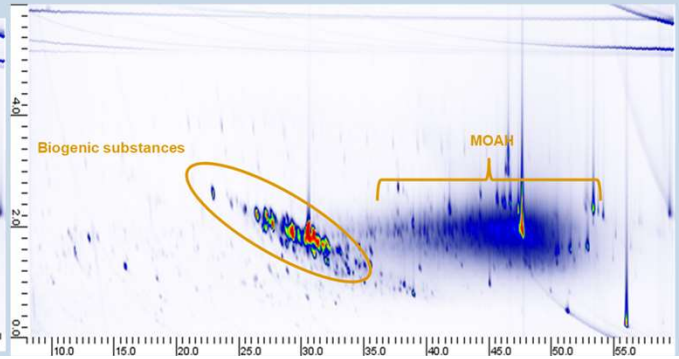


TIC-Image of a standard mixture of different structural mineral oil compounds

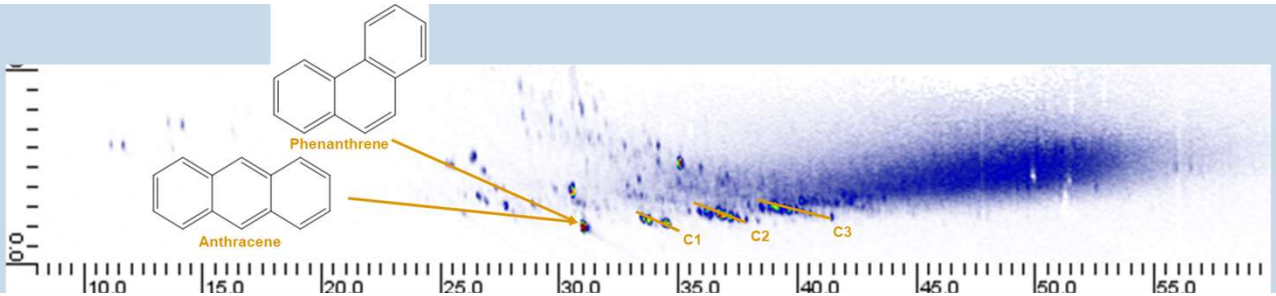
Analysis of charcoal treated samples by GCxGC-MS



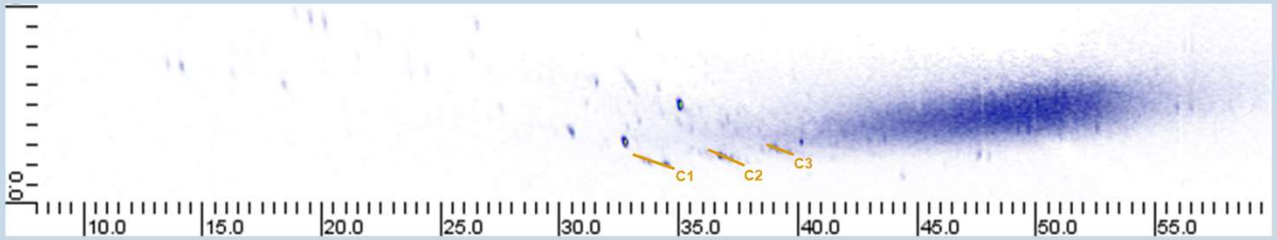
TIC image of an oil spiked with gear oil



Same oil after charcoal treatment



SIM image of phenanthrene/anthracene and alcylated isomers before treatm.



SIM image of phenanthrene/anthracene and alcylated isomers after treatm.

Removal of MOH by adsorbtion and winterisation

- X The adsorbtive materials tested did **not** achieve any significant selective removal of MOSH and MOAH with the exception of active carbon for PAHs

- X Winterisation processes with addition of extra wax of diferent origin did **not** show any significant removal of long chain MOH (data not shown)

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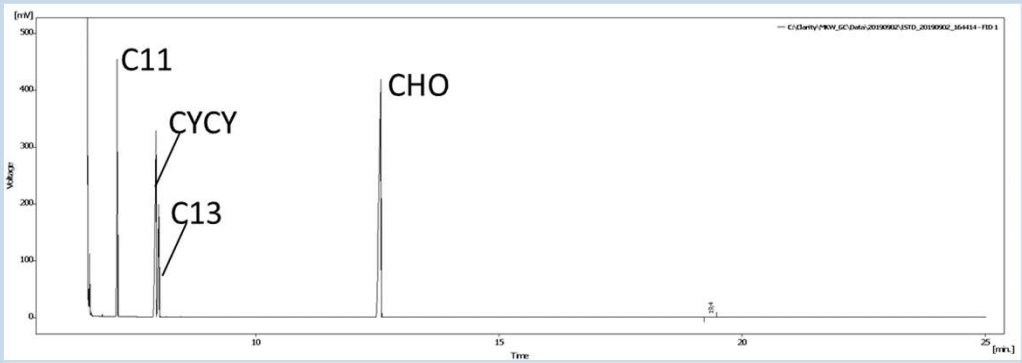
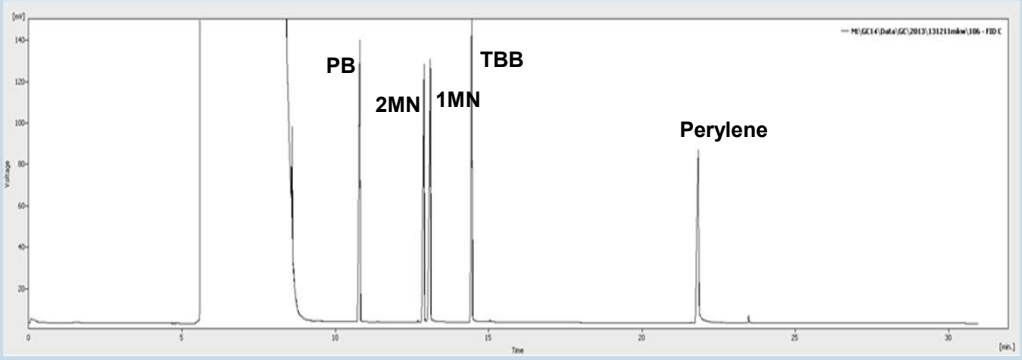
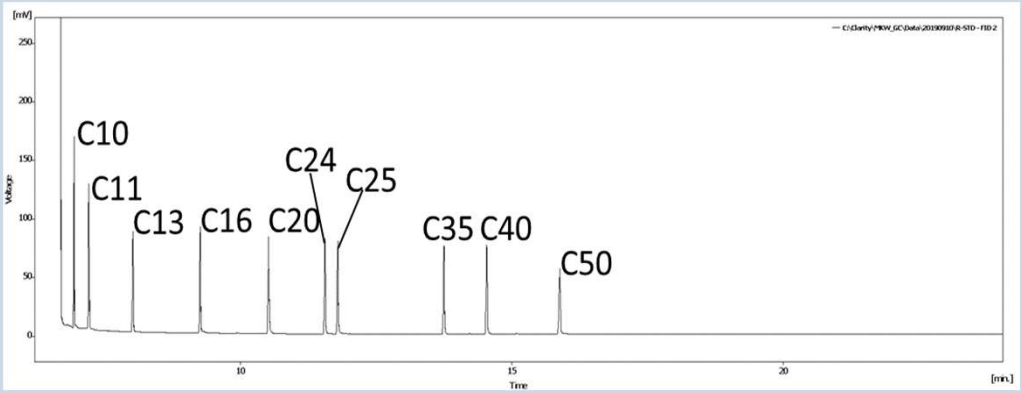
Improvement of the actual LC-GC-Method

System suitability tests

- **Baseline check:**
  - no drift at high temperature.
  - IS shall be separated from solvent
  - ratio baselinedrift:signal hight ≤ 1:4
- **Blank check:**
  - humps and interfering substances from solvents, reagents, system and previous samples less than 30 % of LOQ
  - presence & ratio of qualifying standards
- **Test for discrimination:**
  - Ratio C10:C20 0.8<x<1.2
  - Ratio C20:C50 0.8<x<1.2







Enrichment of the analytes

- Measures in order to safely achieve a limit of quantification at 1 mg/kg:
- Increase of sample weight would result in an overload of the HPLC-column due to triacylglycerides.
- Saponification is a simple and robust measure to enrich hydrocarbons
- Foaming has to be avoided by using ethanol in the washing solvent mixture

Reaction mixture after saponification

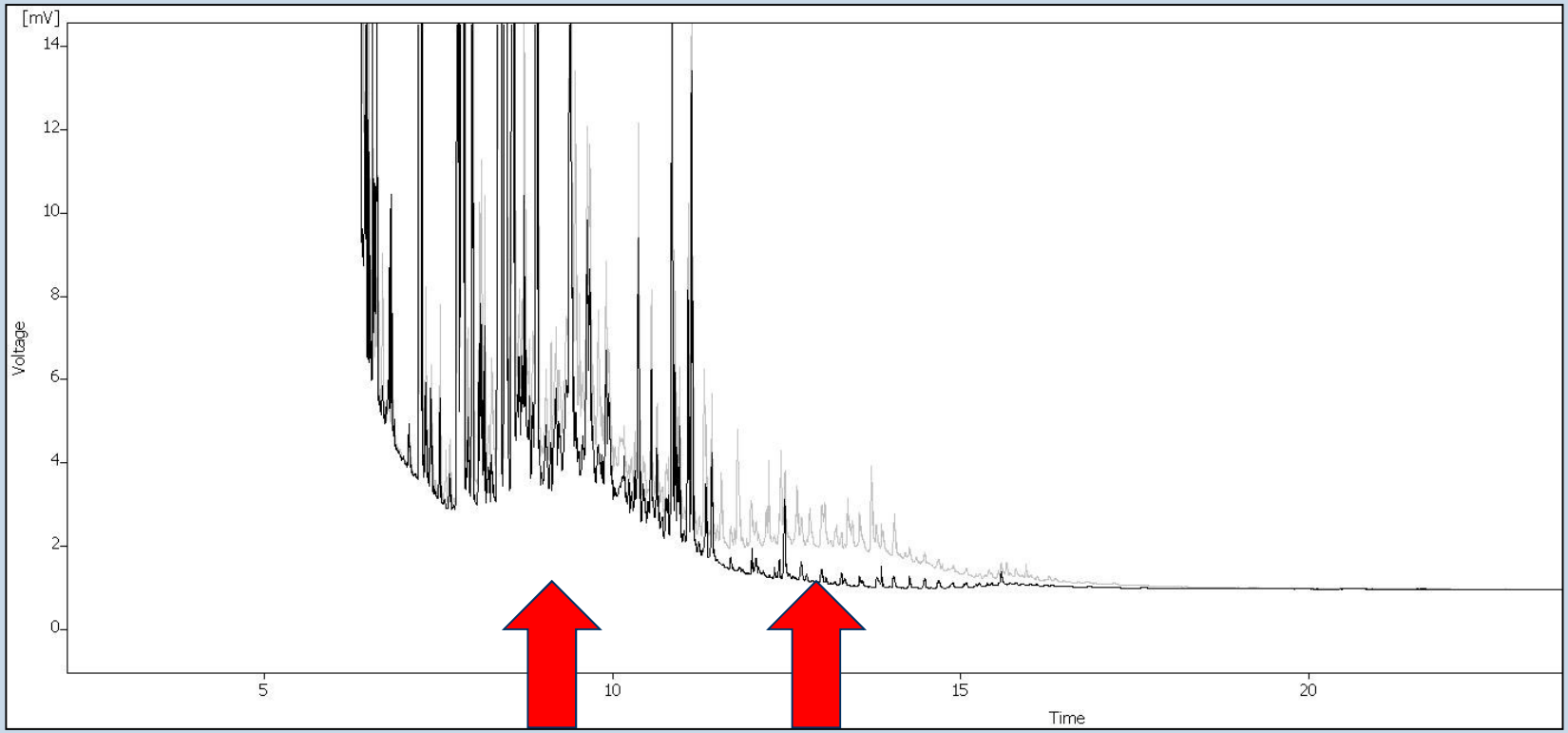
Reaction mixture with addition of ethanol

Clean-up before epoxidation

- Removal of interfering polar substances before the epoxidation step
- Reduces the amount of m-CPBA needed for complete epoxidation
- Improves blank values due to less m-CPBA reagent.
- Enables an automated further sample preparation

Clean-up of a saponified palm oil sample

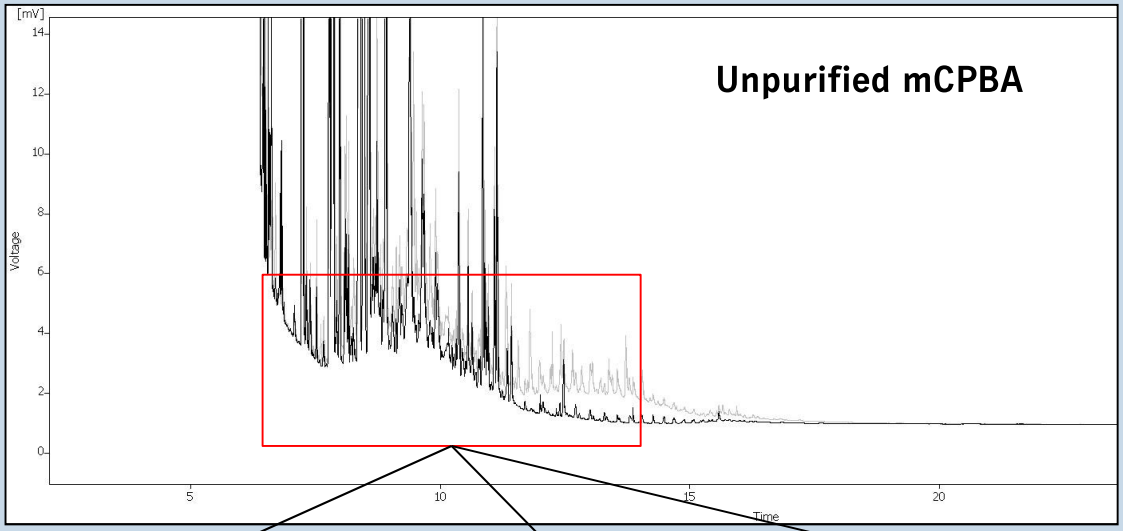
Problems with m-CPBA purity

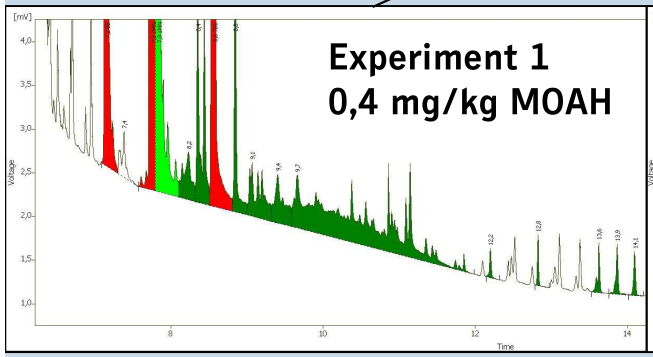


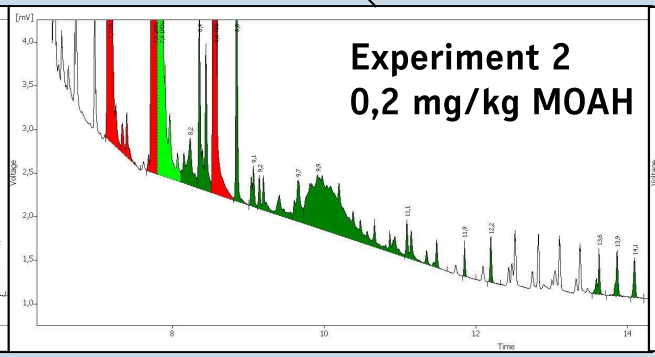
Impurities spiked hump

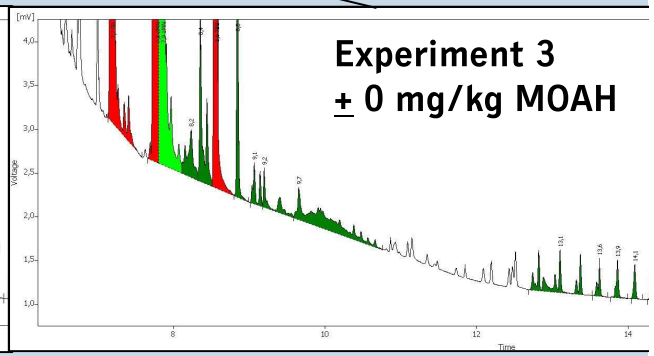
Overlay of m-CPBA blank and olive oil sample spiked with (MOAH 1mg/kg)

Purification of m-CPBA









Deactivation and removal of m-CPBA/m-CBA excess

For epoxidation of almost all interfering substances a sufficient amount of m-CPBA has to be used. The reaction leads to the formation of m-CBA.

After the epoxidation is completed the reagent excess has to be deactivated or/and removed.

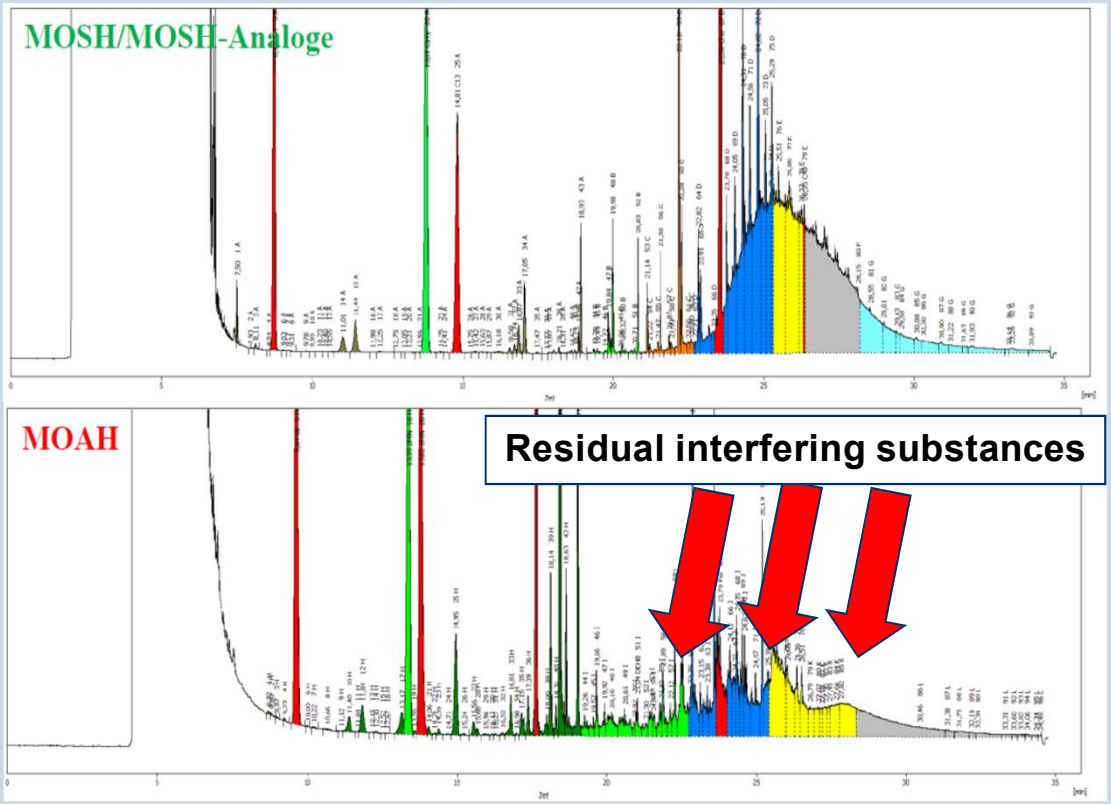
Deactivation improves comparability of results even after prolonged storage on the autosampler tray or in the fridge.

Complete removal of m-CBA ensures less down-time of LC-GC system due to blockage.

Testing several matrices for suitability

A number of 20 samples of different oils (rapeseed, sunflower, palm and olive oils) were tested in order to safeguard that the amount of epoxidation reagent is sufficient to remove almost all interfering substances

Residual interfering substances after epoxidation



Residual interfering Substances, which can not be removed by epoxidation have to be taken into account in the calculation of the limit of quantification.

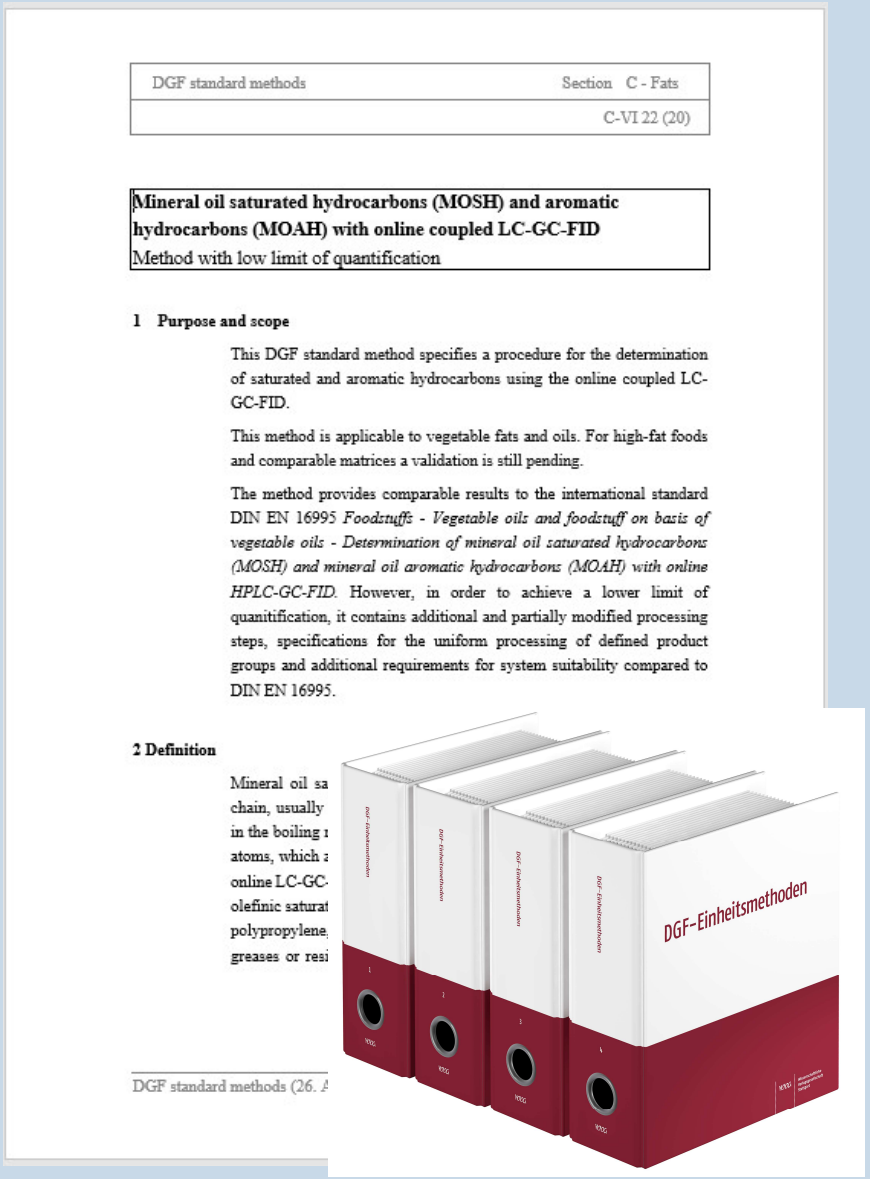
The LOQ must be increased by the amount of MOH corresponding to the area, when the shape of the real hump is not identifiable any more.

Finalisation of the standard method

- The method was finalised after several discussions with all participating laboratories
- A collaborative trial was organized with 6 samples in coded double portions
- Cocoa butter, sunflower oil, spiked rapeseed oil, spiked olive oil, spiked sunflower oil and spiked palm oil was analysed
- The evaluation was executed according to ISO 5725
- MOSH and MOAH was determined in the elution range from C10 to C50 and in addition according to the JRC fractions

Results

- Precision data improved significantly
- Good repeatability and reproducibility of MOSH and MOAH was achieved also at levels at about 1 mg/kg of oil or fat.
- However, some samples show residual interfering substances, which led to a necessarily increase of the limit of quantification.



DGF standard methods | Section C - Fats

C-VI 22 (20)

Mineral oil saturated hydrocarbons (MOSH) and aromatic hydrocarbons (MOAH) with online coupled LC-GC-FID
Method with low limit of quantification

1 Purpose and scope

This DGF standard method specifies a procedure for the determination of saturated and aromatic hydrocarbons using the online coupled LC-GC-FID.

This method is applicable to vegetable fats and oils. For high-fat foods and comparable matrices a validation is still pending.

The method provides comparable results to the international standard DIN EN 16995 *Foodstuffs - Vegetable oils and foodstuff on basis of vegetable oils - Determination of mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH) with online HPLC-GC-FID*. However, in order to achieve a lower limit of quanitification, it contains additional and partially modified processing steps, specifications for the uniform processing of defined product groups and additional requirements for system suitability compared to DIN EN 16995.

2 Definition

Mineral oil sa
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DGF standard methods (26. A

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Conclusion

- It was a challenging task to improve the repeatability and reproducibility
- A group of experienced laboratories had successfully gathered to improve the procedure to make it more robust and to safeguard comparable results.
- After several laboratory comparison studies a standardized method was agreed on with all participating laboratories.
- Crucial points are a strict sample preparation protocol with a minimum of options, changes of the epoxidation reaction, the chromatogram evaluation and checks for system suitability.
- We reached improved precision data to ensure a better basis for the minimization of mineral oils in vegetable oils.
- Further collaborative trial at CEN level is scheduled soon.

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Thank you for your attention!

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