Alternative purification workflow for MOAH analysis in food:
using a silica HPLC column to separate biogenic interferences from the MOAH hump and fractionate into 1-2 and 3+ aromatic rings

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INTRODUCTION

CONTEXT

Mineral oil saturated (MOSH) and mineral oil aromatic (MOAH) hydrocarbons are two sub-classes of petroleum-derived **food contaminants** [1].

Among them, **MOAH** pose a particular concern due to their potential **genotoxicity**, especially compounds with **three or more aromatic rings (AR)**.

Currently, a recommended maximum threshold of 2 mg/kg MOAH in fats and oils is applicable [2].

In practice, the analysis of MOSH and MOAH is typically conducted using **HPLC-GC-FID**, which is preceded by sample preparation steps aimed at extracting, concentrating, and purifying these contaminants.

PROBLEM

A significant challenge in MOAH analysis is the **presence of matrix-derived interferences that coelute with the MOAH hump**, raising the limit of quantification above the regulatory threshold.

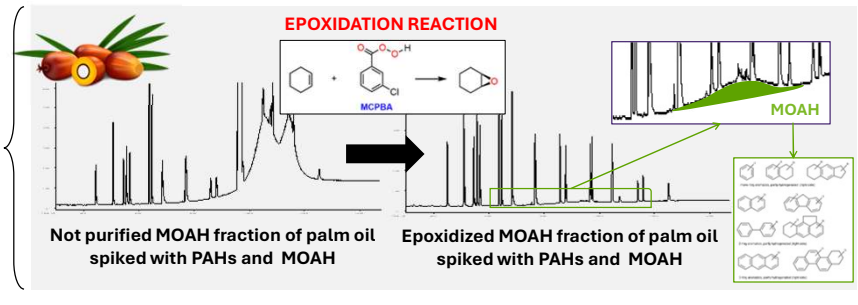
These interferences primarily consist of natural terpenes, such as squalene and carotenes, which are often present at much higher concentrations than MOAH.

The **conventional approach** to reduce these interferences involves chemical **epoxidation**, which alters their polarity and facilitates separation during HPLC elution.

However, this method can lead to **unpredictable MOAH losses**, particularly for compounds with a higher number of aromatic rings, due to unintended epoxidation.

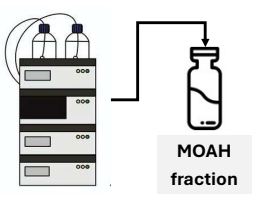
GOAL

This work presents an alternative purification method for MOAH analysis using the same HPLC system (column and eluents) employed in standard HPLC-GC-FID procedures.

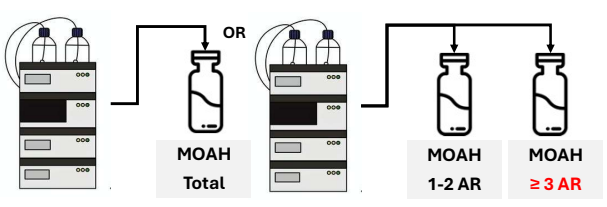


MATERIALS & METHODS

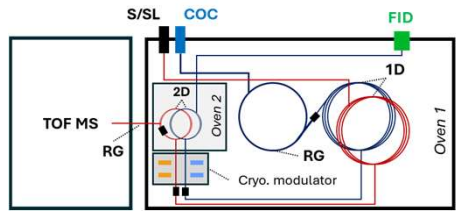
SPIKED VEGETABLE OIL EXTRACT



SAMPLE FRACTIONATION [A] & COLLECTION OF MOAH



HPLC FRACTIONATION [B] & COLLECTION OF PURIFIED MOAH (TOTAL OR 1-2 AR & ≥3 AR) NEW



GC(×GC)-FID/(TOFMS)
MID POLAR × APOLAR COLUMN SET [3]

PREPARATION OF THE EXTRACT

- Unsaponifiable extract from 1 g of vegetable oil (palm, sunflower, olive, or coconut) in 1 mL *n*-C6 obtained following [4]
- Spiked with 16 EPA 610 PAHs (Restek #31264) and coronene (Cor) + MOSH/MOAH internal standard (Restek #31070) + mineral oil

HPLC SYSTEM

- **HPLC**: Agilent 1260 Infinity II HPLC with an isocratic pump G7110B and a VWD acquiring at 230 nm
- **Column: Allure silica 250 mm × 2.1 mm i.d. × 5 µm d_p, 60 Å (Restek)**
- **Autosampler**: PAL3 Autosampler (CTC, Switzerland) equipped with backflush valves and an HPLC collection tool

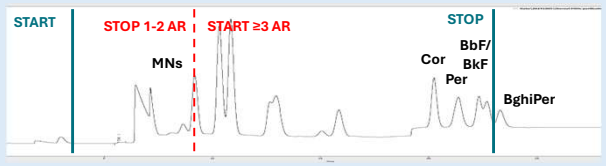
HPLC FRACTIONATION [A]

- **Elution gradient**: 0 min 100% *n*-C6; 1.5–6 min 65% *n*-C6 35% DCM at 0.3 mL/min.
- **Column backflush**: 6-15 min 100% DCM at 0.5 mL/min.
- **Reequilibration**: 15-25 100% *n*-C6 at 0.5 mL/min and 25-30 min at 0.3 mL/min.
- **MOAH** collected between 4.4 & 5.9 min

HPLC FRACTIONATION [B]

- **Elution gradient**:0 min 100% C6; 1.5–14.5 min 98% *n*-C6 2% DCM; 14.5–23 min 95% *n*-C6 5% DCM at 0.3 mL/min
- **Column backflush & reequilibration**:see [A]
- **Purified MOAH**: collected between 3.5 and 20.5 min. For a separation in 1–2 and ≥3 AR ring fraction, the cut was performed at 9.5 min.

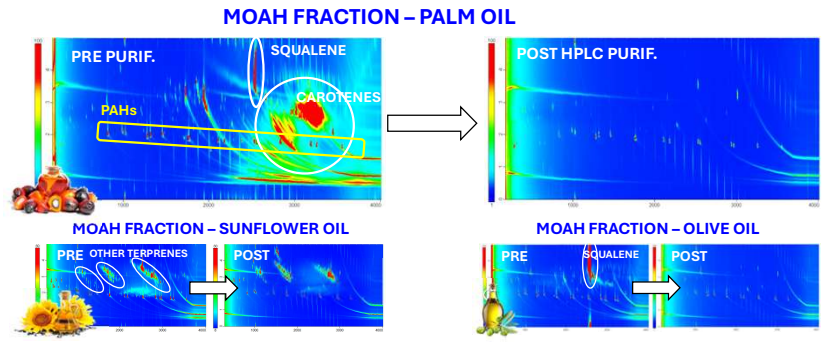
HPLC-UV (230 NM) CHROMATOGRAM OBTAINED DURING FRACTIONATION [B]



The eluting peaks are PAHs or MOSH/MOAH internal standards (#31070)

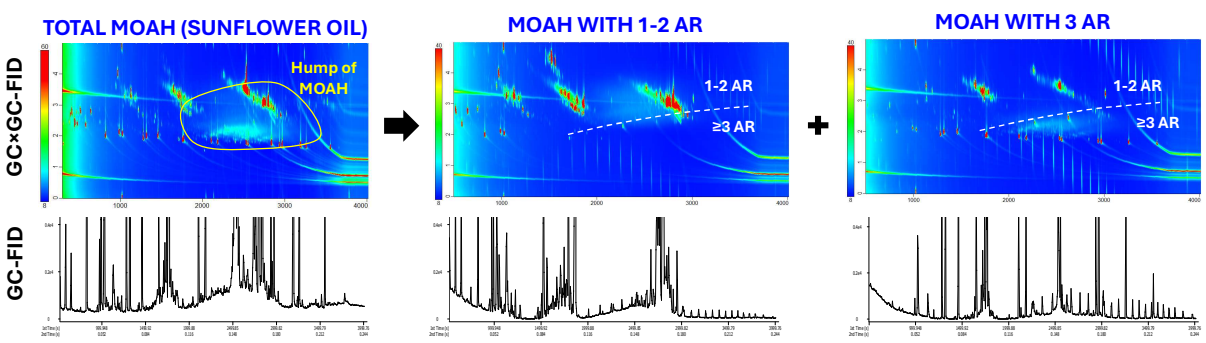
RESULTS & DISCUSSION

I. PURIFICATION EFFICIENCY



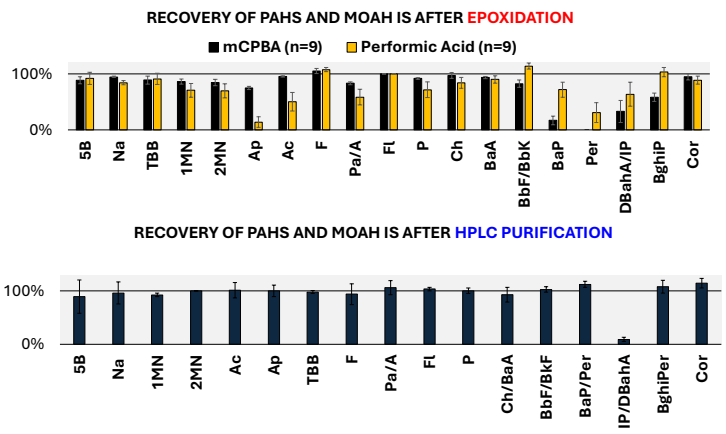
- **The HPLC purification method was efficient at removing carotenes strongly overloading the GC chromatogram of the MOAH fraction of palm, as well as squalene, present both in palm and olive oils.**
- Smaller terpenes present in sunflower oil and coconut oil (not shown as not rich in interferences) are however not removed as they completely co-elutie with MOAH during the elution in HPLC.

II. SEPARATION BY NUMBER OF AROMATIC RINGS



- The used elution gradient allowed to fractionate **MOAH into 1-2 AR and ≥3 AR**, allowing their separate injection into the GC or GC×GC system.
- This fractionation offers **two perspectives**. First, it allows the **quantification of ≥3 AR MOAH (i.e., the genotoxicity-associated fraction) without requiring GC×GC analysis**.
- Second, it provides a preparative approach to obtain **MOAH fractions enriched in either 1–2 AR or ≥3 AR compounds**, enabling, for example, their **separate toxicological assessment**.

III. RECOVERY & COMPARISON WITH EPOXIDATION (ISO 20122:2024 METHOD)



- The **recovery of PAHs and MOAH internal standards was much more consistent** with the proposed HPLC purification method compared to the two epoxidation procedures used as reference.
- In contrast to epoxidation, **no preferential losses of compounds with a higher degree of unsaturation** were observed.
- **MOAH recoveries** showed high consistency across samples and **complied with the requirements of the JRC guidance** for MOSH/MOAH determination in food [5]

Matrix	Palm oil	Sunflower oil	Olive oil	Coconut oil
MOAH recovery (%) after HPLC purification (n=2)	92 ± 8	93 ± 4	98 ± 6	94 ± 2

CONCLUSION

- The proposed HPLC purification method enables the **removal of major terpenic interferences**, such as carotenes and squalene, and allows the **fractionation of MOAH into 1–2 and ≥3 aromatic ring subgroups**.
- Compared to epoxidation, it provides **more consistent recoveries** without selective losses of unsaturated compounds.
- The method meets the **recovery criteria of the JRC guidance** and offers a tool for both quantification and preparative isolation of genotoxicologically relevant MOAH fractions.
- Lastly, it employs the **same HPLC system** (column, eluents) as conventionally used for MOAH analysis.

ACKNOWLEDGMENTS

This work is supported by Fonds de la Recherche Scientifique Belgique (FNRS) PDR projects-ToxAnaMOH T.0187.23 and ACESSS (Academic Center of Excellence for Separation Science and Sensing).

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