Normal-phase HPLC as a superior alternative to epoxidation for biogenic interferences removal in mineral oil aromatic hydrocarbon analysis in food

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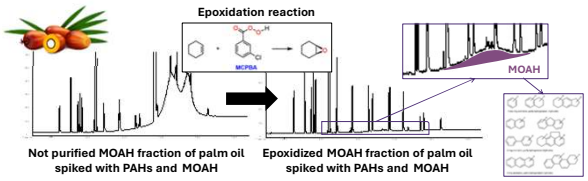
INTRODUCTION

CONTEXT

- Mineral oil aromatic hydrocarbons (**MOAH**) are petroleum-derived **food contaminants** that are associated with **genotoxic** properties, particularly MOAH with **≥3 aromatic rings** (AR) [1].
- Currently, there is a *de facto* limit on total MOAH in food ranging from 0.5 to 2.0 mg/kg depending on the fat content of the matrix [2]
- MOAH analysis is performed using **HPLC-GC(×GC)-FID** after a more or less complex sample preparation procedure, depending on the matrix
- The reference method for MOAH analysis if vegetable oils is the **ISO 20122:2024**.

CHALLENGE

- Food matrices contain terpenes, which share structural similarities to MOAH, but are often more abundant than MOAH → **overload GC chromatogram, making MOAH determination difficult**
- These interferences are usually (partially) removed by **epoxidation**
- **Epoxidation** is not selective and leads to **20-40 % losses of MOAH** (particularly of MOAH with a high number of double bonds)



Not purified MOAH fraction of palm oil spiked with PAHs and MOAH

Epoxidized MOAH fraction of palm oil spiked with PAHs and MOAH

PRELIMINARY OBSERVATION

When eluted on a **silica column**, MOAH:

1. **Show an elution order by number of aromatic rings**
2. **Elute earlier than some terpenic interferences (squalene, carotenes)**

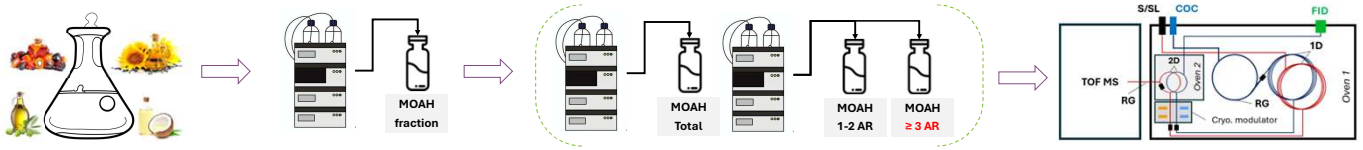
GOAL OF THE WORK

To validate an HPLC fractionation method ...

- That removes interferences from MOAH
- That separates MOAH based on their number of AR

MATERIALS & METHODS

WORFKLOW FOR THE PURIFICATION OF TERPENIC INTERFERENCES FROM MOAH BY HPLC ON SILICA [3]



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

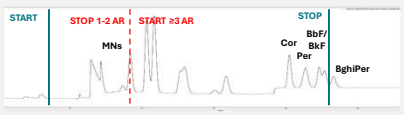
HPLC FRACTIONATION [A]

- **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]

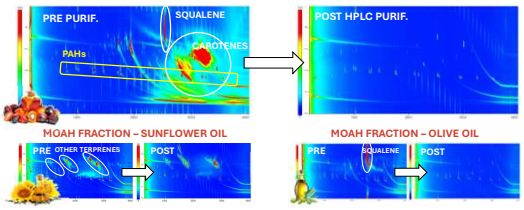


The eluting peaks are PAHs or MOSH/MOAH internal standards (#31070)

RESULTS & DISCUSSION

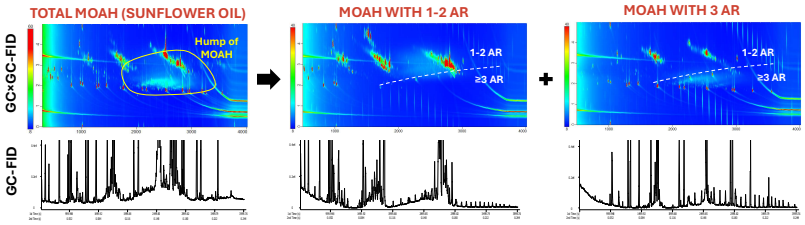
I. PURIFICATION EFFICIENCY

MOAH FRACTION – PALM OIL



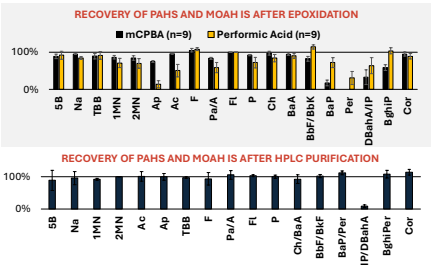
The HPLC purification method was efficient at removing carotenes strongly overloading the GC chromatogram of the MOAH fraction of palm, as well as squalene, present both in palm and olive oils. Smaller terpenes present in sunflower oil and coconut oil (not shown as not rich in interferences) are however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

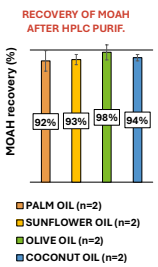
II. SEPARATION BY NUMBER OF AROMATIC RINGS



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 FOR MOSH/MOAH IN VEGETABLE OILS)





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]

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.**

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