

Improved microwave-assisted saponification for reliable MOSH and MOAH determination in edible fats and oils

Aleksandra Gorska¹, Donatella Ferrara^{1,2}, Paula Albendea¹, Chiara Emilia Cordero² and Giorgia Purcaro¹

¹ Analytical Chemistry Laboratory, Gembloux Agro-Bio Tech, University of Liège, Chemistry for Sustainable Food and Environmental Systems, Passage des Déportés 2, Gembloux, 5030, Belgium.

² Dipartimento di Scienza e Tecnologia del Farmaco, Università degli Studi di Torino, Via Pietro Giuria 9, I-10125 Torino, Italy

INTRODUCTION

- DEFINITION** – Mineral oil saturated hydrocarbons (**MOSH**) and mineral oil aromatic hydrocarbons (**MOAH**) are frequently detected **contaminants** in **foods**, particularly in lipid-rich matrices such as edible fats and oils [1].
- METHOD** – The official method for their determination in these matrices is ISO 20122:2024, which includes a **saponification/extraction** step, purification from interferences, and on-line HPLC-GC-FID analysis [2].
- LIMITATION** – The ISO 20122:2024 protocol presents limitations related to **inconsistent recoveries of MOAH internal standards (ISs)** during sample preparation, affecting quantitative reliability.

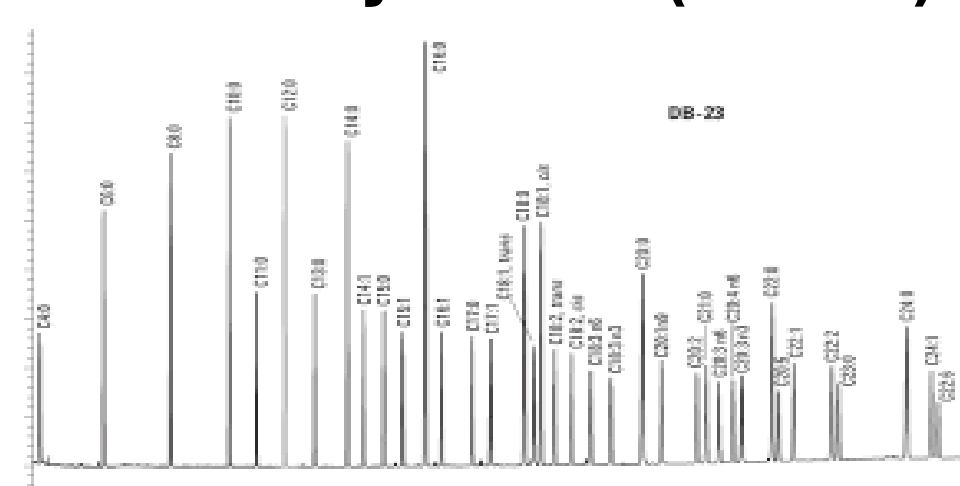
- PAST WORKS** – A microwave-assisted saponification/extraction (**MASE**) method has previously been proposed to improve the consistency of internal standard recovery through optimized solvent composition [3].
- GAPS** – The original conditions (60 °C, 30 min) were **insufficient to ensure complete saponification of some matrices**, particularly hydrogenated or high-melting fats. In addition, **matrix-dependent differences** in ISs recoveries were observed but not previously explained.

AIM OF THE WORK

- ✓ Propose an **optimized MASE method** that:
 - (1) Reduces the partitioning differences among MOAH ISs (in particular **5B, TBB, 2MN, Per**);
 - (2) Allows the saponification of matrices containing high melting fats, notably hydrogenated vegetable fats.
- ✓ Correlate the **fatty acid composition** with **ISs relative recoveries**.

MATERIALS & METHODS

Derivatization of lipids to fatty acid methyl esters (**FAMES**)



& evaluation by GC-FID



+ MOSH/MOAH ISs (Restek #31070)
MOAH ISs: 5B, TBB, 1MN, 2MN, Per



Tested conditions

- ISO 20122:2024 [2]
- 2M KOH in EtOH/H₂O (1:1, v:v) [3]
- at $\neq (T [^{\circ}\text{C}]/t [\text{min}])$

On extra virgin olive oil:
Response surface methodology
(Doehlert design [4]): effect of T [°C]
and t [min] on residue post MASE

- Gravimetric determination of residue
- Evaluation of **ISs** ratios by HPLC/**GC×GC**-FID analysis

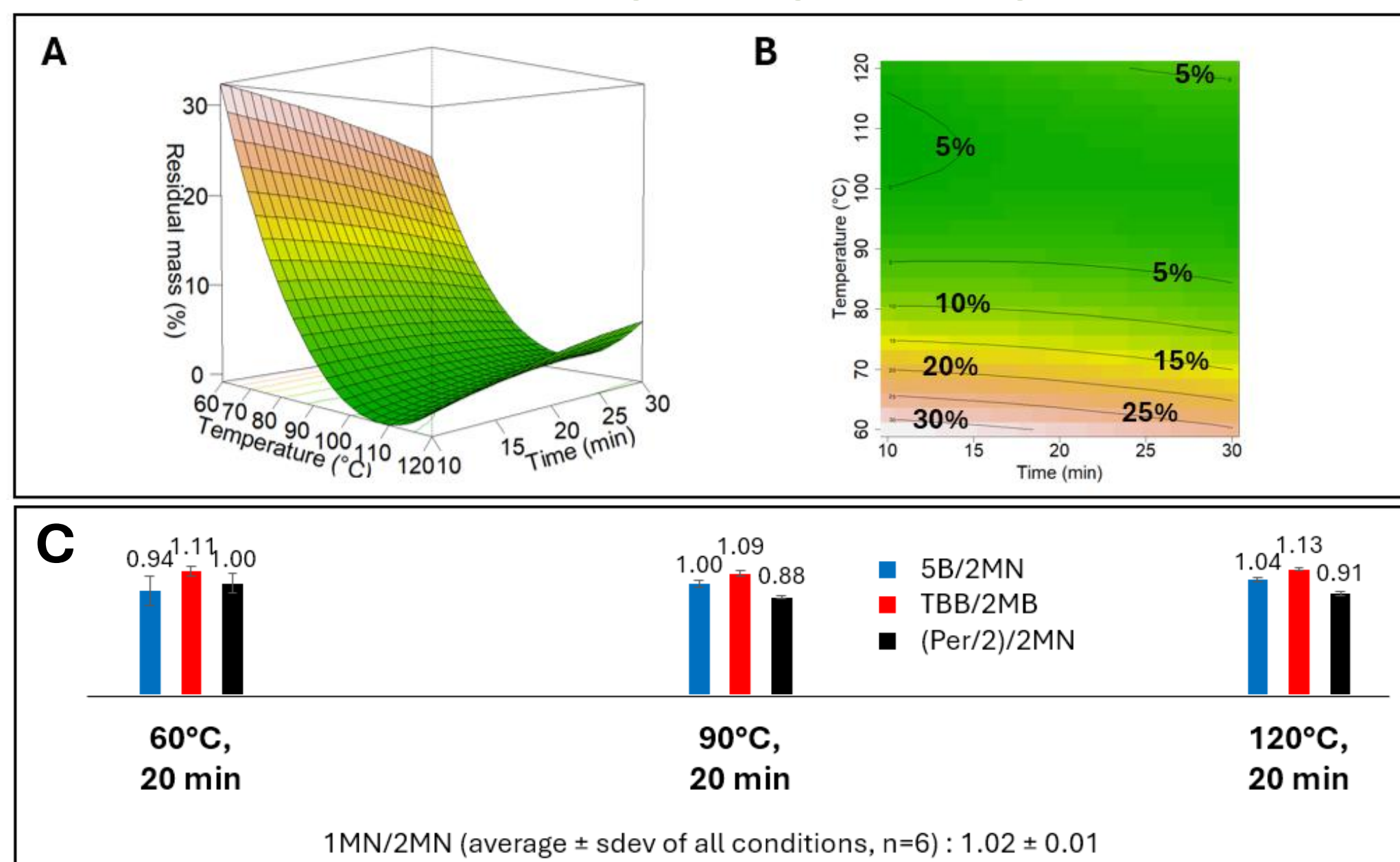
Ideal ratios: 5B/2MN, TBB/2MN, 1MN/2MN, (Per/2)/2MN = **1.00**

CORRELATION

RESULTS & DISCUSSION

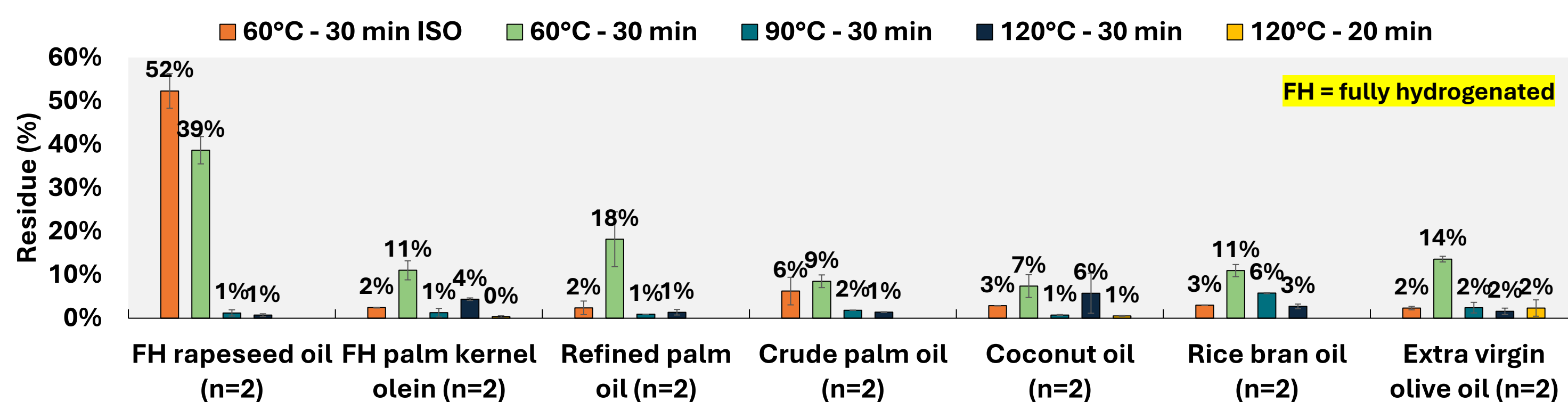
I. Effect of temperature and time on residue and/or ISs ratios

i) Response surface methodology using extra virgin olive oil



A and B: residue post-saponification (%) versus temperature [°C] and time [min]. C: MOAH ISs ratios calculated based on the peak area normalized by the concentration (TBB/2MN, 5B/2MN, (Per/2)/2MN)

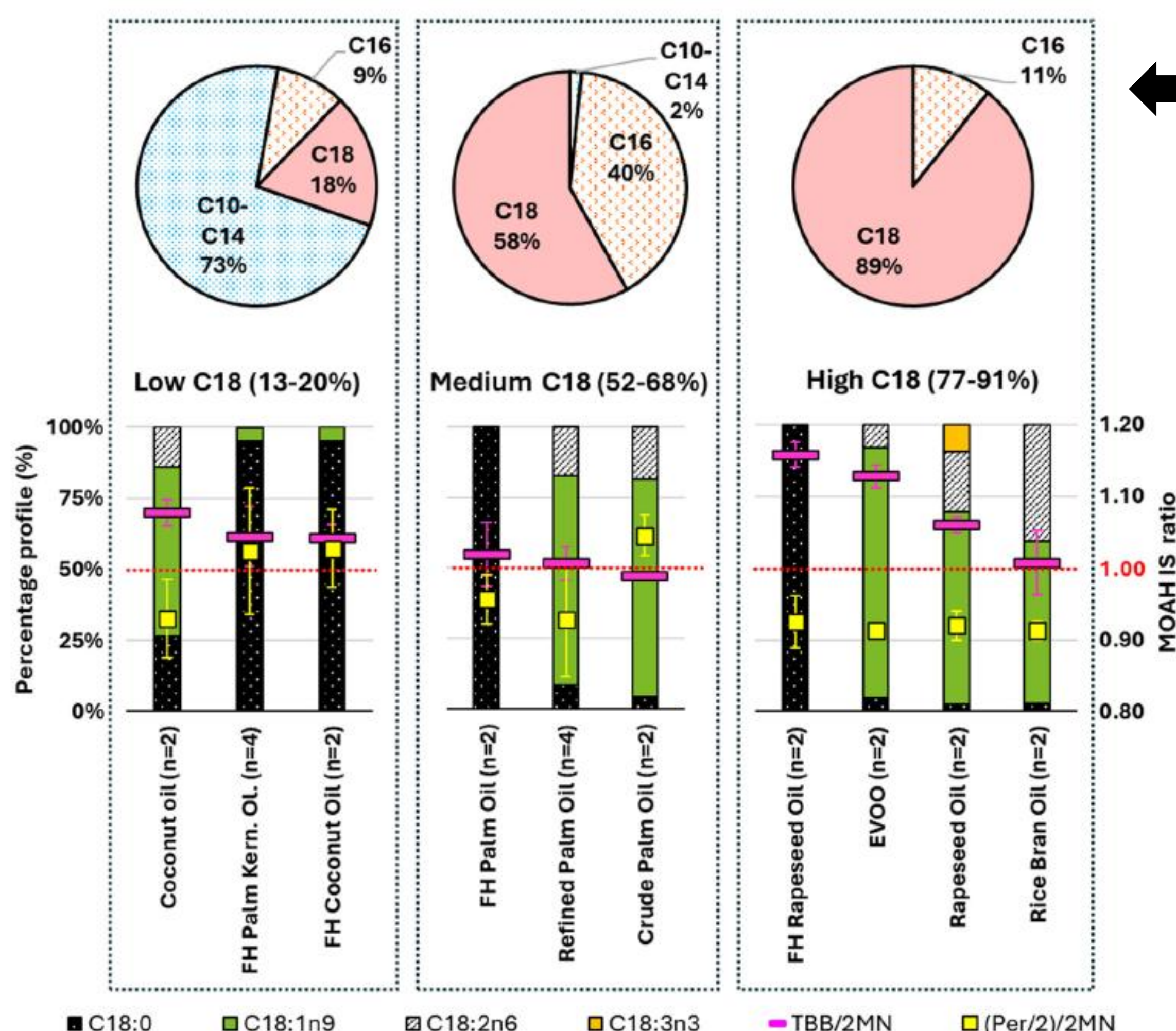
ii) Evaluation of post-saponification residue in various oils



MOAH ISs ratios obtained using the MASE method at 120°C for 20 min. « n » refer to the number of replicates for the ISs evaluation.

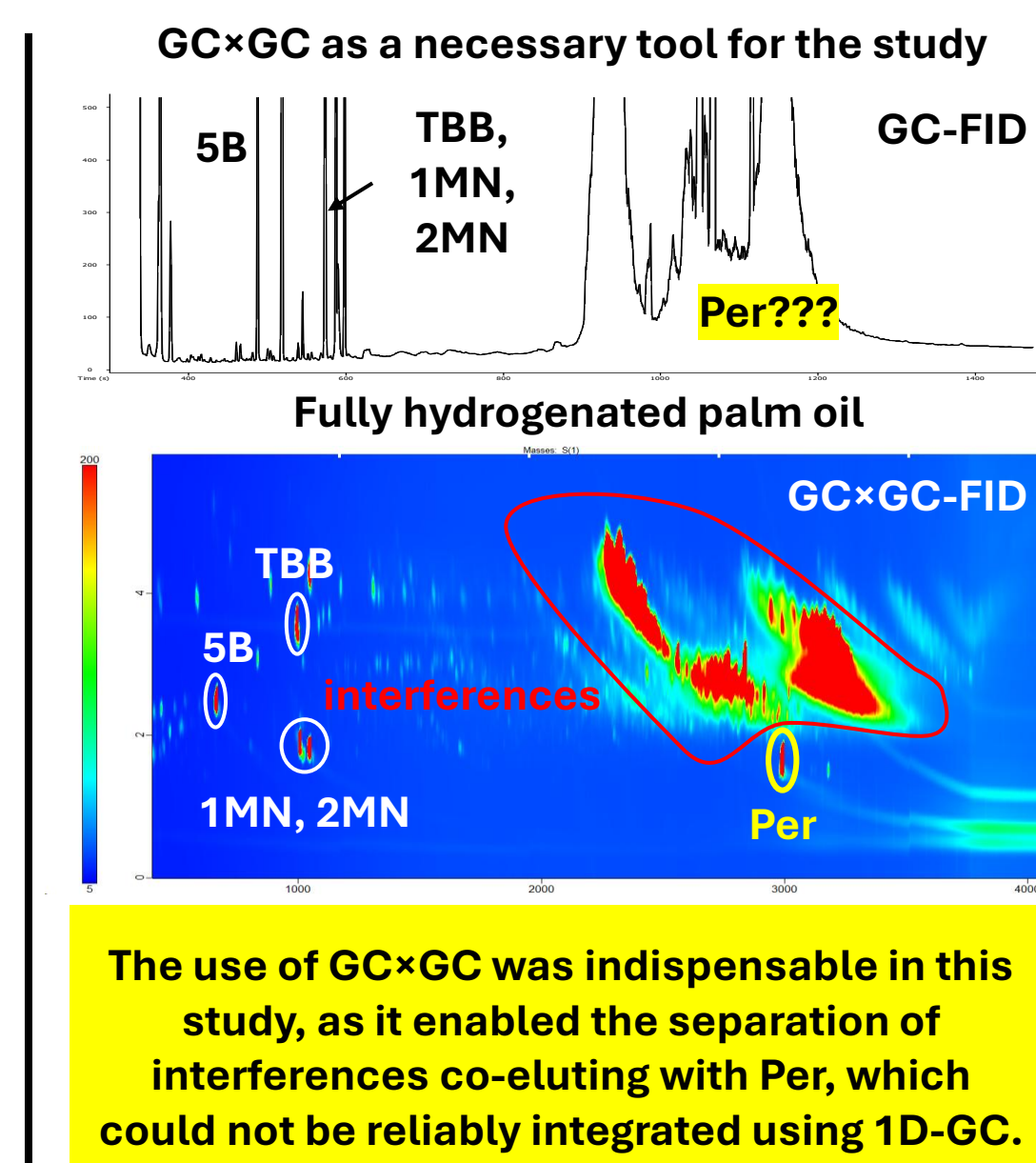
- **MASE temperature had a significant effect on the residue**: increasing the temperature decreased the amount of non-saponified material. MASE time, instead, had a less critical effect.
- Temperature and time **modification did not strongly affect MOAH ISs ratios** (all data not shown),
- Although sufficient residue reduction was observed already at 90°C, **120°C for 20 min was selected as optimal conditions** in order to have enough margin to saponify more recalcitrant matrices. Residues at this temperature were <2% (i.e., usual unsaponifiable residue for vegetable fats and oils).

II. Evaluation of FAMES profiles & correlation with ISs ratios obtained with new MASE



Classification of oils based on the total C18 fatty acid content (sum of C18:0, C18:1n9, C18:2n6, C18:3n3): average FAME profile within each group, individual C18 fatty acid distribution per oil, and the corresponding MOAH IS ratios. The optimal IS ratio is indicated in red.

- Matrices containing **abundant C18** showed the **highest discrepancies** for both ratios. Saturated C18 gave worse results than unsaturated C18 (C18:1 or a mix of C18:1 and C18:2) in that group.
- Matrices containing a **balanced mix of C18 and C16** gave **ratios closest to 1.0**. FA saturation had a lower impact on the results.
- Matrices containing **low levels of C18 and abundant C12:0** gave **intermediate ratios**. FA saturation seemed to improve ISs ratios (e.g., comparison of coconut oil and FH coconut oil).



The use of GC×GC was indispensable in this study, as it enabled the separation of interferences co-eluting with Per, which could not be reliably integrated using 1D-GC.

III. Comparison of ISs ratios using new MASE vs ISO 20144:2024

Saponification method	Average ± SDEV TBB/2MN	Average ± SDEV Per/2MN	Number of samples*
MASE	1.06 ± 0.05	0.96 ± 0.06	34
ISO 20122:2024	1.19 ± 0.09	0.93 ± 0.09	18

* Covered matrices: FH rapeseed oil, FH coconut oil, FH palm oil, FH palm kernel oil, fish oil, rapeseed oil, extra virgin olive oil, refined palm oil, coconut oil, rice bran oil, crude palm oil, procedural blank

- TBB/2MN and (Per/2)/2MN ratios were affected by matrix composition (individual data not presented)
- TBB/2MN was much more affected by saponification conditions than (Per/2)/2MN.
- **TBB/2MN showed to always be lower using the optimized MASE compared to the ISO 20122:2024 method.**

CONCLUSION

The proposed MASE method, using a **2M KOH in EtOH/H₂O (1:1,v:v)** and performed at **120 °C for 20 min** has shown to **(1) bring MOAH ISs ratios (TBB/2MN and (Per/2)/2MN closer to 1.00** compared to the ISO 20122:2024 procedure), despite matrix effects, **(2) saponify and extract MOSH/MOAH from high-melting fats**. Matrix effects seemed related to the FAMES profiles, with highest discrepancies observed for C18-rich matrices.

REFERENCES

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Accessed 14th Jul 2025;

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