Optimized saponification for MOSH and MOAH analysis: addressing high-melting fats and internal standard variability

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INTRODUCTION

CONTEXT

- Mineral oil saturated and aromatic hydrocarbons (**MOSH** & **MOAH**) are petroleum-derived **food contaminants prone to accumulate in fats and oils** at (often) mg/kg levels [1].
- To standard approach for MOSH & MOAH analysis consists of various sample preparation steps, followed by HPLC-GC-FID analysis, such as outlined in the official ISO 20122:2024 method [2].
- One of these steps, **saponification** (followed by liquid-liquid extraction), is commonly applied to **remove triacylglycerols (composing >98 % of fats and oils)** that get coextracted with MOSH & MOAH. The removal of these lipids allows the injection of a higher sample quantities in chromatographic systems, **improving limits of detection and quantification**.

PROBLEMS

- ISO 20122:2024 saponification introduces substantial variability (15-25%) in MOAH quantification due to **inconsistent partitioning of internal standards** (ISs) during saponification, affecting the ratio between 1,3,5-tri-tert-butylbenzene (**TBB**) and 2-methylnaphthalene (**2-MN**) [2].
- In fact, **the expected TBB/2MN ratio is 1.00**, while it often goes >1.15, causing **discrepancies in quantification**.
- In addition, the ISO saponification conditions (60°C, 30 min) are insufficient for some high-melting fats, as the method was validated primarily on low-melting vegetable oils.

PAST OPTIMIZATION

- Our previous work addressed the inconsistent partitioning of ISs by modifying solvent proportions and reagents concentrations [3]. Obtained TBB/2-MN: **1.05 ± 0.01.** It however showed to not be efficient on some high-melting matrices.

GOAL OF THE WORK

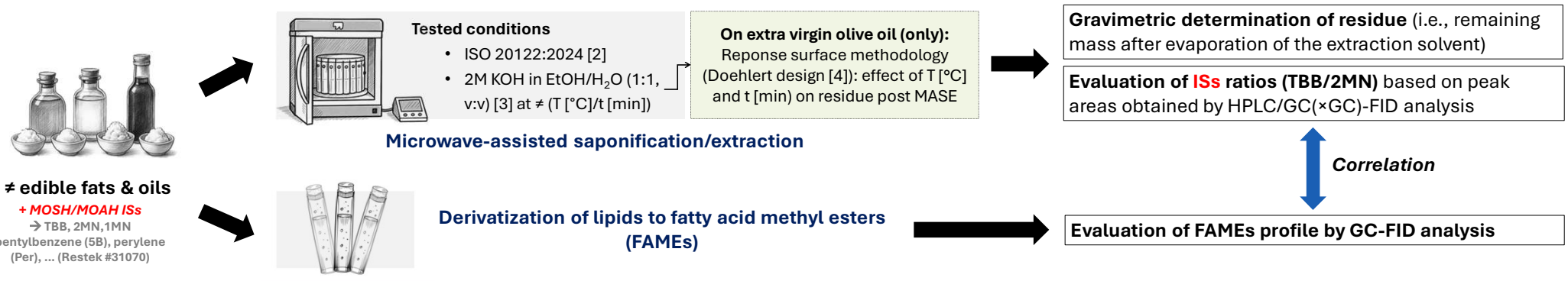
To propose an **optimized microwave-assisted saponification/liquid-liquid extraction (MASE) method** that:

(1) Reduces the partitioning differences among MOAH internal standards, targeting an **ideal TBB/2-MN ratio of 1.00**,

(2) Allows the saponification of matrices containing high melting fats, notably hydrogenated vegetable fats.

Additionaly, the works correlates the fatty acid composition with the ISs ratios.

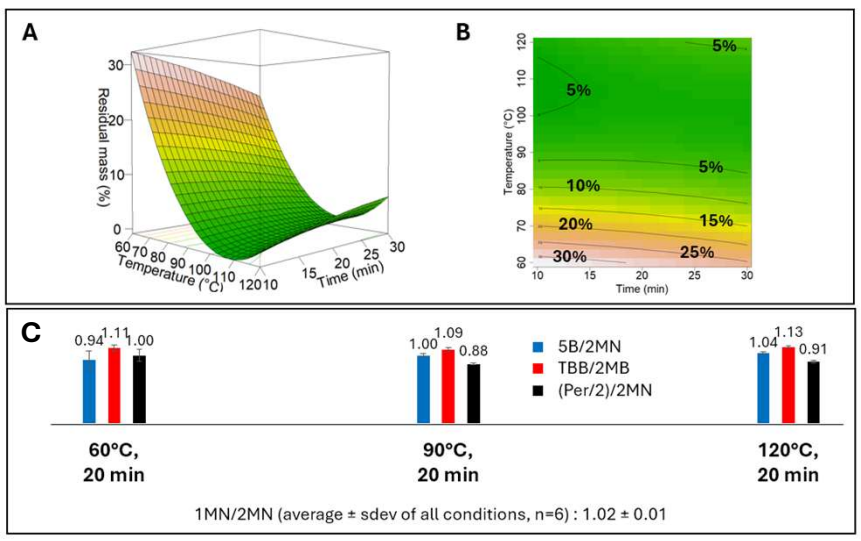
MATERIALS & METHODS



RESULTS & DISCUSSION

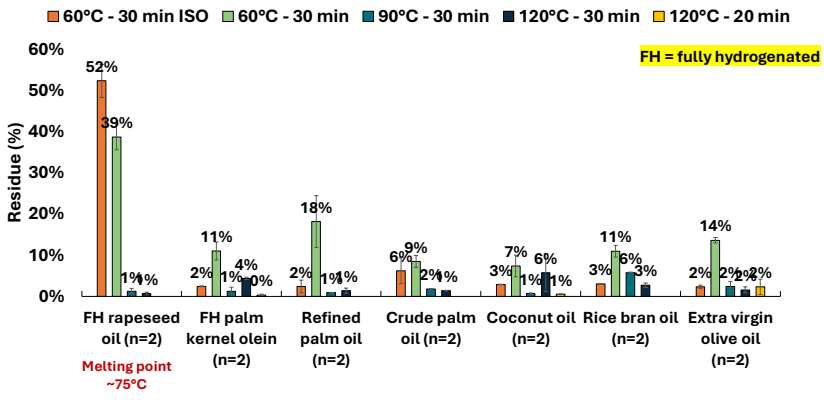
I. Effect of temperature and time on residue and/or ISs ratios

a) Response surface methodology using extra virgin olive oil



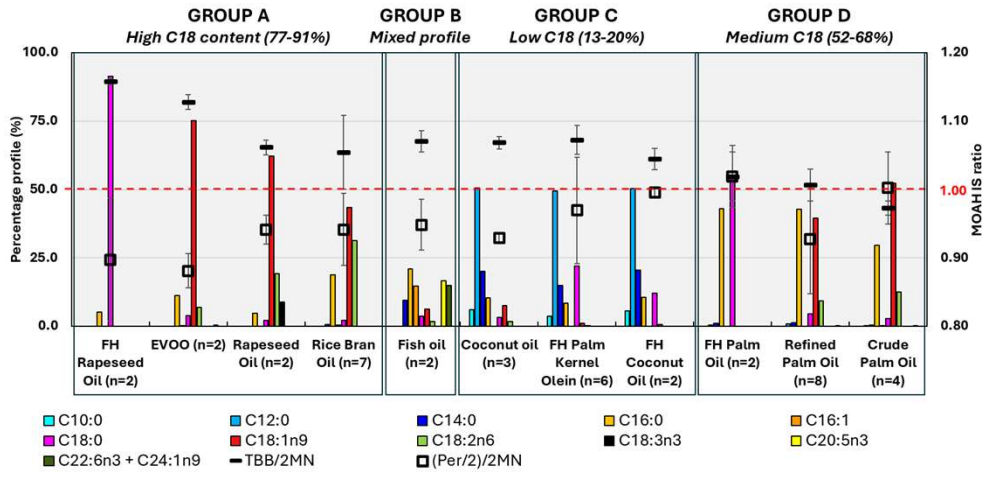
A and B: residue post-saponification (%) versus temperature [°C] and time [min]. C: MOAH ISs ratios calculated based on the peak area normalized by the concentration (TBB/2MN, 5B/2MN, (Per/2)/2MN)

b) Evaluation of post-saponification residue in various oils



- **MASE temperature had a significant effect on the residue**: increasing the temperature decreased the amount of non-saponified material (a & b). MASE time, instead, had a less critical effect (a).
- Temperature and time modification **did not strongly affect MOAH ISs ratios** (a; 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 recalitrant 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



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.

During saponification, triglycerides are hydrolysed to glycerol and fatty acid salts (soaps). These soaps are mainly soluble in the hydroalcoholic medium used for the reaction. Their solubilisation changes the polarity of the medium and can affect how other compounds, including internal standards, partition between phases.

- In terms of FAMEs profile, the tested fats and oils could be classified into 4 compositional groups which correlated with TBB/2MN and (Per/2)/2MN.
- **Matrices containing abundant C18 (group A) 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 (group D) 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 (group C) gave intermediate ratios between group A and D.** FA saturation seemed to improve ISs ratios (e.g., comparison of coconut oil and FH coconut oil).
- Fish oil, having a mixed FA profile, showed similar results to group C.

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

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 olein, 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/H_2O (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.

ACKNOWLEDGMENTS

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