

Application of an optimized microwave-assisted saponification/extraction method for MOSH/MOAH analysis in food and food contact matrices

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INTRODUCTION

- Mineral oil saturated hydrocarbons (**MOSH**) and mineral oil aromatic hydrocarbons (**MOAH**) are frequently detected contaminants in foods, particularly in lipid-rich matrices [1]. Determination of MOSH and MOAH in lipid-containing matrices requires a **saponification/extraction step** in order to reach regulatory-complaint limits of quantification (LOQs), i.e., **<2 mg/kg** [2]. However, saponification/extraction can induce **discrepancies** in MOSH/MOAH internal standard (IS) recoveries, particularly for MOAH ISs, with potential impact on quantitative accuracy.
 - Ideally**, MOSH IS ratios should correspond to $n\text{-C}_{11}/\text{CyCy} = (2 \times n\text{-C}_{13})/\text{CyCy} = (\text{Cho}/2)/\text{CyCy} = 1.00$, while **MOAH IS ratios** should correspond to $5\text{B}/2\text{MN} = 1\text{MN}/2\text{MN} = \text{TBB}/2\text{MN} = (\text{Per}/2)/2\text{MN} = 1.00$.
 - In practice, MOAH IS ratios, especially TBB/2MN, are often shifted above the theoretical value.
- A microwave-assisted saponification/extraction (**MASE**) method has previously been optimized to improve the consistency of ISs recovery through optimized solvent composition, **reaching TBB/2MN ratios closer to 1.00** [3]. Variants were later proposed for **high-melting fats** [4], **meat samples** [5], and **miniaturized extraction of adipose tissue** [6].
 - The final protocol uses 1 g sample, 10 mL KOH 2 M in EtOH/H₂O 1:1 (v/v), and 10 mL *n*-hexane (*n*-C₆), at 120 °C for 20 min. **Maintaining the EtOH/H₂O ratio is important to limit IS discrepancies** [3].
- OBJECTIVE OF THE WORK** – To assess the transposability of the MASE method across diverse matrices, including food additives, seafood, insects, and jute bags, and to propose practical variants for matrix-specific challenges.

MATERIALS & METHODS

FOOD OR Food Contact Material	ORIGINAL METHOD (ORIG)								EVALUATION • Practicality of the workflow • IS behaviour LOQs • Sample representativeness
SAPONIFICATION/EXTRACTION		VARIANT 1 (EMUL)		MASE 120 °C 20 min		Wash and re-extraction			
		FOR MATRICES FORMING HYDROPHOBIC SOAPS	Sample	2M KOH in EtOH/H ₂ O 1:1	<i>n</i> -C ₆	Post-MASE addition of H ₂ O (directly to MW vessel)	Re-extraction	Wash of combined <i>n</i> -C ₆ extracts with H ₂ O	
		VARIANT 2 (MINI)							
HPLC/GC×GC-FID ANALYSIS	FOR LIMITED SAMPLE	ORIG	1 g	10 mL	10 mL	Yes, +20 mL	5 mL <i>n</i> -C ₆	No	
		EMUL	1 g	10 mL	10 mL	No	5 mL <i>n</i> -C ₆	Yes, + 3×10 mL	
		MINI	0.25 g	2.5 mL	2.5 mL	Yes, +5 mL H ₂ O	1 mL <i>n</i> -C ₆	No	

RESULTS & DISCUSSION

I. PRACTICAL CHALLENGES ENCOUNTERED WITH THE ORIGINAL METHOD AND SOLUTIONS

ISSUE NO. 1 WITH THE ORIGINAL METHOD

ORIGINAL METHOD

n-C₆

Hydroalcoholic phase after post-MASE addition of H₂O (20 mL)

Ideal situation

E472e

Formation of a dispersion* at the interface

* Other matrices gave a liquid viscous middle phase

HYPOTHESIS

The dispersed solid (or 3rd phase) are saponification products (soaps) insoluble in the hydroalcoholic phase.

Note: E472e is an emulsifier consisting of mono- and diacetyl tartaric acid esters of mono- and diglycerides of fatty acids

SOLUTION

➤ the polarity of the hydroalcoholic phase.

Do not increase EtOH/H₂O ratio above 1.0 in the vessel, as leads to discrepancies in ISs recoveries.

In practice: avoid the extra addition of H₂O to the MW vessel after MASE.

E472e

Neat phase separation

Note: the organic phase needs to be washed after collection in a separate flask

EMUL METHOD

ISSUE NO. 2 WITH THE ORIGINAL METHOD

Limited sample availability prevented proportional miniaturization, as solvent volumes could not be reduced due to microwave system constraints. This could have lowered the sample-to-solvent ratio, increasing concentration factors and the relative impact of procedural blanks, which compromised low LOQs.

SOLUTION

Proportional 4× scale-down of sample mass and solvent volumes using synthWAVE microwave system (Milestone Srl, Sorisole, Italy).

250 mg per extraction tube (instead of 1 g)

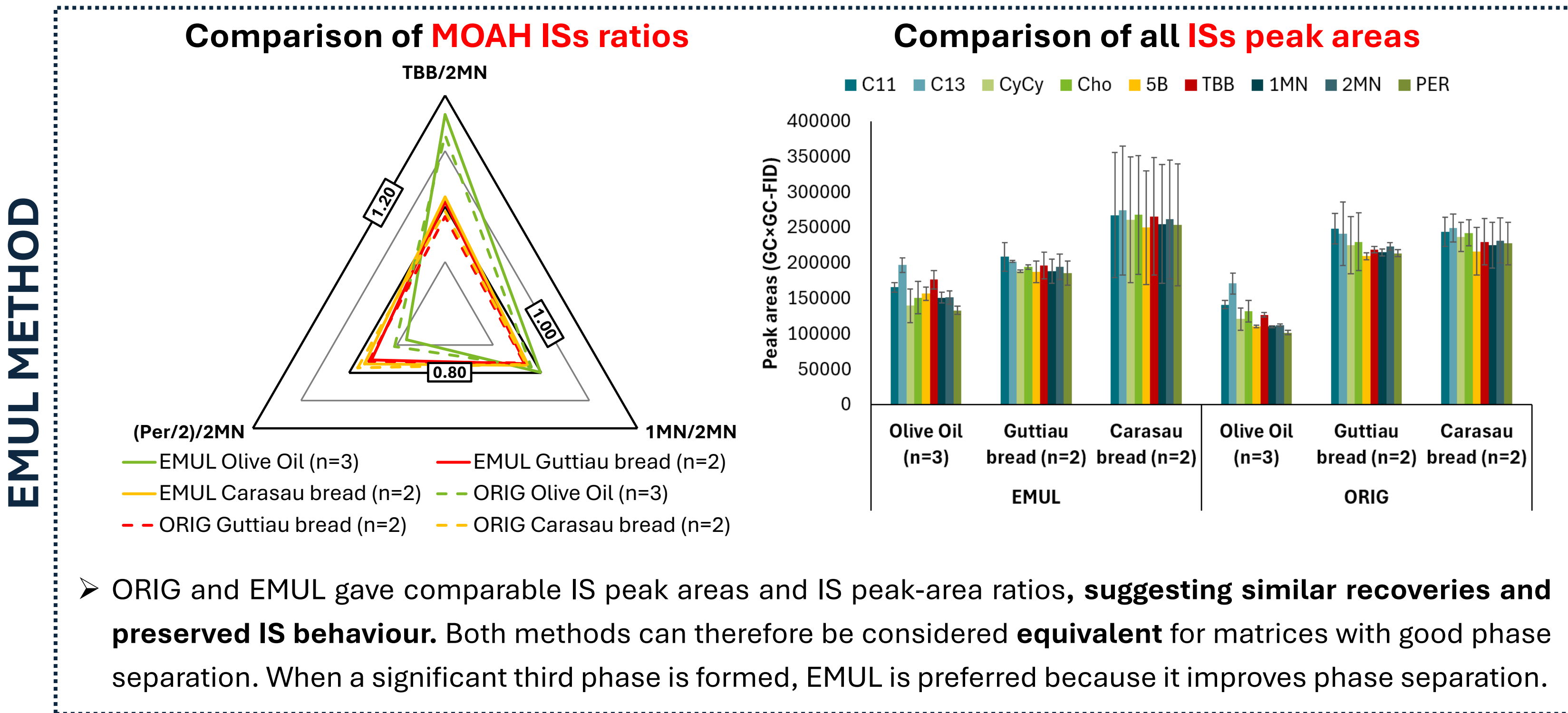
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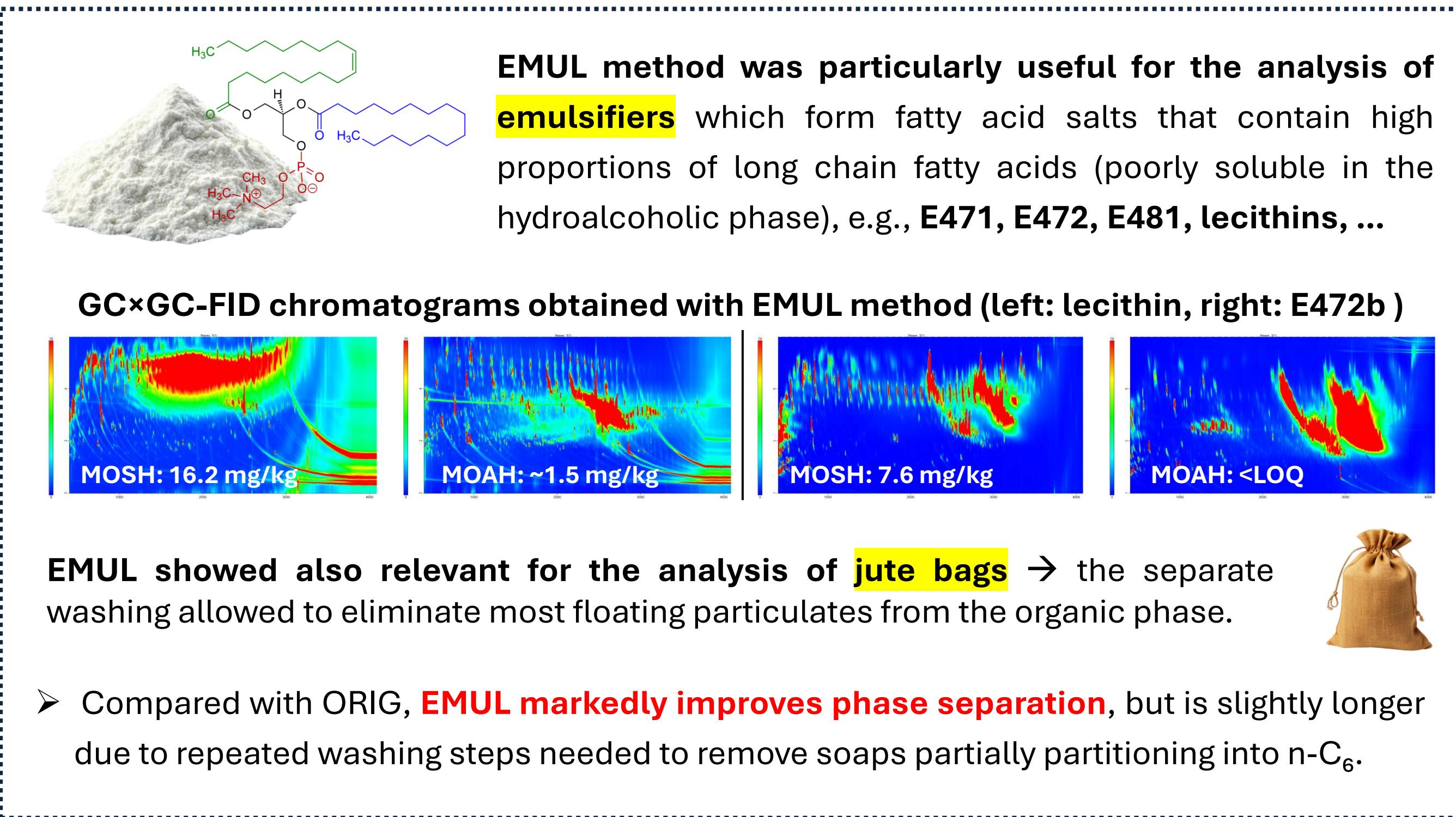
MINI METHOD

II. EVALUATION OF METHOD VARIANTS

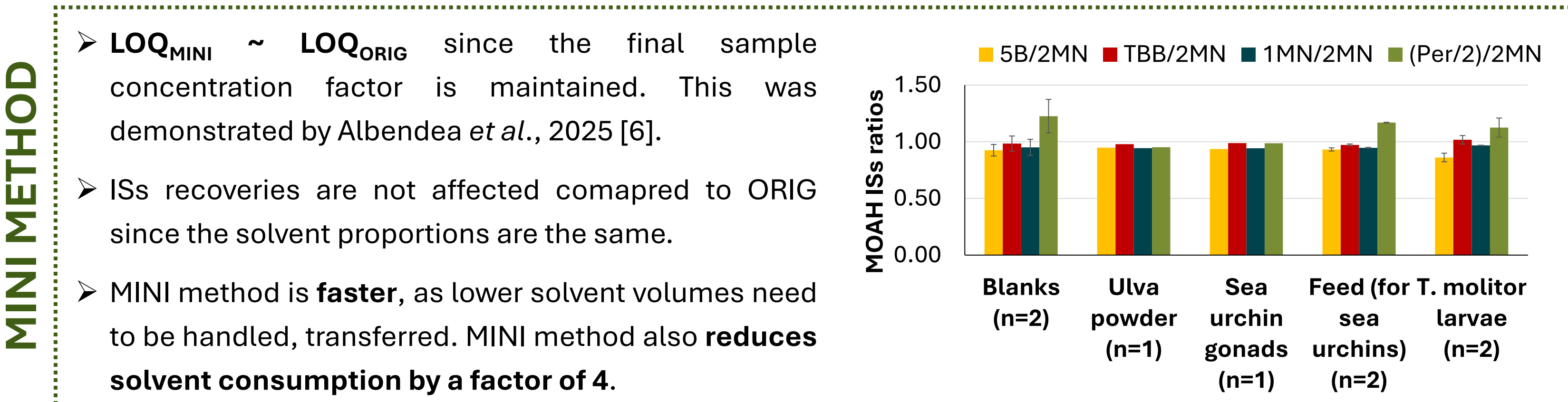
PERFORMANCES OF EMUL vs ORIG?



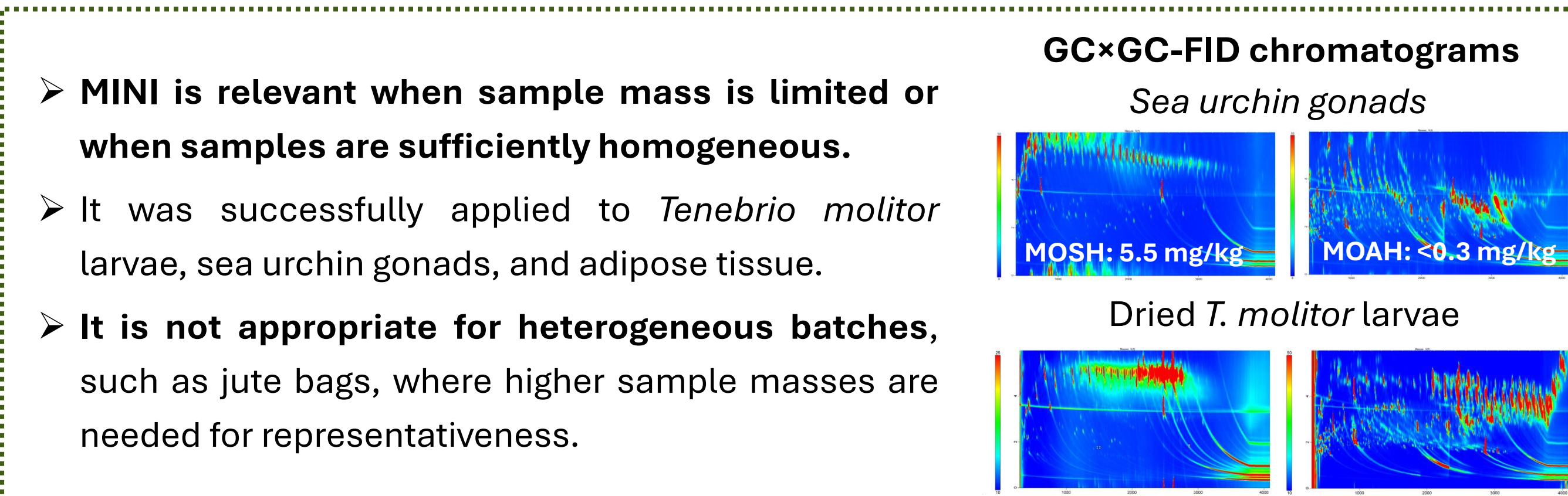
FOR WHICH MATRICES IS EMUL METHOD RELEVANT?



PERFORMANCES OF MINI vs ORIG?



FOR WHICH MATRICES IS MINI METHOD RELEVANT?



CONCLUSION

- Overall, MASE has so far shown good versatility for MOSH/MOAH analysis across the matrices investigated.
- The ORIG, EMUL and MINI variants allow the workflow to be adapted to different matrix behaviours and sample availability.
- Further work will extend the applicability study to additional matrices, after which the need for additional protocol variants will be evaluated.

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