COMPARISON OF DIFFERENT EXTRACTION METHODS FOR THE DETERMINATION OF FATTY ACID METHYL ESTERs IN MUSSEL SAMPLES

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INTRODUCTION

The most common approach for analyzing Fatty Acids involves the conversion of esterified fatty acids into their corresponding Fatty Acid Methyl Esters (FAMEs) through derivatization.
This process not only facilitates the separation and identification of individual fatty acids but also enhances their detectability using gas chromatography (GC) techniques. However, the extraction and derivatization steps can be challenging, especially when dealing with complex matrices such as mussel samples. The aim of this study is to compare different extraction methods for the identification and quantification of FAMEs in mussel samples. Flow-modulation comprehensive two-dimensional gas chromatography (GC×GC) coupled to flame ionization detection (FID) was utilized for the subsequent analysis of FAMEs, enabling the identification and quantification of individual fatty acids. GC×GC analysis offers enhanced sensitivity compared to one-dimensional chromatography, resulting in improved separation capabilities and a well-structured chromatogram that facilitates the identification of FAMEs.

MATERIALS AND METHODS

ONE-STEP MICROWAVE-ASSISTED EXTRACTION AND DERIVATIZATION

0.5g sample + 10 mL HCl/MeOH + 25 mL CycloHexane

Program: 120 °C × 15min

DIRECT METHYLATION OF LIPIDS IN FOODS BY ALKALI HYDROLYSIS (Official Method Ce 2b-11) [1]

0,5 g sample + 5 mL NaOH /MeOH + 5mL BF_3/MeOH + 5 mL Hexane

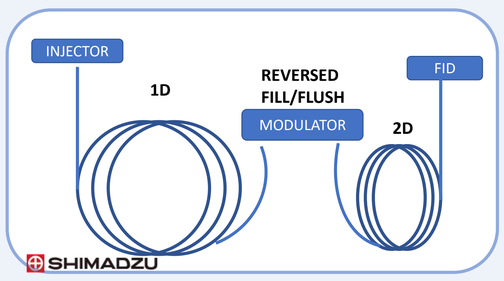
~ 1h

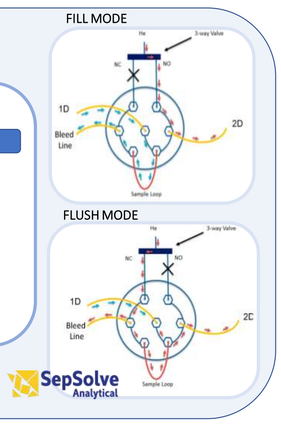
GC x GC – FID and GC-FID CONDITIONS

GCxGC-FID

1D Rtx-2330 (20 m x 0.18 mm x 0.1 µm);
const flow 0.5 mL/min
2D Rtx-5MS (5 m x 0.25 mm x 0.1 µm);
const flow 20 mL/min

Oven program: 40 °C (3min) to 260 °C at 10 °C/min
Modulation time: 3 s
Data were elaborated using: Chromespace software



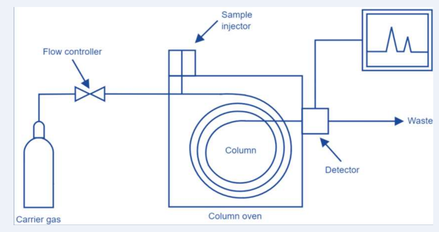


GC-FID

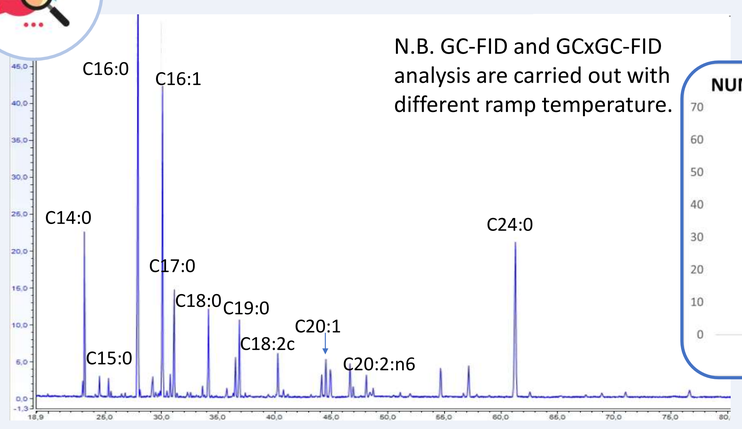
Official Method Ce 2b-11 [1]

SP®-2560 Capillary **GC Column**
L × I.D. 100 m × 0.25 mm, d_f 0.20 µm

Oven program:
60 °C (2 min) to 172 °C (5 min)
to 210 °C (35 min)



RESULTS AND DISCUSSION



N.B. GC-FID and GCxGC-FID analysis are carried out with different ramp temperature.

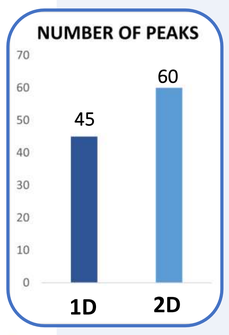


Figure 1. GC-FID Chromatogram of FAMES in mussle sample

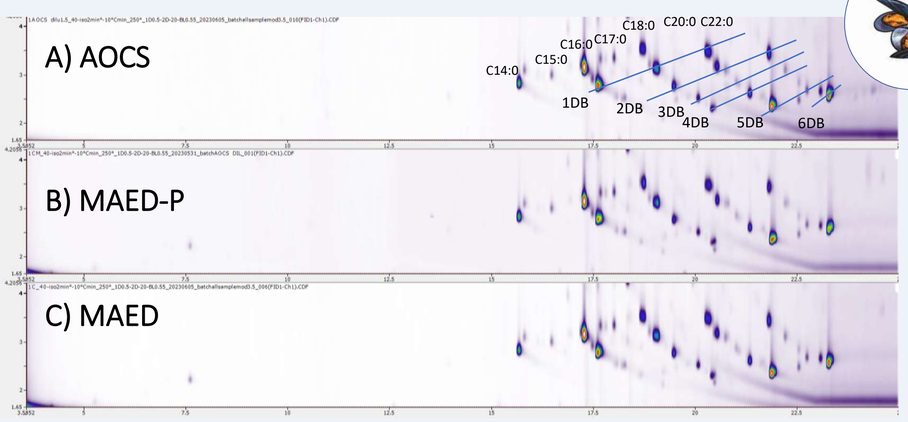
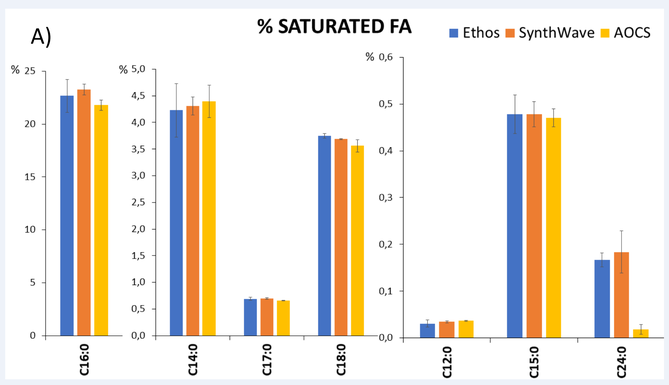
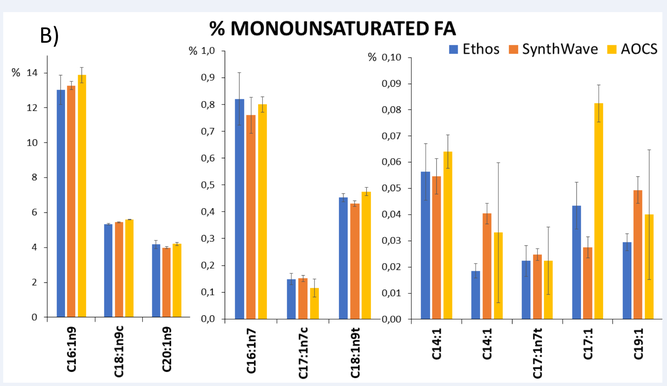
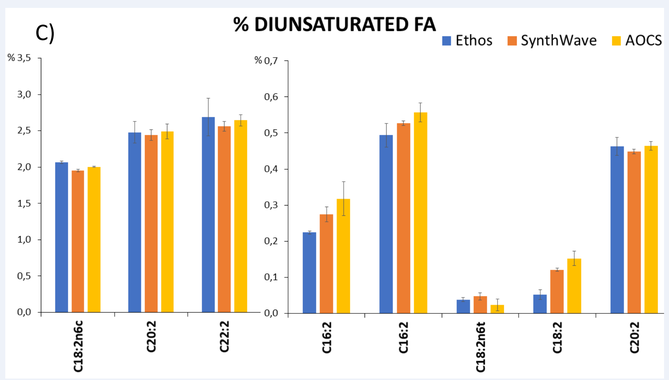


Figure 2. Comparison of GC×GC-FID chromatograms obtained with A) AOCS method; B) MAED-P method and C) MAED method







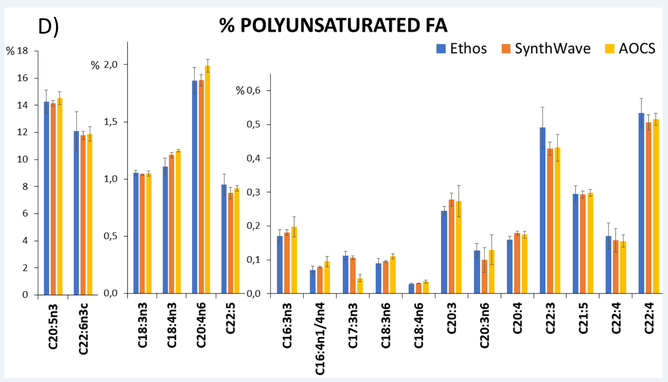


Figure 3. FAMEs profile A) Saturated; B) Mono-Unsaturated; C) Di-Unsaturated and D) Poly-Unsaturated FAMEs obtained performing the different extraction and derivatization methods followed by GCxGC-FID analysis.

The AOCS reference method for the determination of FAMEs [1] was compared with two different types of extraction/derivatization methods: Microwave-assisted extraction/derivatization (MAED) [2] and Pressurized Microwave-assisted extraction/derivatization (MAED-P). In the last case, the extraction/derivatization is conducted in an inert atmosphere. Comparing the results, overall the three extraction and derivatization methods resulted equivalent (Figure 3), except for C24:0 for which significant higher extraction was observed when microwave-based techniques were used, and C17:1 which showed an opposite behaviour.
On the other side, the use of GC×GC compared to 1D GC allowed for the identification of more compounds (60 vs 45 peaks) and at the same time provide a support for the identification thanks to the formation of clear chemical patterns in the 2D space, based on the number of double-bonds and their position.

EVALUATION OF THE GREENNESS OF THE METHODS [3]

With the MAED method there is the possibility of integrating multiple steps into one, promoting automation, minimizing energy consumption, and favouring in-situ procedures.

In this study MAED-P was performed based on the previously optimized MAED [2] just scaling down the overall amount of solvent and sample. On-going studies aim to miniaturize this method.

CONCLUSIONS

Microwave-based extraction and derivatization methods proved to be a valid alternative to the official method for FAMEs analysis. The proposed methodology coupled to GC×GC-FID provided satisfactory results. MAED represents a simple, fast (15' vs 1h [1]) and universal sample preparation step. Moreover, GC×GC-FID is more sensitive and with higher separation power than GC-FID and provides a structured chromatogram that support the identification and separation of the geometrical and positional isomers in a single run. Thus, the proposed methods is highly beneficial for the overall lab throughput.

REFERENCES

[1] AOCS Official Method Ce 2b-11
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