FAST AND GREEN ANALYSIS BY MICROWAVE-ASSISTED SPONIFICATION AND EXTRACTION, FOLLOWED BY SOLID PHASE EXTRACTION

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INTRODUCTION

The official method proposed by the International Olive Council (IOC) [1] analyzes the sterol fraction through a multi-step process: initial saponification followed by liquid-liquid extraction and isolation of the fraction using thin-layer chromatography (TLC). The final analysis is carried out with gas chromatography coupled with a flame ionization detector (GC-FID). To streamline and accelerate this process, the sample is processed using a Microwave-Assisted Saponification and Extraction (MASE) method, with purification achieved through solid-phase extraction (SPE) prior to analysis in one-dimensional chromatography (^{1}D GC). This study focuses on improving the greenness of the method by reducing solvent volumes, increasing sample throughput, and enhancing the separation of sterols from interfering compounds (Des A, B, and C).

MATERERIALS & METHODS

IOC METHOD

1 SAPONIFICATION

5 g of olive oil + 50 mL 2M KOH (**EtOH/H2O 80:20** v/v)

2 LIQUID-LIQUID EXTRACTION

3 times using: 80 mL +70 mL +70 mL of ethyl ether

3 WASHING

With water untill neutrality, almost 200 mL

4 TLC

Preparation of the basic thin-layer chromatography plates and development in Hexane: ethyl ether 65:35 v/v

IMPROVED METHOD

1 MASE

1 g of olive oil + 10 mL 2M KOH (**EtOH/H_2O 50/50** v/v) + 10 mL Hexane

2 WASHING

With water untill neutrality, almost 200 mL

3 SPE

1g silica gel basified with 0,2 M KOH.
CONDITIONING: 6 mL of Hexane.
LOADING: Sample dissolved in 1 mL of Hexane.
WASHING: 40 mL of Cyclohex/Et_2O 98/2 v/v,
ELUTION: 7 mL of Cyclohex/Et_2O30/70 v/v.

DERIVATIZATION AND GC-FID

Rxi 5MS 30 ×0,25 mm i.d ×0,25 µm
Flow: 1,8 mL/min Ramp: 80°C held 1 min

Total time: 7h 40 min

Total time: 3h 30 min

RESULTS

MASE

Temperature and Time were the two variables to optimize for the MASE. We used an inscribed central composite design (Figure 1) to explore the **temperature** in the range of 60°C to 140°C and the **time** in the range of 10 minutes to 30 minutes.

The model incorporated first-order (linear) and quadratic terms for both time and temperature.

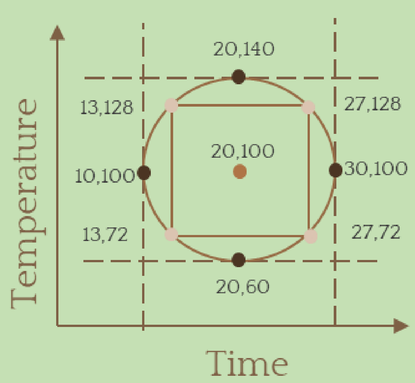

Figure1, Central Composite design Inscribed

A response surface methodology (RSM) was applied to model the relationship between residues (response variables) and the two independent variables.

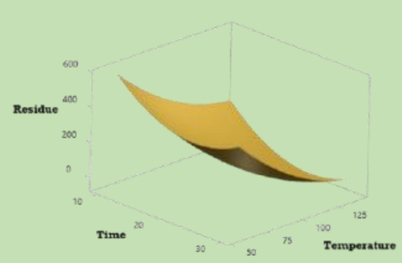

Figure2, Response surface

The design of the experiment outcome (Figure 2) shows that increasing temperature and time the response (residue) decrease till a certain point. In this case, the optimal condition were found targeting the same residue as the official method. Under this requirement, the chosen conditions were 20 min and 120 °C.

RESULTS

SPE

To optimize the solid-phase extraction procedure, we started from the method developed by Mascrez et al. [2], with the goal of reducing solvent volumes and improving the co-elution of sterols with certain interfering compounds, hereafter referred to as Des A, B, and C (Figure 3). The initial step involved replacing hexane with cyclohexane, monitoring how changes in solvent strength of the washing mixture affected elution, and adjusting accordingly (Figure 4).

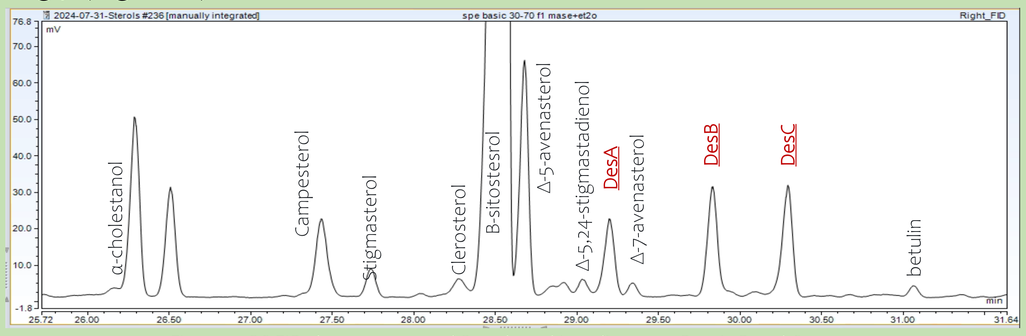

Figure 3. GC-FID chromatogram of Sterols and Interfierences Des A, B and C

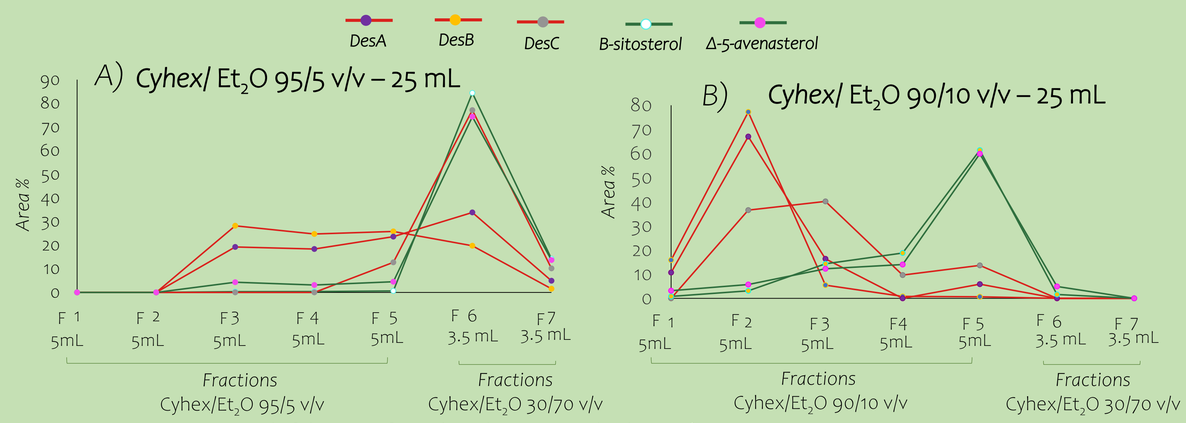

Figure 4. Trend of the area of sterols and Des A, B and C during A) *Cyhex/* Et_2O 95/5 v/v and B) *Cyhex/* Et_2O 90/10 v/v

The fraction of washing were divided in sub-fractions (%F1-%F5) and the same for the elution fraction (%F6-%F7) to identify the exact point where the interferences (red) and the sterols (green) could be separated. However, simply switching to cyclohexane did not lead to better separation of Des A, B, and C and sterols . The condition Cyclohexane/Et_2O 90/10 v/v was almost separating sterols and interferences but not as efficiently as needed. Based on the work of Mathiason (Figure 5 A)[3], we tested basified silica, which showed promising improvements (Figure 5B). The procedure is still under optimization to reduce more the volume of solvents and totally separate the interferences Des A, B and C from sterols during the SPE procedure.

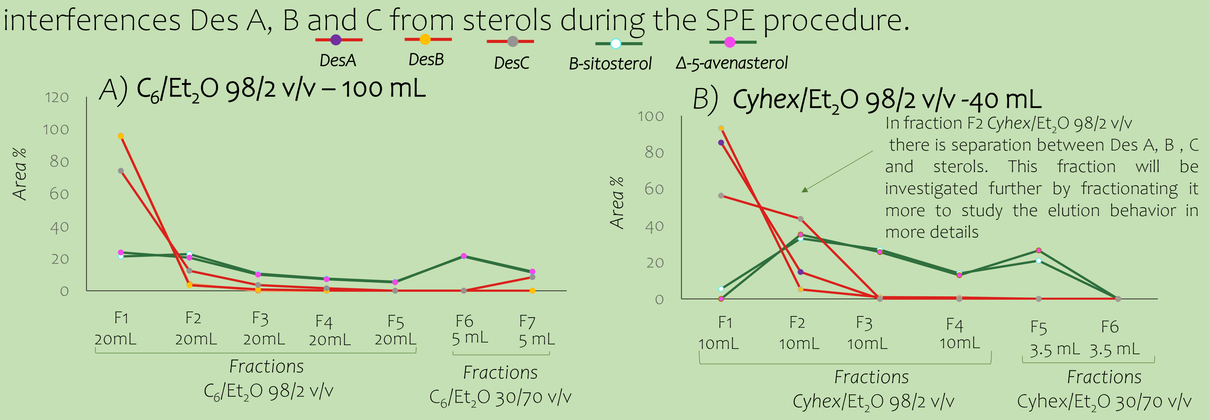

Figure 5. Trend of the area of sterols and Des A, B and C with basified SPE. A) Mathianson, B) Improved method

GRENNEESS

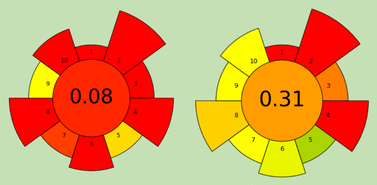

Figure5. Greenness od A) IOC, B) Proposed method

As it is shown in Figure 5, the greenness is clearly improved in the proposed method. Particularly important is the throughput of sample thanks to the MASE, the reduced volume of solvent used and the automation promoted by the integration of the two steps.

CONCLUSIONS

The optimized MASE method offers a faster approach to sample preparation for sterol analysis. In this method, two steps that are performed separately in the official protocol are combined, leading to reduced solvent consumption and shorter processing time. Additionally, the amount of ethanol used for saponification is decreased in MASE, enhancing the environmental sustainability of the procedure.
In particular, the solid-phase extraction (SPE) step will be further optimized to lower solvent volumes.

ADKNOLEDGEMENT AND REFERENCES

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[1] International Olive Council (IOC). International Olive Council: Madrid, Spain, 2017.
[2] Mascrez et al Foods 2021 Feb 18;10(2):445
[3] Mathison et al. A Rapid Method To Determine Sterol, Erythrodiol, and Uvaol Concentrations in Olive Oil. Journal of Agricultural chemistry, 2013