Microwave-assisted extraction and saponification to increase the throughput in food quality analysis

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INTRODUCTION

Sample preparation is often the most time-intensive step in the analytical workflow, yet it remains critical to prevent contamination, enhance accuracy, and minimize the risk of data distortion. Despite these benefits, many methods—particularly those used for high-fat foods—are still lengthy and demand significant time and solvent use. This is especially the case when saponification is needed to enrich minor compounds. To streamline and accelerate this process, microwave-assisted solvent extraction (MAE) and microwave-assisted saponification (MAS) provide a reliable and efficient alternative. By utilizing microwave heating, these techniques reduce time and solvent consumption, allowing for a greener, more cost-effective lab approach. This study introduces an optimized Microwave-assisted saponification and extraction (MASE) method for the rapid, robust analysis of sterols in lipids. Additionally, tedious TLC-based sterol purification is replaced by a faster, more practical SPE purification step, followed by derivatization and final GC-FID analysis.

MATERIAL AND METHOD

OFFICIAL METHOD IOC [1]

SAPONIFICATION	LIQUID-LIQUID EXTRACTION	WASHING	THIN LAYER CHROMATOGRAPHY	DERIVATIZATION and GC-FID	Total time
5 g of olive oil + 50 mL 2M KOH (**EtOH/H2O 80:20** v/v) **40 MINUTES**	3 times using: 80 mL +70 mL +70 mL of ethyl ether **30 MINUTES**	Till neutrality: almost 200 mL of H_2O **30 MINUTES**	Preparation of the basic thin-layer chromatography plates and development in Hexane: ethyl ether 65:35 v/v **5 HOURS**	Pyridine and BSTFA **15 MINUTES** COLUMN Rxi-5MS 30 m 0.25 mm i.d. 0.25 m. FLOW He 1.3 mL/min OVEN PROGRAM 80°C, ramp to 160°C at 20°C/min, and ramp to 340°C at 5°C/min, hold 1 min **30 MINUTES**	**7 H 40 min**

PROPOSED MASE-SPE METHOD

MICROWAVE-ASSISTED EXTRACTION AND SAPONIFICATION	WASHING	SOLID PHASE EXTRACTION [2]	Total time
1 g of olive oil + 10 mL 2M KOH (**EtOH/H2O 1:1** v/v) + 10 mL Hexane **20 MINUTES**	Till neutrality: almost 50 mL of H_2O **30 MINUTES**	1g silica gel. CONDITIONING: 6 mL of Hexane. LOADING: Sample dissolved in 1 mL of Hexane. WASHING: 5 mL of Hexane/diethyl ether 98/2, 5 mL Hexane/diethyl ether 96/4, 25 mL of Hexane/diethyl ether 95/5. ELUTION: 7 mL of Hexane/diethyl ether 30/70. **2 HOURS**	**3 H 30 min**

EXPERIMENTAL DESIGN

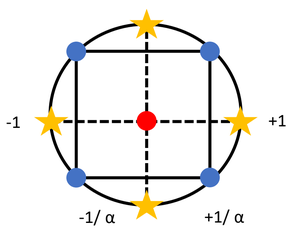

Figure 1. Inscribed central composite design

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.

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

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

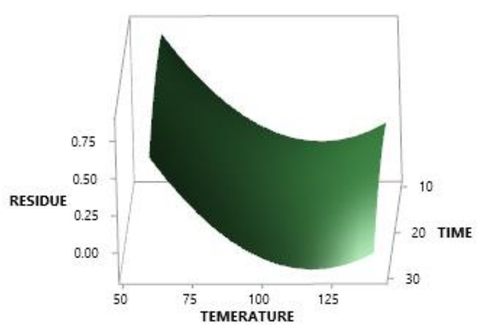

Figure 2. Response surface

The design of the experiment outcome (Figure 2) shows that temperature is the primary factor influencing the residue. While, time does not significantly affect the residue within the tested range. To have a residue as similar as possible to a reference method, the chosen conditions are 20 min and 120 °C.

RESULTS AND DISCUSSION

The optimized MASE method proposed an easy and fast sample preparation for the characterization of sterols. Two steps that are carried out separately in the official method are merged in the MASE. As a consequence, the solvent consumption and the processing time are reduced. Moreover, the amount of ethanol used for saponification has been decreased in MASE, looking forward to the green aspect of the procedure.

Figure 3 shows that the IOC method and the MASE followed by no matter which purification step (TLC or SPE) gave aligned results. However, we propose the SPE to simplify the process. Thanks to the MASE-SPE method the throughput of the process increases significantly. Microwaves allow for more efficient heating, processing more samples simultaneously, and in this case, integrating saponification and extraction in one step.

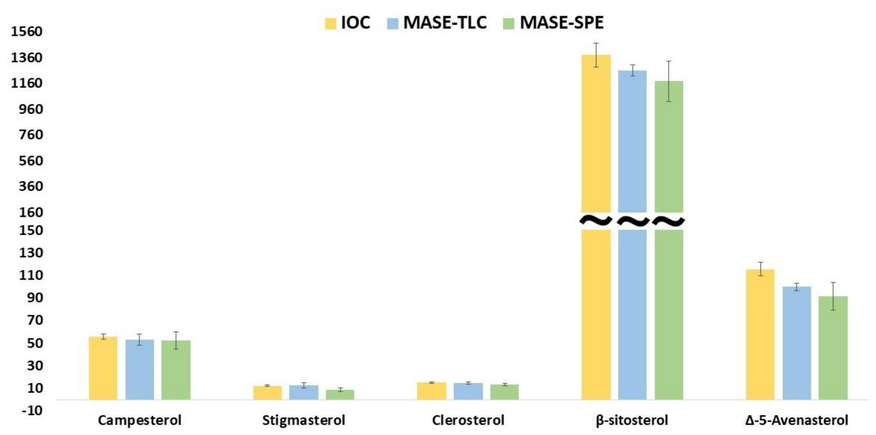

Figure 3. Barplot of sterols

CONCLUSION AND FUTURE PERSPECTIVE

The optimized MAS-SPE method proposed a single solution for the characterization of sterols in an easy sample preparation procedure. A future perspective is to optimize SPE to further reduce volumes and minimize the co-elution of other compounds along with sterols.

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[1] International Olive Council (IOC). International Olive Council: Madrid, Spain, 2017. [2] Mascrez et al Foods 2021