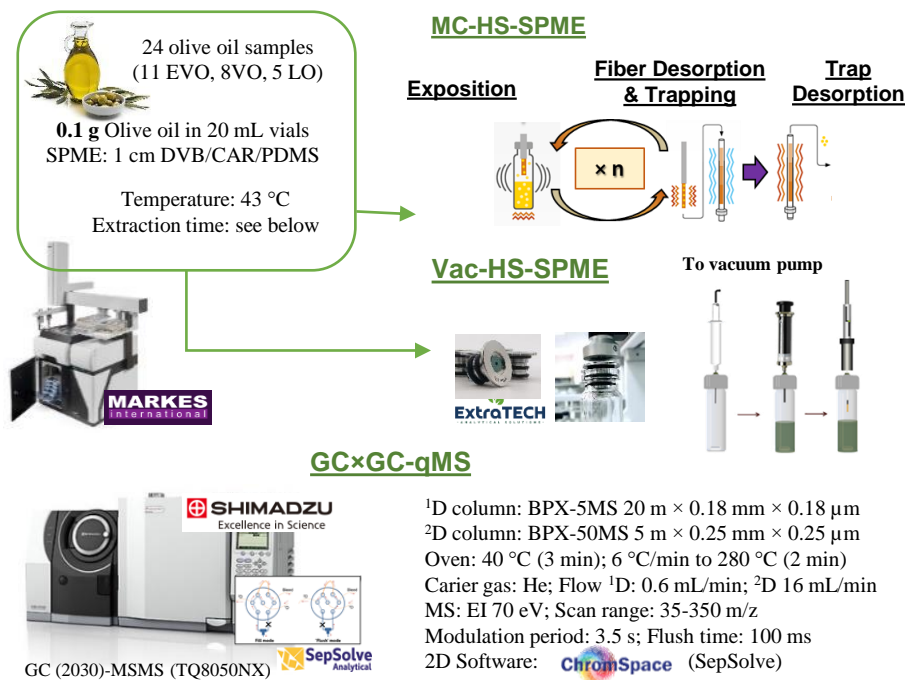


## Introduction

In this study, we assessed vacuum (Vac) and multiple cumulative trapping (MCT) in headspace solid-phase microextraction (HS-SPME) as potential alternative or combined methods for highly informative virgin olive oil volatile profiling. All three techniques, i.e., Vac-HS-SPME, MCT-HS-SPME, and Vac-MCT-HS-SPME demonstrated enhanced semi-volatile extraction performance. The synergistic combination of Vac and MCT exhibited up to a 5-fold increase in extraction efficiency for less volatile compounds. Integrating these techniques with comprehensive multidimensional gas chromatography (GC×GC) facilitated a thorough analysis of the volatilome.

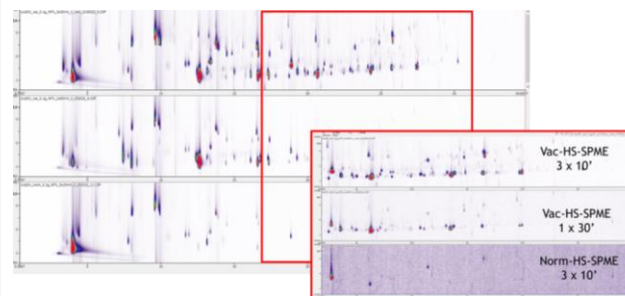
Initially, we examined 18 targeted quality markers, previously identified through traditional HS-SPME-GC-MS, to compare the performance of the three techniques and later to assess their effectiveness in categorizing virgin olive oil in its commercial categories. Using the proposed alternative techniques, the selected markers provided biased results towards geographical origin over the commercial categories, while using the data matrices in an untargeted way allowed for a less biased clusterization based on the commercial categories. Notably, no misclassifications were observed, except for one instance where one extra virgin olive oil was erroneously classified as VV in the 3×10 min Vac-MCT-HS-SPME.

## Material and Methods

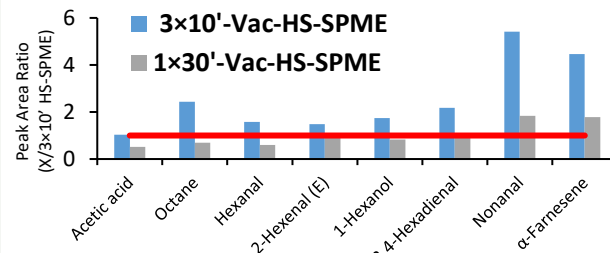


## Sampling evaluation

The repeated MCT provide a much higher extraction yield, in particular when performed in vacuum conditions (**Figure 1**). Vac-HS-SPME and atmospheric pressure MCT-HSSPME show similar extraction responses, being their ratio slightly below 1 for the most volatile compounds and slightly above 1 for less volatile compounds (max. 1.8 for nonanal, **Figure 2**). The recovery gain using Vac-MCT-HS-SPME is remarkably, reaching ratios compared to 3 × 10 min HS-SPME above a value of 4 for the less volatile compounds (5.4, and 4.5 for nonanal and α-farnesene, respectively). This behaviour is a synergic combination of the effects observed for Vac and MCT-HS-SPME alone.



**Fig 1.** Comparison of the GC×GC chromatograms obtained using MCT-HS-SPME (HS-SPME 3×10'); Vac-HS-SPME (1×30') and Vac-MCT-HS-SPME (3×10').



**Fig 2.** Change in extraction efficiency for 3 × 10 min-Vac-MCT- and 1 × 30 min-Vac- HS-SPME versus 3 × 10 min-MCT-HS-SPME. The red line indicates the value of 1.

## References:

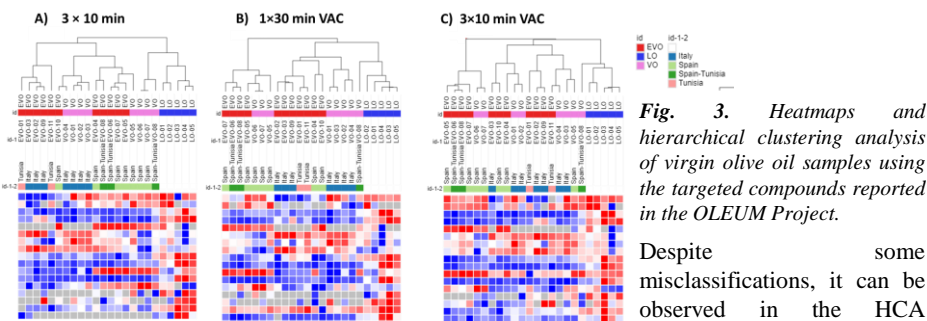
- [1] S. Mascrez, E. Psillakis and G. Purcaro, Anal Chim Acta, 2020, 1103, 106-114.
- [2] S. Mascrez and G. Purcaro, Anal Chim Acta, 2020, 1122, 89-96.
- [3] S. Mascrez et al. Food Chem 442 (2024)138409.

## Results and Discussion

### Cross-Sample comparison

A cross-sample comparison based on the pattern recognition technique was carried out to assess the improvement in the overall information extractable using the MCT-HS-SPME with or without vacuum and single Vac-HS-SPME.

A first study was performed targeting the quality markers identified and used in the European OLEUM project to evaluate the ability of these compounds to discriminate among commercial categories despite the sampling approach applied (**Figure 3**).

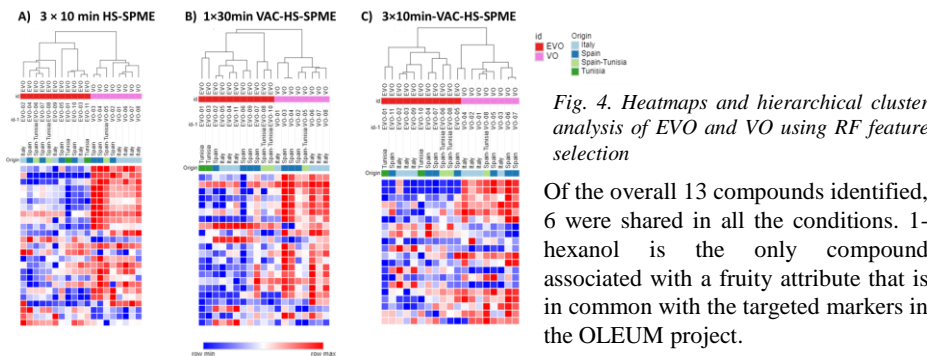


**Fig. 3.** Heatmaps and hierarchical clustering analysis of virgin olive oil samples using the targeted compounds reported in the OLEUM Project.

Despite some misclassifications, it can be observed in the HCA obtained from the three

studied sampling techniques that a general clustering is obtained based on the geographical origin (mainly Spain and Italy; Tunisia is generally clusterized with Italy).

On a second study, the same datasets were elaborated to select the most discriminating features to maximize the clustering among the commercial categories. As the LO were heavily defected overfitting the separation they were removed (**Figure 4**).



**Fig. 4.** Heatmaps and hierarchical cluster analysis of EVO and VO using RF feature selection

Of the overall 13 compounds identified, 6 were shared in all the conditions. 1-hexanol is the only compound associated with a fruity attribute that is in common with the targeted markers in the OLEUM project.

As aforementioned, it is the authors' opinion that to achieve the goal of supporting the panel test in the classification of EVO and VO, it is vital to select markers able to perform robustly despite the analytical technique applied and the sample set investigated.

## Acknowledgments:

The authors thank Markes, SepSolve, Shimadzu, Supelco, and Extratech for their support to this project.