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ANALYTICAL SOLUTIONS

# Comparison of Vac-HS-SPME and HS-SPME coupled to GC-MS for virgin olive oil aroma analysis

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## ABSTRACT

The effectiveness of vacuum-assisted headspace solid-phase microextraction (Vac-HS-SPME) was evaluated in contrast to conventional headspace solid-phase microextraction (HS-SPME) when analyzing virgin olive oil. Vac-HS-SPME demonstrated notable advantages, especially concerning semi-volatile compounds, markedly enhancing extraction kinetics. Furthermore, for viscous samples, such as olive oil, the combination of sample heating and vacuum application proved advantageous by lowering sample viscosity, enhancing compound diffusivity in the liquid phase, and improving the volatilization of less volatile compounds.


## INTRODUCTION

Virgin olive oil (VOO), renowned for its high value and health benefits, is a key component of the Mediterranean diet. European regulations classify physically extracted olive oil into different commercial categories—extra virgin oil (EVO), virgin oil (VO), and lampante oil (LO)—based on various criteria, including physicochemical properties and sensory characteristics. The sensory evaluation process faces challenges such as time consumption, costliness, and potential inconsistency in results along different panels around the world, prompting the need for improvement. A significant focus has been placed on understanding the intricate aroma profile of olive oil, which is influenced by many factors like cultivar, geography, harvesting year, and processing methods. The need for a standardized analytical method to support or even replace the sensory evaluation has been highlighted several times, and it is the focus of many ongoing researches. The challenge is to find a robust and highly informative method to sample the characteristic aroma of virgin olive oil.

The gold technique used in the literature for characterizing virgin olive oil is headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography-mass spectrometry (GC-MS). SPME is based on partition equilibria between the sample, the headspace, and the sorbent immobilized on the SPME fiber. Reaching equilibrium may vary from a few minutes to several hours, especially in the case of semi-volatile compounds. Stirring and temperature are usually applied to accelerate the kinetics. Nevertheless, using high temperatures for a long extraction time can create artifacts and alter the volatile profile as perceived by the human nose. An exciting alternative investigated in this application is the use of vacuum-assisted (Vac-) HS-SPME to accelerate the extraction kinetics and, consequently, the coverage of semi-volatile compounds in a single analysis. A systematic comparison of regular and Vac-HS-SPME was performed before investigating the capability of VAC-HS-SPME in clustering VOO based on its commercial category.

## EXPERIMENTAL

Table 1 and Table 2 describe the final optimized Vac-HS-SPME and standard HS-SPME methods, respectively. Table 3 gives details on the GC-MS method.

*Table 1. Optimized Vac-HS-SPME method.*

| | |
|---|---|
| **Sample:** | 1.5/0.1 g of olive oil in 20 mL crimp top vial; ExtraTECH Vac-closure (PN: 20-101) |
| **Air- evacuation** | 1 min before sample introduction, pumping unit with 7 mbar ultimate vacuum |
| **SPME Fiber:** | DVB/CAR/PDMS, 50/30 µm/ 1 cm |
| **Incubation:** | 5 min, 30 or 43 °C, agitation |
| **Extraction:** | 10 and 30 min, 30 or 43 °C, agitation |

*Table 2. Optimized regular HS-SPME method.*

| | |
|---|---|
| **Sample:** | 1.5/0.1 g of olive oil |
| **Fiber:** | DVB/CAR/PDMS, 50/30 µm/ 1 cm |
| **Incubation:** | 5 min, 30 or 43 °C, agitation |
| **Extraction:** | 10 and 30 min, 30 or 43 °C, agitation |

*Table 3. GC-MS method.*

| | |
|---|---|
| **Column:** | SLB5MS (30 m × 0.25 mm i.d. × 0.5 µm df) |
| **Oven:** | 35 °C (2 min), 3 °C/min to 250 °C, 25°C/min to 300°C |
| **Inj. Temp.:** | 250 °C |
| **Carrier Gas:** | Helium, 1 mL / min constant flow |
| **Detector:** | MS, scan 35-500 m/z range |
| **Injection:** | 1:5 split |
| **Desorption:** | 2 min at 250 °C |

## RESULTS

The advantages of using Vac-HS-SPME are clearly visible in Figure 1. Indeed, it appears evident that the chromatogram obtained evacuating the air from the vial (blue one at the bottom) is much richer, particularly in semi-volatile compounds. The change in the overall kinetic of extraction can also be clearly seen from the surface responses obtained investigating the same time and temperature ranges in the two conditions (i.e., regular and Vac-HS-SPME), plotted against the total area of the chromatogram. Indeed, the extraction equilibrium was not reached under regular conditions, while a clear maximum can be observed using Vac-HS-SPME.

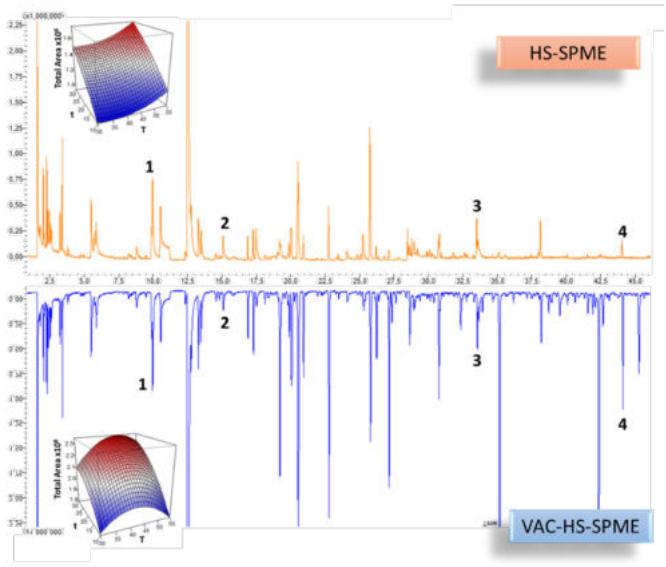


*Figure 1. Comparison between the TIC chromatograms obtained using regular and Vac-HS-SPME as well as the surface responses obtained for their optimization. Peak numbers correspond to compounds reported in Figure 2.*

It is interesting to notice the synergic effect of vacuum and temperature when dealing with high-viscous samples such as edible oils. Indeed, the increased temperature reduces the viscosity, thus increasing the diffusion coefficient. This allows the analytes to reach the liquid-gas interface rapidly and replenish the HS.

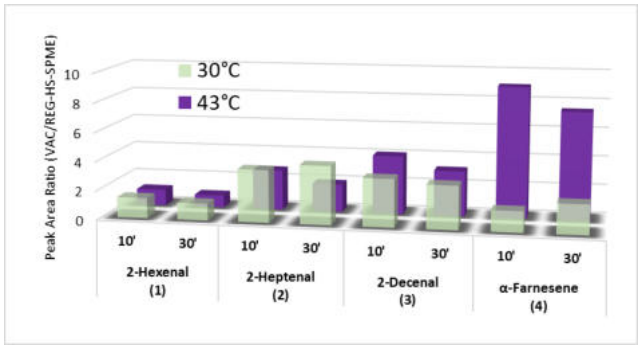


*Figure 2: Relative response increase in Vac-HS-SPME and regular HS-SPME at 30 °C and 43 °C from virgin olive oil. Compounds correspond to the peak nulbered in Figure 1.*

Indeed, a decrease of about 40% in viscosity was observed by heating the sample from 30°C to 43°C, thus improving the liquid-phase diffusivity and, with it, the overall extraction yield, especially for semi-volatiles. This process of 'replenishment' becomes even more evident in Vac-HS-SPME. This combined effect can be clearly seen in less volatile compounds such as decenal (3) and farnesene (4) in Figure 2.

### Cross-sample analysis of virgin olive oil

In a following study, the optimized Vac-HS-SPME was used to evaluate the ability to classify virgin olive oil based on the commercial category. The optimized Vac-HS-SPME was used to analyze 24 samples, certified for their commercial class, sensory evaluation, and geographical origin (for the virgin and extra virgin oils). A very good clusterization of the different samples was obtained as shown in Figure 3.

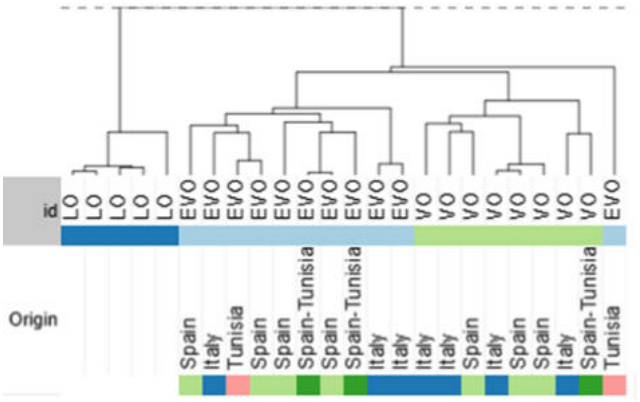


*Figure 3. Heatmap and hierarchical cluster analysis obtained by analyzing 24 samples of certified commercial class and geographical origin.*

## CONCLUSIONS

The benefits of employing Vac-HS-SPME over regular HS-SPME, as well as the synergic benefit of increased temperature and reduced pressure when dealing with highly viscous samples such as olive oil were demonstrated. Moreover, the application of Vac-HS-SPME in untargeted studies can significantly enhance the level of information attainable and the efficiency of cross-sample comparisons when employing pattern recognition algorithms. This enhancement allows for more precise identification of markers relevant to quality and authenticity studies.

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**REFERENCE:** S. Mascrez et al., Anal. Chim Acta 1103 (2020) 106-114 (https://doi.org/10.1016/j.aca.2019.12.053)

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