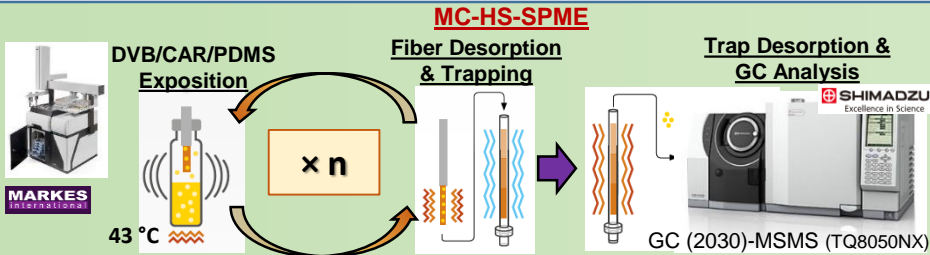


Introduction

Multiple-cumulative (MC) HS-SPME is a very powerful technique to enhance the level of information on the volatile profile of foods. A fully automated multi-mode sample preparation system, containing a sorbent based focussing trap to retain and preconcentrate analytes, was used. The novel approach was investigated for the analysis of olive oil aroma profile using a pattern recognition approach. Different extraction parameters were investigated, e.g. extraction time, numbers of cumulative extraction, and, last but not least, the sample volume. Shorter cumulative extraction times proved to be more effective than a single longer extraction to provide more comprehensive profile of volatiles and semi-volatiles. Fundamental was the choice of a proper sample volume avoiding headspace saturation in order to maximize the extraction of the semi-volatile compounds. This technique was successfully applied for the distinction of extra virgin olive oil, from the less expensive virgin olive oil and lampante oil [1,2]. Finally, the coupling of MC-SPME with GCxGC-MS provided a powerful platform for the detailed characterization of the extra-virgin olive oil aroma profile.

Material and Methods



GC-qMS

GC: Column : SLB – 5ms 30 m x 0.25 mm x 0.5 µm; Oven : 30 °C (5.5 min) to 310°C at 10 °C/min; 250 °C to 300 °C at 25 °C/min; Carrier gas: He, 35 cm/s (Constant µ)
MS: Mass range: 35 – 500 m/z; MS ionization : EI 70 eV

GCxGC-qMS

¹D column: BPX-5MS 20 m x 0.18 mm x 0.18 µm
²D column: BPX-50ms 5 m x 0.25 mm x 0.25 µm
Oven: 40°C (1 min); 4 °C/min to 280 °C (4 min)
Carrier gas: He; Flow ¹D: 0.5 mL/min; ²D 12 mL/min
Modulation period: 4.0 s; Flush time: 300 ms
2D Software: ChromSpace (SepSolve)

Table 1. Sampling design for MC-SPME. In italic conditions applied for the cross-sample comparison.

Sample amount (g)	Extraction time (min)	N cumulative extraction			Sample amount (g)	Extraction time (min)	N cumulative extraction		
0.1	<i>10</i>	<i>1</i>	<i>3</i>	<i>6</i>	1	<i>10</i>	<i>1</i>	<i>3</i>	<i>6</i>
	30	1	3	6		30	1	3	6
0.25	<i>10</i>	<i>1</i>	<i>3</i>	<i>6</i>	1.5	<i>10</i>	<i>1</i>	<i>3</i>	<i>6</i>
	30	1	3	6		30	1	3	6
0.5	<i>10</i>	<i>1</i>	<i>3</i>	<i>6</i>		<i>10</i>	<i>1</i>	<i>3</i>	<i>6</i>
	30	1	3	6					

Sample volume study

The use of peak area intensity as indicator of the absolute concentration is widely applied in cross sample comparisons, but HS linearity condition needs to be verified. According to the theory, when the HS linearity condition is verified, multiple headspace extractions (MHE) from the same vial determine an exponential decline of the chromatographic area recorded, which reflects in a logarithmic increased in MC-SPME.

Figure 1 shows the R^2 obtained with the linear and exponential models, when performing MC-SPME for 10 or 30 min with different sample volumes. The linear model fits better the cumulative curve when HS was saturated. Contrarily, the R^2 of the exponential model was maximized with 0.1 g of sample.

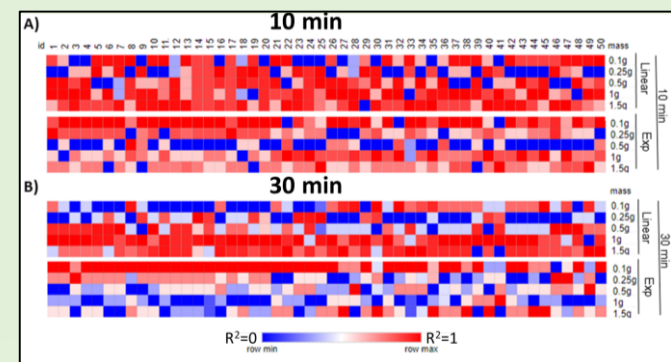


Figure 1: Heat-maps representing the distribution of R^2 obtained applying a linear or an exponential model in the MC-SPME.

The increase of the sample volume improved the extraction of high volatile compounds, contrarily, low volatile compounds are almost not affected (**Figure 2**). On the other hand, performing multiple-10 min extraction improved the sensitivity of the low volatile compounds compared to a single longer extraction (**Figure 3**).

Results and Discussion

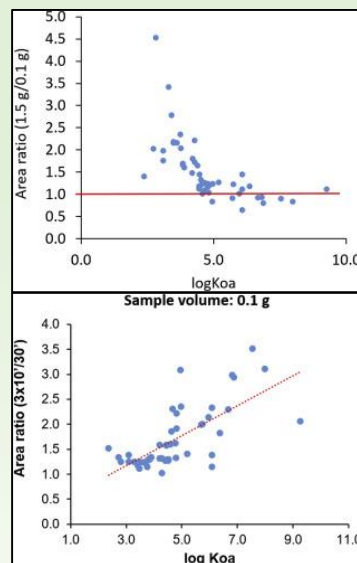


Figure 2: Change in extraction efficiency as a function of the $\log K_{oa}$ when extracting for 30 min at 43 °C.

Figure 3: Peak area ratio between performing 3 x 10-min-MC-SPME and 1 x 30-min versus $\log K_{oa}$.

Cross-sample comparison

Saturation of the HS hinders differences in the total concentration among samples, leading to a less informative fingerprint. The MC-HS-SPME approach improves the cross-sample comparison amplifying the differences and enhancing the sensitivity. 12 samples (6 EVO, 2 VO, and 4 LO) were tested under the italics conditions in **Table 1**.

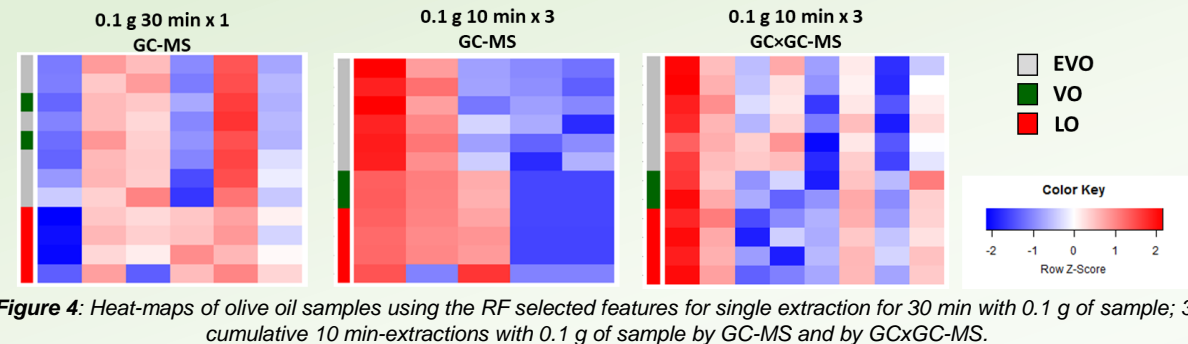


Figure 4: Heat-maps of olive oil samples using the RF selected features for single extraction for 30 min with 0.1 g of sample; 3-cumulative 10 min-extractions with 0.1 g of sample by GC-MS and by GCxGC-MS.

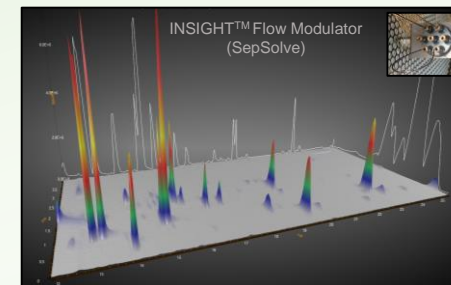


Figure 5: expansion of EVO by GCxGC-MS.

The MC-HS-SPME improved the clustering using both 1.5 g or 0.1 g of samples. The clustering capability is maximized performing 3 x 10 min MC-SPME of 0.1 g, also compared to 1 x 30 min SPME, allowing a perfect discrimination between EVO, VO and LO (**Figure 4**). The use of GCxGC-MS allowed to improve the separation power facilitating the identification of the compounds of interest, saving the discrimination capability (**Figure 5**).