ENHANCED FINGERPRINTING OF EXTRA VIRGIN OLIVE OIL BY MULTIPLE-CUMULATIVE SPME AND GC×GC

Steven Mascrez, Giorgia Purcaro

Gembloux Agro-Bio Tech, University of Liège, Gembloux, 5030, Belgium

LIÈGE université Gembloux Agro-Bio Tech

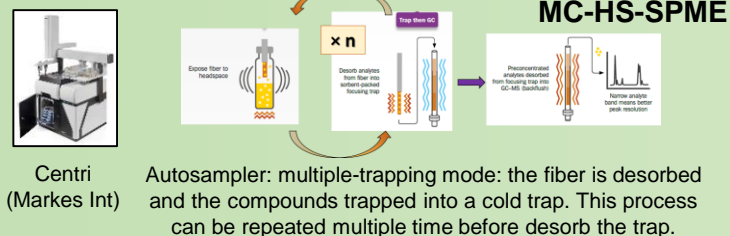12TH Multidimensional Chromatography Virtual Workshop

Introduction

The use of multiple-cumulative headspace-solid-phase microextraction (named MC-SPME) was explored to enhance the volatile profiling of extra-virgin olive oil (EVO). The SPME extraction was performed using an automated multi-mode sample preparation system containing a sorbent based focussing trap to retain and preconcentrate analytes. 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 sample volume to maximize the sensitivity and the sample throughput, important factor in large cross-sample studies. This technique has been successfully applied for the distinction of extra virgin olive oil (EVO), from the less expensive virgin olive oil (VO) and lampante oil (LO). The coupling of MC-SPME with GCxGC-MS generates a powerful platform for the detailed characterization of the extra-virgin olive oil aroma profile, with high potential to be extended towards different fields of applications.

Material and Methods

MC-HS-SPME

Centri (Markes Int)

Autosampler: multiple-trapping mode: the fiber is desorbed and the compounds trapped into a cold trap. This process can be repeated multiple time before desorb the trap.

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	*10*	*1*	*3*	*6*	1	10	1	3	6
	30	1	3	6		30	1	3	6
0.25	10	1	3	6	*1.5*	*10*	*1*	*3*	*6*
	30	1	3	6		30	1	3	6
0.5	10	1	3	6					
	30	1	3	6					

GC (2030)-MSMS (TQ8050NX) (Shimadzu)

GC×GC-qMS

Injection: Split 1:5
^{1}D column: BPX-5MS (SGE) 20 m × 0.18 mm × 0.18 µm
^{2}D column: BPX-50ms (SGE) 5 m × 0.25 mm × 0.25 µm
Oven program: 40°C hold 1 min; 4 °C/min to 280 °C hold 4 min
Career gas: He; Flow: 0.5 mL/min in ^{1}D; 12 mL/min in ^{2}D
MS: SCAN mode; range: 35-350 m/z; Ion source: 200 °C; Interface: 280 °C
Modulation period: 4.0 s; Flush time: 300 ms
2D Software: ChromSpace (SepSolve)

INSIGHT™ Flow Modulator (SepSolve)

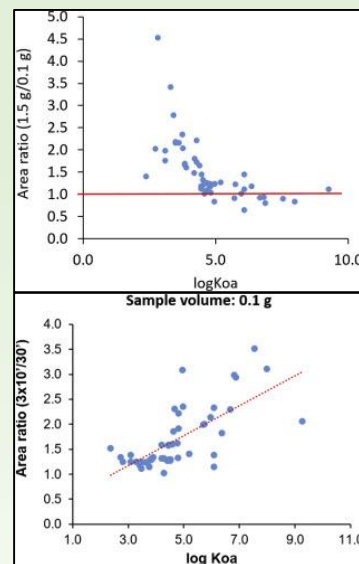## Results and Discussion

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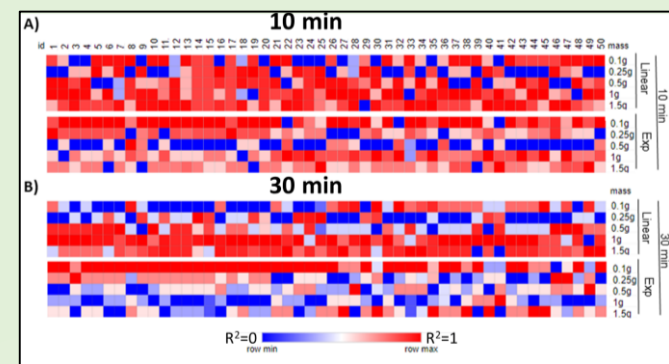
Figure 1: Heat-maps representing the distribution of R^2 obtained applying a linear or an exponential model in the MC-SPME.


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

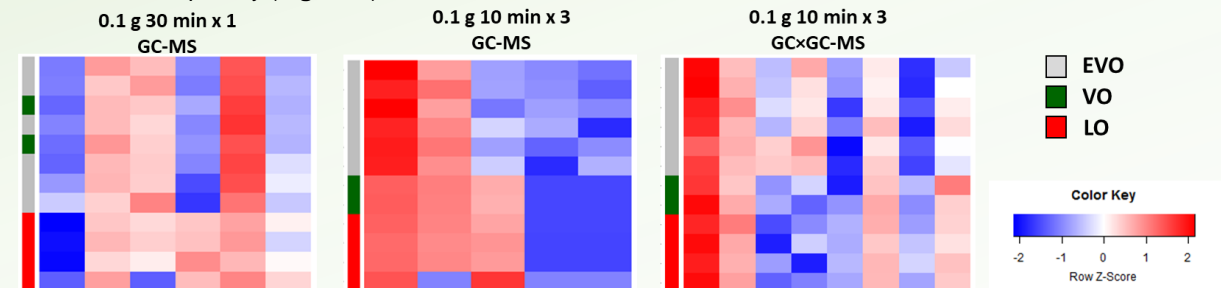*Figure 3: Peak area ratio between performing 3 x 10-min-MC-SPME and 1 x 30-min versus log K_{oa}.*

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**).

Cross-sample comparison

Differences in the total concentration among samples are hindered by the maximum capacity of the HS, leading to a less informative fingerprint. We hypothesized that the use of the MC-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 conditions reported in italics in **Table 1**. The MC-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 GC×GC-MS allowed to improve the separation power facilitating the identification of the compounds of interest, saving the discrimination capability (**Figure 5**).
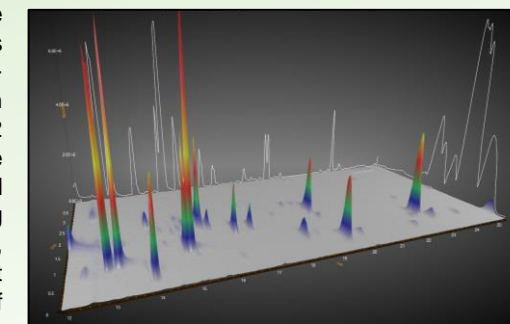
Figure 5: Expansion of EVO-1 sample obtained by GC×GC-MS.

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.

References
S. Mascrez, G. Purcaro, J Sep Sci, 43 (2020) 1934-1941
S. Mascrez, G. Purcaro, Anal Chim Acta, 1122 (2020) 89-96