



Application Note 274

Enhanced olive oil aroma profiling by solid-phase microextraction (SPME) using the novel approach of multi-step enrichment (MSE®)

Multi-step enrichment (MSE®) enhances the performance of headspace-SPME-trap (HS-SPME-trap). The aroma profile of olive oil was analysed using GC-MS to identify volatile and semi-volatile organic compounds (VOCs/SVOCs). SPME extraction was performed using an automated multi-mode sample extraction and enrichment platform (Centri®) containing a sorbent-based focusing trap to retain and preconcentrate analytes. Multiple sequential extractions were loaded onto the same trap from the same vial to produce an enriched sample profile. The technique was used to distinguish between olive oil samples using ChromCompare+, software that transforms complex data into meaningful results.

Introduction

SPME is a solvent-free sample preparation technique in which samples are extracted by immersive or headspace sampling. SPME is used for a wide range of sample classes and applications, including foods and beverages, environmental, clinical, industrial and defence. This is made possible by the availability of a variety of SPME fiber phases (including PDMS, polyacrylate and multi-phase DVB/CAR/PDMS), which allow analyte selectivity to be optimised. However, workflows for conventional ('direct') SPME sometimes suffer from its limited sensitivity. This stems from the small volume of sorptive phase on the fiber (typically ~0.5 µL of PDMS), as well as from the relatively slow heating rate of commonly used GC injection ports, resulting in broad peaks.

The primary focus of this work was to assess the incremental gain in peak area, and therefore sensitivity, for a range of representative analytes in olive oils when using the automated Centri sample extraction and enrichment platform. Linear retention index values were calculated to confirm peak identification with MS results from the NIST17 database. A commonly used multi-phase fiber for edible oils (divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS)) was selected for extraction and, as such, both absorptive and adsorptive processes took place with the potential for lower volatility compound displacement using longer sampling times. A balance between extraction time and sensitivity (across the broad volatility range of compounds in the aroma profile) needed to be achieved. A single sample extraction was compared to cumulative three-fold and six-fold extractions from the same vial using various incubation times. Furthermore, the advantage of using shorter extraction times for MSE compared to an equivalent single extraction time is shown; e.g. 3 x 10 minutes (total extraction time 30 mins) is compared to a single 30-minute extraction time. The successful application of MSE to the distinction of extra virgin

Background to Centri®

Markes' versatile Centri automation platform combines extraction, enrichment and injection for a wide range of complex GC-MS applications including solid, liquid and gaseous samples.

Centri uses leading GC robotics to maximise instrument usage and throughput, with automated extraction options including HiSorb™ high-capacity sorptive extraction (immersive or headspace), SPME, headspace and tube-based thermal desorption. All of these options offer sample enrichment on a cryogen-free, sorbent-packed focusing trap, before injection of the analytes into the GC-MS as a narrow band of vapour for optimum sensitivity.

Additional features offered by Centri include:

- Multi-step enrichment: combining multiple extracts onto the same trap for greater sensitivity.
- Re-collection: Quantitative trapping of the split flow from any sample extraction mode on a sorbent tube, for re-analysis without needing to repeat lengthy sample extraction procedures, or archiving in a stable form.



For more on Centri, visit www.markes.com.

olive oil, virgin olive oil and lampante olive oil has been extensively shown by research conducted by Purcaro *et al.*^{1,2} and is presented here.

Experimental

Section A: An extra virgin olive oil (EVO) sample purchased from a supermarket was used for a comparative analysis of single and multi-step SPME-trap extraction and enrichment (Figure 1).

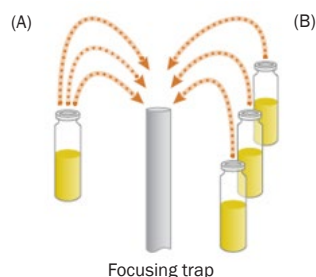


Figure 1: An illustration of the multi-step enrichment (MSE) process, which can be performed from a single vial (A) or from replicate samples in multiple vials (B). Sample extractions are loaded onto the same focusing trap for a single analysis.

Section B: Five extra virgin (EVO), two virgin (VO) and four lampante (LO) olive oils were analysed as part of a case study based on the MSE method developed in Section A.

Sample preparation:

Samples (1.5 g \pm 0.05 g) were weighed into 20-mL screw-top headspace vials, which were then sealed.

Extraction and enrichment:

Instrument: Centri (Markes International)

Headspace-SPME-trap:

Fiber:	Multi-phase DVB/CAR/PDMS, 10 mm, 50/30 μ m d _f (MilliporeSigma)
Sampling depth:	30 mm
Pre-sampling:	43°C (5 min)
Extraction:	43°C (10 min or 30 min)
Agitation:	300 rpm
Fiber desorption:	250°C (2 minutes)
Enrichment:	3 and 6 extractions (same vial)
Enrichment delay:	5 min
Replicates:	3 for each cumulative analysis
Flow path:	180°C
Focusing trap:	'Material emissions' (part no. U-T12ME-2S)
Purge flow:	50 mL/min (1 min)
Trap low:	0°C
Trap high:	300°C (10 min)
Split ratio:	5:1

GC-MS:

Column:	SLB®-5ms (silphenylene polymer), 30 m x 0.25 mm x 0.5 μ m
Oven program:	30°C (5.5 min), 10°C/min to 310°C (0 min)
Constant velocity:	35.9 cm/second (He)
Transfer line:	280°C
Ion source:	200°C
Mass range:	m/z 50-450

Data analysis:

Data mining and chemometrics in ChromCompare+ (SepSolve Analytical)

MS library: NIST17 database

Experimental linear retention index within ± 15 units

Results and discussion

Section A: Comparing the sensitivities of single extraction and multi-step enrichment

The extra virgin olive oil (EVO) sample was extracted with a multi-phase porous fiber (DVB/CAR/PDMS). When using this fiber, short extraction times are recommended to reduce competitive adsorption between the VOCs.³ The EVO sample was extracted once, then three and six times from the same vial (allowing 10 minutes for each extraction) and injected each time onto the same focusing trap of the Centri platform, such that the multiple extractions were then analysed in a single GC-MS run. The focusing trap was maintained at a sub-ambient temperature for all extractions and then rapidly heated to release the compounds as a narrow band into the capillary column. Figure 2 shows a comparison between the three extraction modes, *i.e.* single extraction and the three and six extractions, which demonstrates an increase in abundance that is proportional to the number of extractions. The gain in sensitivity using MSE was evaluated by dividing the peak area of each compound by the results obtained after a single extraction. The overall extraction ratio showed an average 3.01- and 5.96-fold increase (for the three and six extractions, respectively) in abundance compared to the single extraction. A representative selection of 10 compounds is shown in Figure 3 and Table 1 to further highlight this.

Compound	CAS no.	t _R	Incremental response to the 1 x 10-minute value	
			3 x 10 min	6 x 10 min
1-Penten-3-one	1629-58-9	3.47	3.11	5.86
Hex-(2E)-enal	6728-26-3	8.78	2.96	5.80
Hex-(2E)-enol	928-95-0	9.21	3.05	6.20
Hex-(3Z)-enyl acetate	3681-71-8	12.43	2.92	5.90
Benzyl alcohol	100-51-6	12.96	2.97	5.67
(3E)-4,8-Dimethyl-1,3,7-nonatriene	19945-61-0	14.48	2.90	5.99
1-Dodecene	112-41-4	15.97	2.98	5.83
α -Copaene	138874-68-7	18.71	3.07	5.78
2-Pentadecanone	2345-28-0	22.74	2.92	7.01
1-Hexadecanol	36653-82-4	24.80	3.30	5.55
Mean			3.01	5.96

Table 1: Peak area ratios using MSE SPME-trap. The 3 \times 10-minute and 6 \times 10-minute enrichment values are divided by the single SPME-trap (1 \times 10 min) peak area to give the incremental ratios. Averages of 3.01 and 5.96 were calculated for the compounds listed.

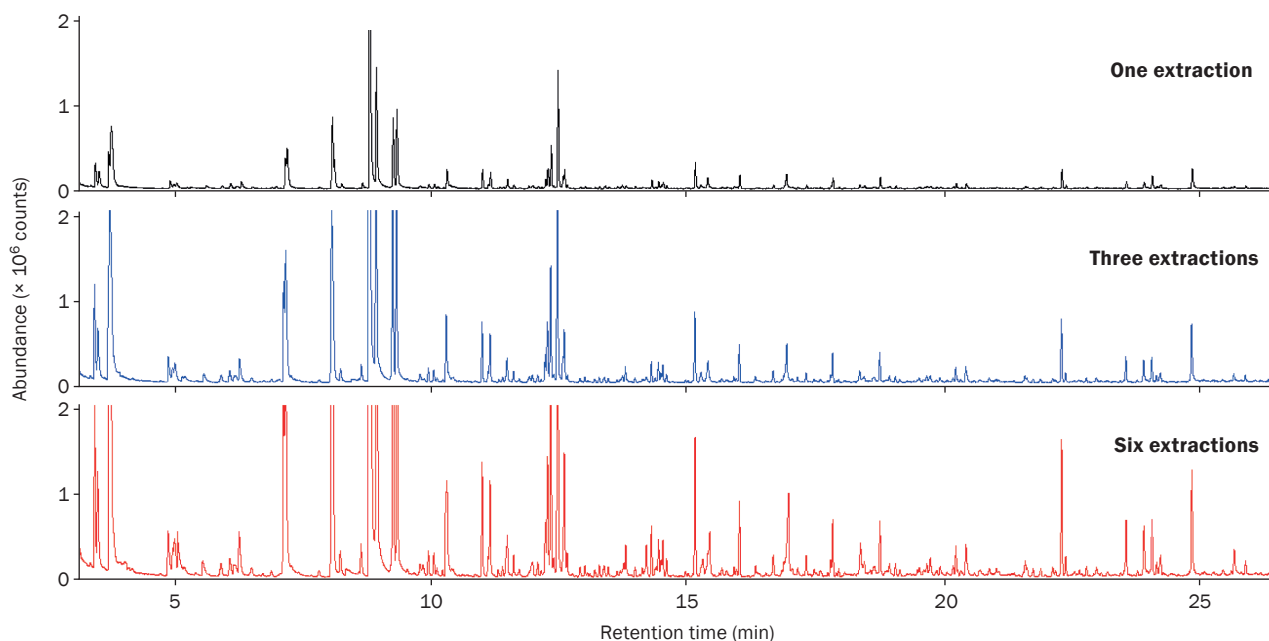


Figure 2: Total ion chromatograms (TICs) for the EVO sample using a single extraction (1 × 10 min; black upper trace), three extractions (3 × 10 min; blue middle trace) and six extractions (6 × 10 min; red lower trace). The increase in abundance is proportional to the number of extractions.

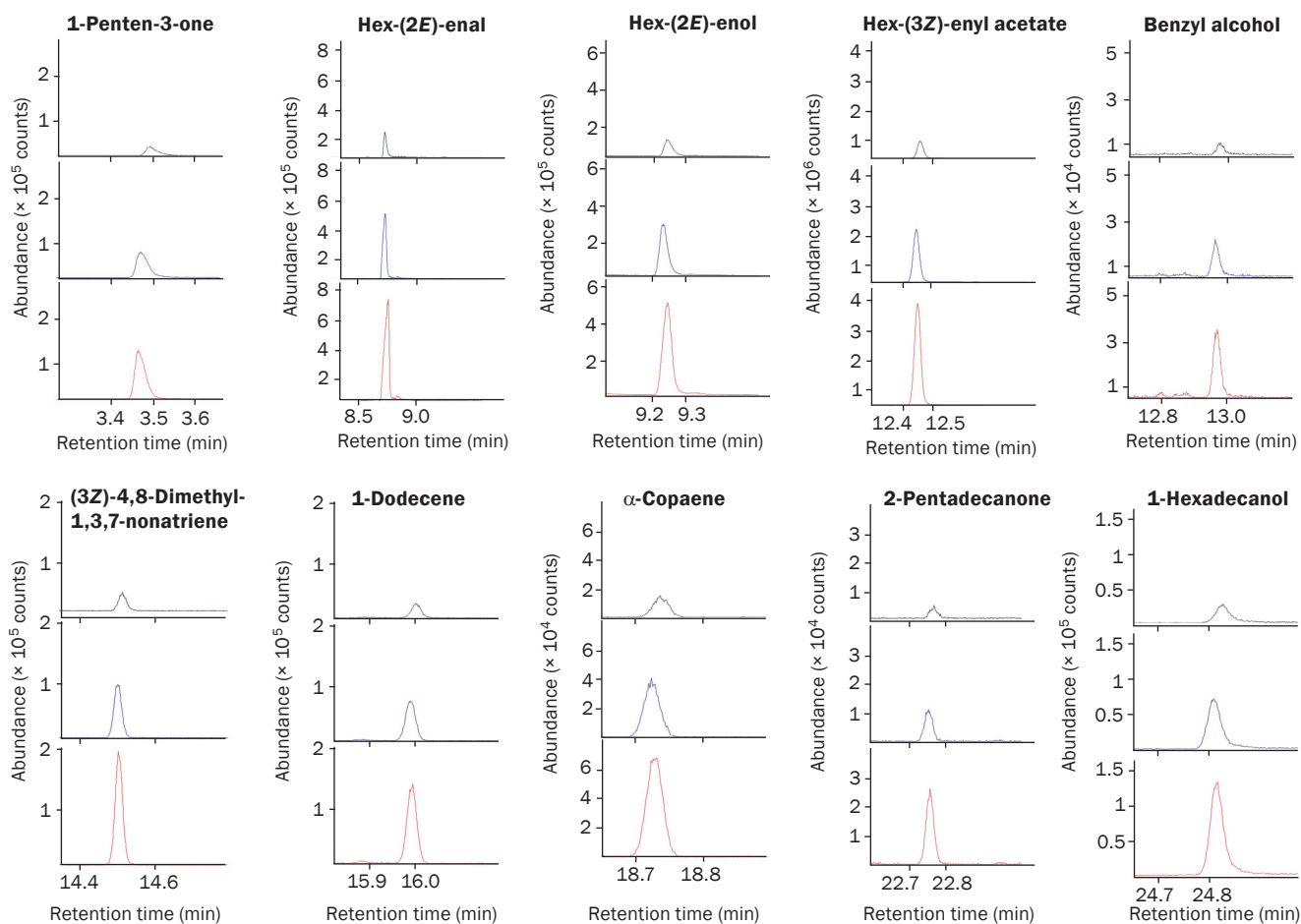


Figure 3: TIC profiles to compare the incremental responses of compounds with a single extraction (black upper trace), three extractions (blue middle trace) and six extractions (red lower trace).

Comparison between MSE and a single extraction using the same total sampling time

When designing an analytical system, the sampling method is an important consideration because the characteristics of some compounds, such as polarity or volatility, can affect the profile of a sample. For example, when sampling with a SPME fiber, profiles are altered when competitive adsorption processes occur, which may be related to the time taken to extract samples. A longer extraction time facilitates this displacement effect, which occurs with some heavier analytes. To better understand the effect of sampling time, a comparison between one extraction for 30 minutes and three extractions for ten minutes (to give the same total sampling time) was carried out. Figure 4 shows that the single

extraction (1×30 min) did not necessarily provide better results than the shorter repeat extractions. Differences are more evident with the SVOCs, for which, in some cases, a 10-minute extraction provided a significantly higher yield (inset in Figure 4(a)). For 2-pentadecanone, dibutyl adipate, n-butylbenzenesulfonamide and 1-hexadecanol, the abundances for the 3×10 -minute extractions are between four and six times higher than those of the 1×30 -minute extractions (Figure 4(b)). This suggests that short repeat samplings enable the detection of more compounds in the volatile profile of extra virgin olive oil than the single extraction. Dibutyl adipate and n-butylbenzenesulfonamide are plasticisers, likely to have leached from the oil's packaging.

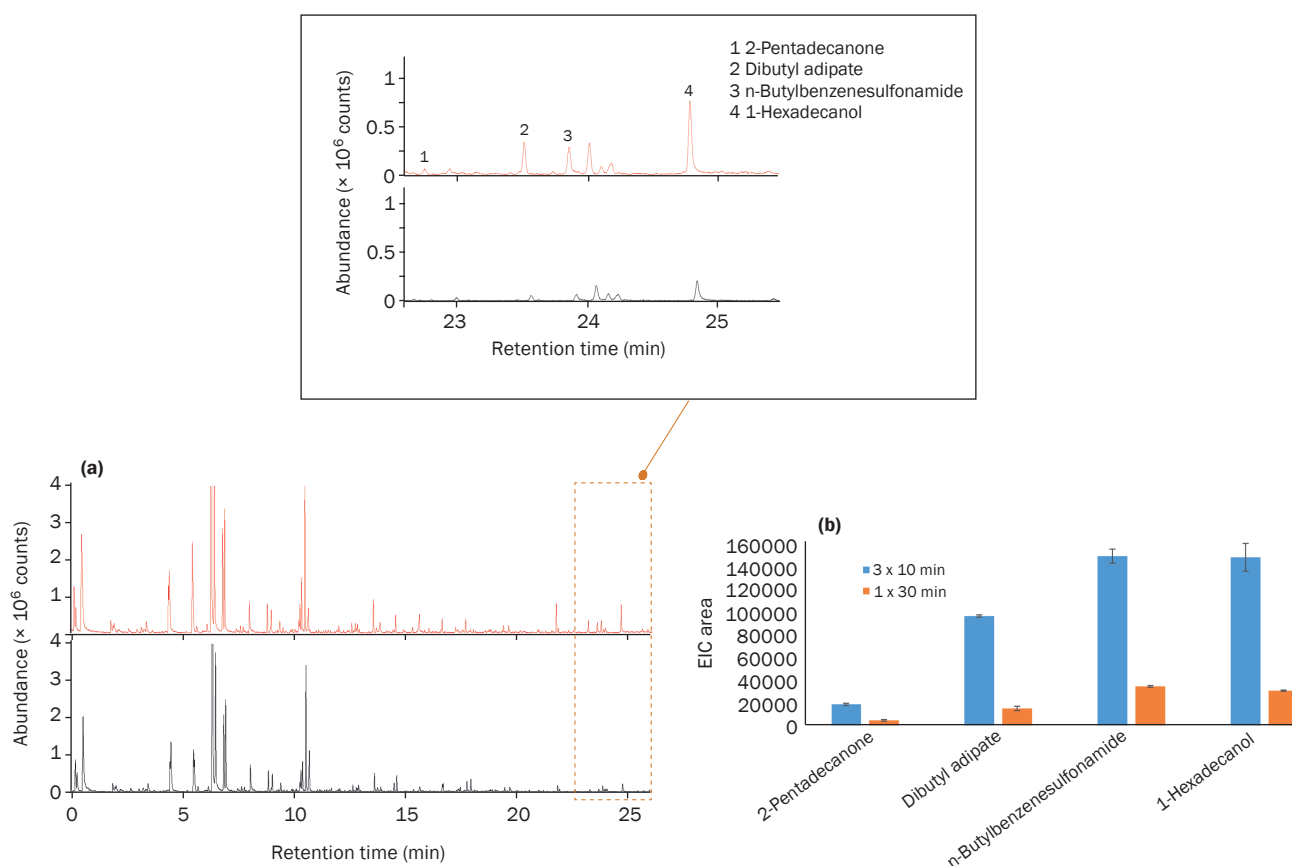


Figure 4: (a) Comparison between a 3×10 -min MSE (top panel, red) and a 1×30 -min extraction (bottom panel, black). The inset shows areas in both chromatograms that include the heavier SVOC compounds. (b) Comparison between EIC peak areas for the 3×10 -min MSE and the 1×30 -min extraction for four SVOCs.

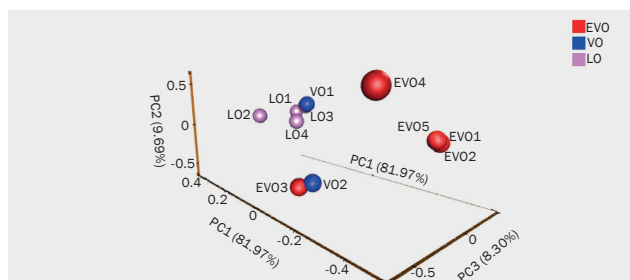


Figure 5: Principal components analysis (PCA) score plot for five extra virgin olive oils (EVOs), two virgin olive oils (VOs) and four lampante oils (LOs).

Section B: Identification of specific VOC markers from different olive oils by multi-step enrichment

There is an increasing interest in the use of VOCs as markers of authenticity in food matrices. Finding a reduced set of volatile markers offers a rapid and potentially inexpensive screening tool. Here, VOC profiles of several olive oils were automatically compared using the ChromCompare+ software platform. ChromCompare+ identified a reduced set of VOCs with the potential to act as markers of quality to distinguish between olive oil samples.

11 samples of olive oils (five extra virgin olive oils (EVOs), two virgin olive oils (VOs) and four lampante oils (LOs)) were analysed. Six SPME-trap extractions were performed, each for 10 minutes, which avoided the competitive adsorption discussed earlier. See Purcaro *et al.*¹ for a statistical analysis of the results. A reduced set of VOCs can be used to distinguish between EVOs (red) and LOs (pink) while the VOs (blue) are at the median position between the two, as shown in the principal components analysis (PCA) score plot (Figure 5). The results correlate with the compositions of the oils: VOs present some compositional defects, but not as strongly as the LOs, and maintain important positive attributes contained in the EVOs.

Interestingly, two of the four compounds in the reduced set of VOCs were not detected when a single extraction was performed (the late-eluting compounds cyclododecanol and octacosane), an observation that highlights another benefit of using MSE with SPME-trap.

Conclusions

This work was carried out to investigate the potential of SPME when combined with multi-step enrichment to provide an enhanced characterisation of the aroma profiles of a range of olive oil samples. The results demonstrate that MSE SPME can improve the overall sensitivity for the VOCs and SVOCs in these products and enhance the understanding of information from cross-sample studies. Using MSE SPME with shorter extraction times is preferable to an equivalent sampling time for a single extraction, both in terms of overall signal response and for limiting the competitive mechanisms that occur when adsorption fibers are used. This study suggests that a reduced number of VOCs could be used to distinguish expensive extra virgin olive oil from other types, so the technique could help confirm authenticity and prevent fraud.

Acknowledgements

Markes International gratefully acknowledges Giorgia Purcaro and colleagues (University of Liège, Belgium) for kindly supplying the data described here.

References

1. S. Mascrez and G. Purcaro, Exploring multiple-cumulative trapping solid-phase microextraction for olive oil aroma profiling, *Journal of Separation Science*, 2020, 43: 9–10, DOI: 10.1002/jssc.202000098.
2. S. Mascrez and G. Purcaro, Enhancement of volatile profiling using multiple-cumulative trapping solid-phase microextraction, Consideration on sample volume, *Analytica Chimica Acta*, 2020, 1122: 89–96.
3. T. Górecki, X. Yu and J. Pawliszyn, Theory of analyte extraction by selected porous polymer SPME fibres, *Analyst*, 1999, 124: 643–649.

Centri®, ChromCompare®, ChromSpace®, MSE® and HiSorb™ are trademarks of Markes International.

SLB®-5ms is a trademark of Supelco.

Applications were performed under the stated analytical conditions. Operation under different conditions, or with incompatible sample matrices, may impact the performance shown.