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ENHANCEMENT OF VOLATILE PROFILING USING MULTIPLE-CUMULATIVE TRAPPING SOLID-PHASE MICROEXTRACTION. CONSIDERATION ON SAMPLE VOLUME

Steven Mascrez ,

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

Giorgia Purcaro *

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

Abstract. In the present work, the performance of the multiple-cumulative trapping headspace solid-phase microextraction technique used in the headspace linearity range and saturated headspace was investigated and compared, with the ultimate goal of maximizing the fingerprinting information extractable using a cross-sample comparison algorithm for olive oil quality assessment. It was highlighted as the use of 0.1 g of olive oil provides comparable or even better profiling than 1.5 g at a little expense of sensitivity. However, the use of multiple-cumulative-solid-phase microextraction, along with the correct sample volume, improved not only the overall sensitivity but significantly burst the level of information for

cross-sample studies.

Keywords. Headspace solid-phase microextraction, Multiple-cumulative trapping headspace, solid-phase microextraction, Olive oil, Sample volume, Pattern recognition.

HIGHLIGHTS

- The use of Multiple-cumulative trapping was explored.
- Different sample volume and sampling time were tested.
- Cumulative extraction times of low sample amount improved the level of information.
- EVO, VO and LO were properly classified using Random Forest with optimized conditions.

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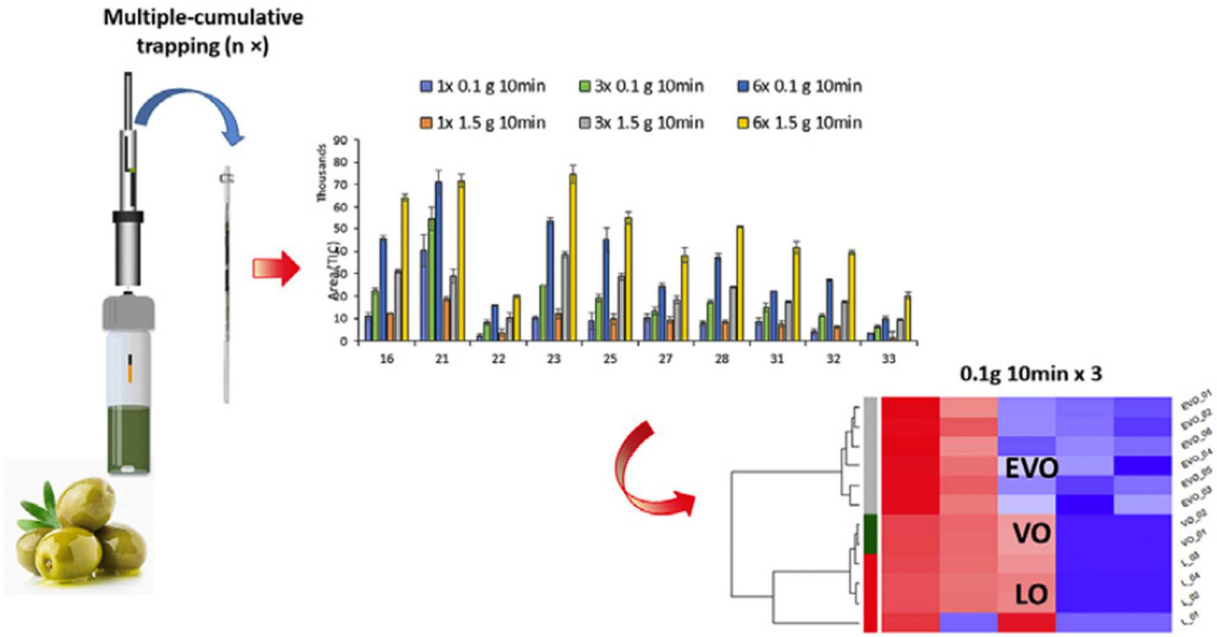
GRAPHICAL ABSTRACT



1. Introduction

Among the different techniques for volatiles profiling of foods, headspace (HS) solid-phase microextraction (SPME) is probably the most widely applied. The main advantages of HS-SPME are easy automation, solvent-free applications, and flexibility due to the different sorbents commercially available [1]. This easy-to-use technique has its theoretical basis on the combination of two equilibria between three-phases [2]. The first equilibrium occurs between the sample and the HS (measured by its distribution constant, K_{hs}), and the second one is between the HS and the fiber (K_{fh}). Both equilibrium or non-equilibrium extractions can be performed; in the former case, the extraction yield is theoretically maximized, but only when liquid-fiber coatings are used (alias when analytes are extracted via absorption mechanism). The situation is more complicated when sorbent coatings exploiting adsorption mechanisms are used. In the latter case, competition may occur, reducing the extraction yield [3,4]. Nevertheless, the amount extracted by SPME (n) is theoretically proportional to the initial concentration (C_0) in the sample both under equilibrium and non-equilibrium conditions, according to equation (1):

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$$n = \frac{K_{hs}\ K_{fh}\ V_f\ V_s\ C_0}{K_{hs}\ K_{fh}\ V_f + K_{hs}\ V_h + V_s} \qquad (1)$$

where C_0: initial concentration; K_{hs} and K_{fh}: distribution coefficient HS/sample and fiber/HS [5], respectively; V_s, V_h, V_f: volume of the samples, the HS, and the fiber coating, respectively. This proportionality can also be expressed as:

$$A = \frac{C_0}{K_{hs} + ß} \qquad (2)$$

Where A is the chromatographic area, and ß the phase ratio (ß ¼ V_h/V_s); when not considering the additional effect of the fiber selectivity and the partition coefficient between the HS and the fiber (K_{fh}) [6]. However, the direct proportionality between the chromatographic area and C_0 is verified only when working in the HS linearity condition, avoiding HS saturation. Verification of the HS linearity is highly mandatory when accurate quantification through calibration is the goal [6]. The calibration procedure then adjusts for other factors, such as matrix effect and response mediated by the fiber. However, verification of HS linearity should not be neglected either when the area intensity of different samples (without calibration) is used as an indicator of absolute concentration, as done when large studies of cross-sample comparisons are performed [7]. The HS linearity range depends on K_{hs} and the activity coefficients of each component. It is generally in the 0.1–1% range of the actual concentration in the sample. It is affected by the sampling temperature, time, and ß. Besides, there are the effects related to the specific sorbent extraction, such as the absorption or adsorption extraction mechanisms (which may lead, in the latter case, to displacement effects), sorbent amount, and sampling temperature and time, which determine the extent of compounds extracted by the fiber as well [6,7]. When multi-component analysis of complex volatile fractions is performed, linearity conditions are a compromise to accommodate both trace and major compounds.

At present, most of the applications involving HS-SPME use adsorption-type fiber, *i.e.*, divinylbenzene/carboxen/polydime thylsiloxane (DVB/CAR/PDMS) [8] with the goal to cover the broaden possible volatility and polarity range of volatiles and to maximize the area response. Moreover, to increase the sensitivity, the trend is the increase of the sample volume, without verifying the HS linearity condition. In this study, olive oil was used as an example matrix. The fingerprintingandprofilingofoliveoilvolatileshavebeenextensively studied for quality and authenticity assessment, with the ultimate goal tosupportthesensoryevaluationanddiscoveryoffrauds[9e–4]. The typical amount of sample used (*i.e.,* ~1.5 g) [10,15,16] leads to HS saturation, as recently showed [7,17].

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Sampling from a saturated HS may reduce the information derivable from the volatile fingerprint, regardless the chemometrics tool applied (*e.g.*, hierarchical cluster analysis, random forest, etc). This limitation can be circumvent using multiple-cumulative SPME (MC-SPME), exploiting the trapping technology. This technique improved the overall sensitivity, amplifying the difference among sample classes, turning the saturation of the HS into an advantage [17]. However, the potentiality of MC-SPME, when working in the HS linearity condition, has never been investigated.

In the present work, the performance of the MC-SPME technique used in the HS linearity range and saturated HS was investigated and compared, with the ultimate goal of maximizing the fingerprinting information extractable using a cross-sample comparison algorithm for olive oil quality assessment.

2. Experimental section

2.1. CHEMICALS AND REAGENTS

Hexane GC grade (MilliporeSigma®, USA) was used to dilute normal alkanes (C_7–C_{30}) mixture (Supelco, PA, USA) used for calculating the linear retention index (LRI) for confirming peak identity. The divinylbenzene/carboxen/polydimethylsiloxane (DVB/ CAR/PDMS) d_f 50/30 mm 1 cm length fiber was kindly provided by Millipore Sigma (USA).

2.2. OLIVE OIL SAMPLES

An extra-virgin olive oil sample was purchased at a local supermarket (Gembloux, Belgium).

Twelve samples of olive oil belonging to different quality categories, namely 6 extra-virgin (EVO), 2 virgin (VO), and 4 lampante (LO) olive oils were kindly provided by Carapelli Firenze SpA - Italy (Deoleo group). The samples were of verified geographical and botanical origin and the data of the sensory evaluation carried out according to the International Olive Oil Council protocol [18] were provided as well (Supplementary Table S1).

2.3. HS-SPME PROCEDURE

Different amounts of EVO (0.1, 0.25, 0.5, 1.0, and 1.5 g) were weighed in a 20 mL screw top vials, metallic caps with a central hole and polytetrafluoroethylene (PTFE)/silicone septa (Restek, USA).

Before the first use, the SPME fiber was properly conditioned, as suggested by the provider. Centri Autosampler (Markes International Ltd, UK) was used to automate the sample extraction. The sample was agitated at 300 rpm and heated at 43°C, after 5 min of equilibration, the fiber was exposed to the HS for 10 or 30 min.

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The fiber was then thermally desorbed at 250°C for 2 min. The trap (U-T12ME-2S, Markes International, general-purpose in the C4eC32 volatile range) was cooled at 0°C during desorption of the fiber in the injector, then 1 min purge at 50 mL/min was performed before heating the trap to 300°C (hold 10 min) at the maximum ramp temperature allowed by system. A 1:5 split was applied after the trap. MC-SPME was carried out with a 5 min enrichment delay (at 43°C) before the following extraction. Three and six cumulative extractions were performed from the same vial. Specific conditions were then selected for the real-world samples analyzed for cross-sample comparison. The complete sampling design is reported in Table 1.

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		
0.1	*10*	*1*	*3*	*6*
	30	*1*	3	6
0.25	10	1	3	6
	30	1	3	6
0.5	10	1	3	6
	30	1	3	6
1.0	10	1	3	6
	30	1	3	6
1.5	*10*	*1*	*3*	*6*
	30	1	3	6

All experiments for investigating the trend of the MC-SPME were run in triplicate. Real-word samples were analyzed in single. Before starting any samples batch, an SPME fiber blank was performed, as well as periodically to ensure the absence of carryover between runs.

2.4. GC-MS ANALYSIS

All analyses were performed on a Shimadzu GCMS-TQ8050 NX (Japan), consisting of a GC2030 coupled to a triple-quadrupole mass spectrometer detector (TQ-MS) (Shimadzu, Germany), equipped with a Centri autosampler (Markes International). The chromatographic column was a 30 m x 0.25 mm i. d. x 0.5 mm d_f SLB-5ms capillary column [(silphenylene polymer, practically equivalent in polarity to poly(5%diphenyl/95% methylsiloxane)] kindly obtained from MilliporeSigma (USA). GC oven temperature program: 30°C for 5.5 min to 310°C at 10 C/min. Carrier gas: helium in constant linear velocity mode at 35.9 cm/s, corresponding to an initial inlet overpressure of 45.6 kPa. The MS was operated in single-quadrupole mode, in EI mode at 70 eV. The ion source and transfer line temperatures were 200°C and 280°C, respectively. The scan mass range was set to 50–450 m/z, with an acquisition frequency of 10 Hz.

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Data were acquired using Shimadzu GCMSolution ver 4.45 (Shimadzu, Japan). NIST17s and FFNSC 3.0 MS commercial libraries were used for identification. Putative identification was based on the combination of the MS similarity with the NIST17 library and the FFNSC library (Shimadzu) (80%) with the confirmation using experimental linear retention index (LRI) within a ±15 range.

2.5. DATA ELABORATION AND STATISTICAL ANALYSIS

The data matrix resulting from the olive oil samples analysis was first normalized using Probabilistic Quotient Normalization (PQN) [19]. The data underwent a logarithmic transformation to stabilize the variance and making the distribution of the variables closer to normal [20].

The number of features still outweighed the number of samples; therefore, to overcome this limitation, a machine learning algorithm, namely the random forest (RF) was applied to build a threeclass model and select the most discriminatory core volatile analytes [21].

The separation performances of the different conditions were evaluated, measuring the inter-group Euclidean distance [20].

All statistical analyses were performed using R v3.6.1 (R Foundation for Statistical Computing, Vienna, Austria), Excel® (Microsoft Office, version 2016), and Morpheus® (https://software.broadinstitute.org/morpheus/).

3. Results and discussion

The HS-SPME extraction from the sample vial was performed multiple times, the fiber was then desorbed into the injector inlet and the compounds released were trapped into a cold trap located between the heated inlet and the head of the analytical column. When the multiple extractions (3 or 6) were completed, the trap was rapidly heated to release the compounds into the analytical column. The use of MC-SPME was explored using decreasing sample volume to define the sample amount that did not cause saturation of the HS. Moreover, the difference in the overall gain of information on the volatile profile in single extraction or MC-SPME was evaluated when working in the HS linear range or with HS saturation.

For the first comparison study, 49 compounds were selected, covering a wide range of polarity and volatility [10,11,13,17,22,23] (Supplementary Table S2).

All the data were acquired in triplicate; the average was considered for comparison purposes. Very good repeatability was obtained at each condition tested with an average relative standard deviation percentage (RSD %) of 8.5 [median 8.5; min: 3.8% and max: 30% (for the less intense compounds)].

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3.1. EFFECT OF DIFFERENT SAMPLE AMOUNT ON THE VOLATILE PROFILE USING MC-SPME

As mentioned in the introduction, to use the area intensity as an indicator of the absolute concentration in the sample, the HS linearity condition should be verified [6,7]. Therefore, the sample volume to be used needs to be appropriately optimized. 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, according to equation (3):

$$A_i = A_1\, e^{-q(i-1)} \qquad (3)$$

Where A is the chromatographic area; $_i$ indicates the number of extraction steps; *q* is a constant.

To determine *q*, equation (3) is transformed into a linear equation in the following form:

$$\ln A_i = -q(i-1) + \ln A_1 \qquad (4)$$

For an analogy, the application of MC-SPME generates a cumulative curve described by a logarithmic equation as (5):

$$A_i = A_1 \times \ln[q(i-1)] \qquad (5)$$

Which is transformed into a linear curve to determine *q* according to the exponential equation (6):

$$e^{A_i} = q(i-1) + e^{A_1} \qquad (6)$$

Thus, when the HS linearity condition is verified, the exponential model should give a better coefficient of determination (R^2 ~1), while when the HS is saturated, the same amount is extracted each time, thus obtaining a linear model. The R^2 obtained with the two different models (*i.e.* linear and exponential), extracting for 10 or 30 min different sample volumes (*i.e.*, 0.1, 0.25, 0.5, 1.0, and 1.5 g) are plot in heat-maps depicted in Fig. 1.

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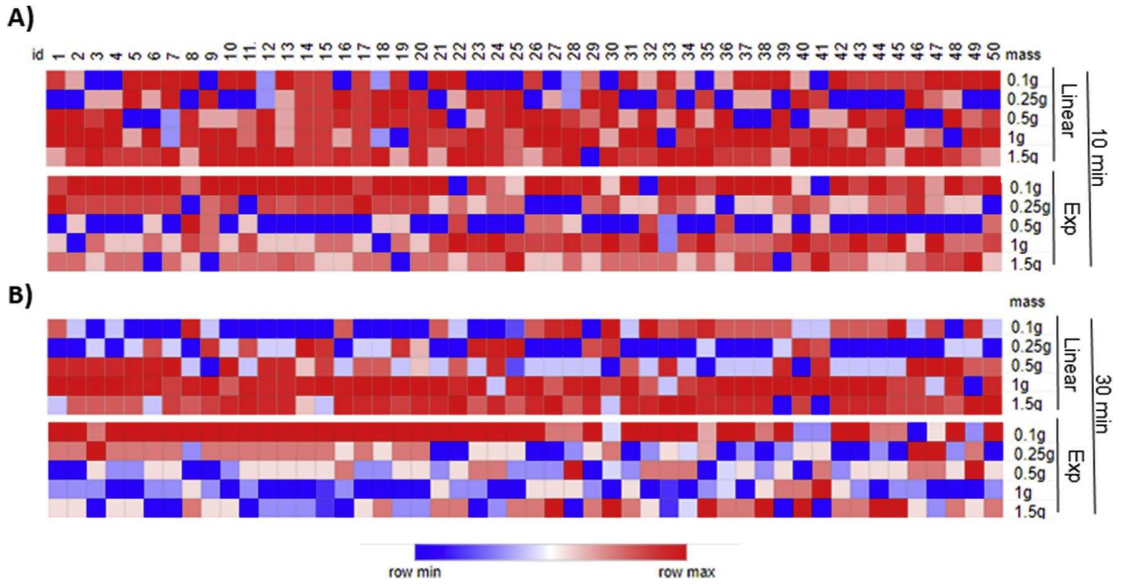

Fig. 1. Heat-maps representing the distribution of the coefficients of determination (R^2) obtained applying a linear or an exponential model on the MC-SPME extraction of different amounts of sample (0.1, 0.25, 0.5, 1.0, 1.5 g) extracting for A) 10 min or B) 30 min. Data were column scaled

Reddish color in the heat-maps represents an R^2 closer to 1, while more blueish colors mean values progressively farther from 1. Applying the linear model R^2 closer to 1 were obtained for the majority of the compounds when higher amounts of sample (*i.e.*, 1 and 1.5 g) were extracted (both at 10 and 30 min extraction time); while, the linear model was less suitable when lower amounts of sample were extracted (*i.e.*, 0.1 g). As aforementioned, the linear model fits better the cumulative curve when the HS was saturated with the compound of interest. On the other hand, when transforming the variables according to the exponential model, R^2 was maximized when 0.1 g of sample volume was extracted. This behavior denoted that a much lower amount of sample allowed to verify the HS linearity condition for most of the compounds considered, at both 10 or 30 min of extraction.

However, the sample volume impacts the sensitivity differently according to the partition coefficient of the analyte. The increase of the sample volume improved the extraction of compounds characterized by high volatility; contrarily, low volatile compounds are almost not affected [6]. Unfortunately, data on solute partitioning between olive oil and air are sparse. Predictive theoretical models have been proposed correlating partition coefficients in the air-olive oil to those in the air-octanol (K_{oa}) system [24e26]. However, olive oil is far to be a homogenous and reproducible product since its composition is highly cultivar, year of harvesting, and geographical origin dependent. Therefore, in this context, the impacts of the sample volume on the sensitivity was evaluated by correlating the area response with K_{oa} (Fig. 2). Precisely, the ratio between the chromatographic peak area obtained extracting 1.5 g and 0.1 g was plotted against K_{oa} (visualize against $\log K_{oa}$ for scaling reason). It has to be specified, as showed above, that

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when 1.5 g of sample is analyzed, the HS of most of the compounds resulted saturated; thus, the chromatographic area recorded is a function of the K_{fh} rather than K_{hs}, mainly when a single extraction is performed. However, it was observed that even in this extreme case, the improvement in sensitivity was limited showing a median over the 49 compounds of 1.3 (min: 0.4; max: 1.8) and 1.5 (min: 0.1; max: 2.3) extracting for 10 and 30 min, respectively. However, no trend in relation to K_{oa} was clearly observed when a single extraction was performed. Different was the situation when 6-MCSPME were performed. In the latter case, we can assume that, when starting from saturated HS, after a certain number of extractions a decrease in the HS concetration might occur, at least for some compounds, resembling more the ideal situation of HS linearity. Indeed, after 6-MC-SPME, a clear trend was observed, especially when 30 min extraction was performed (Fig. 2). Here the theory was confirmed with a not significant increment towards higher K_{oa}. A similar trend, although less evident, was observed when extracting multiple times for 10 min (median: 1.2; min: 0.7; max: 2.9).

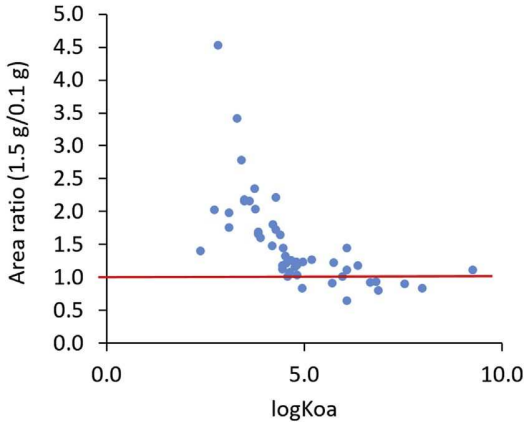

Fig. 2. Change in extraction efficiency as a function of the log K_{oa} when extracting for 30 min at 43°C. The red line highlight the threshold of 1 above which increase in the sensitivity is recorded for extraction of 1.5 g. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Finally, the extraction efficiency was compared at 10 and 30 min of extraction for both 0.1 g and 1.5 g. When porous fibers are used (as DVB/CAR/PDMS), shorter extraction time is suggested to reduce the competitive adsorption [3,27]. This means that, for most of the compounds, the possibility of sampling not at the equilibrium is increased (as also shown in the surface responses reported in our previous work [15]), which reflects in a lower sensitivity. To evaluate the potential loss of sensitivity, the ratio between the chromatographic area obtained extracting 30 and 10 min was calculated. Independently from the number of multiple extractions, as well as from the sample volume, the ratios of the targeted analytes showed medians in the 1.5–2.1 range, with a maximum 3.3–fold increase at 30 min. A slight decreasing trend of

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the increment can also be observed towards less volatile compounds. This means that while longer extraction times are beneficial for more volatile compounds, shorter times increase the uptake of the less volatile ones. This is even more evident when MC-SPME is performed. The same total extraction time was compared when performed as a single extraction or multiple shorter one. Therefore, a 30 min single extraction was compared with 3 x 10 min MC-SPME. A significant increment was observed, both for 0.1 g and 1.5 g of sample volume, ranging between almost no-increment (ratio ¼ 1) and ~4-fold. Fig. 3 depicted the overall trend in the sensitivity increment when 0.1 g of sample was extracted.

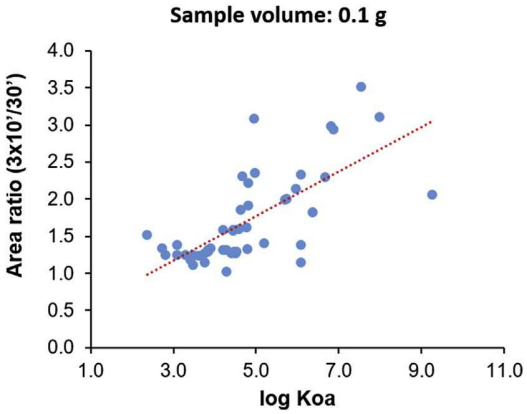

Fig. 3. Chromatographic peak area ratio between performing 3-times 10-min-MCSPME and a single 30-min extraction versus log K_{oa}. The dotted red line highlights the general trend. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

It can be concluded that sampling for a multiple-short sampling time provides a better overview of the volatile and semi-volatile profile of extra virgin olive oil, covering a more comprehensive range of compounds. The application of MC-SPME may be beneficial to enhance even more the overall information extractable from HS profiling, with the main advantage of not impacting the sample throughput.

Fig. 4 reports the comparison among the chromatographic traces obtained by performing single or MC-SPME at 10 or 30 min with 0.1 g sample volume. In the box, an expanded area to emphasize the gain in sensitivity obtained by performing 3-time 10 min-MC-SPME.

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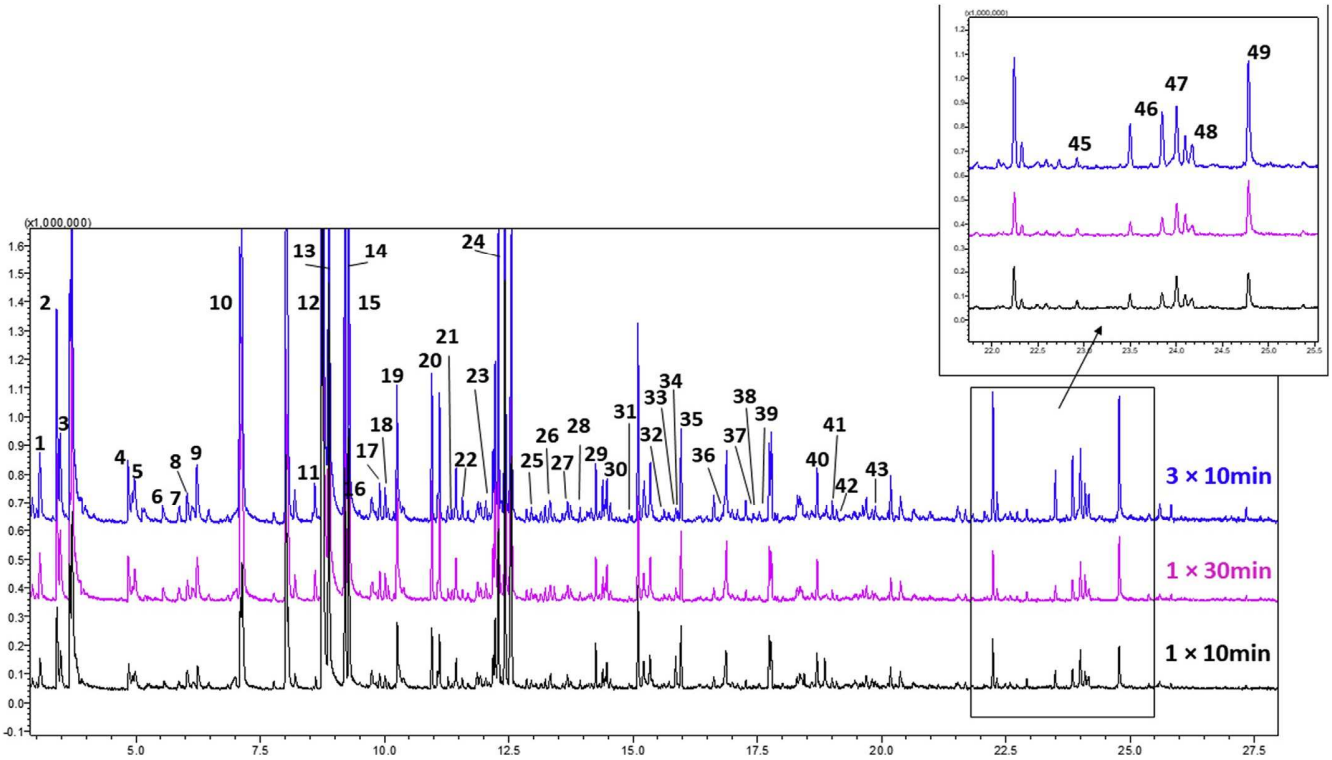

Fig. 4. Total ion chromatogram of an EVO sample (0.1 g in a 20 mL vial at 43 C) extracted 1-time for 10 min, 1 time for 30 min or 3-time for 10 min using MC-SPME. In the box expansion of the chromatographic area within the box.

3.2. CROSS-SAMPLE COMPARISON USING MC-SPME

To evaluate the enhancement in the overall information extractable using the MC-SPME, a cross-sample comparison was performed based on pattern recognition technique. As it has been discussed, the generally accepted approach to treat the GC-MS peak area information as a concentration indicator can be misleading [7], especially when working outside the HS linear range, alias in HS saturation condition. Under the latter conditions, differences in the total concentration among samples are hindered by the maximum capacity of the HS, leading to a less informative fingerprint. Such a situation is very commonly encontered in the literature, where the usual sample volume for EVO analysis is ~1.5 g or more [10,13,16,28,29]. However, due to the high complexity of the EVO volatile profile and the wide dynamic range of its components, the use of lower sample volume, although beneficial for the most abundant constituent, can be detrimental for the sensitivity of important trace components. In light of this, we hypothesized that the use of the MC-SPME approach improves the cross-sample comparison amplifying the differences and enhancing the sensitivity, in particular, when working in the HS linear range.

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Twelve samples of olive oils (6 EVO, 2 VO, and 4 LO) (Table S1) were selected as a proof-of-concept. The experimental design is highlighted in italic in Table 1. Two different sample amounts were tested, namely 1.5 and 0.1 g. Single extraction and 6-MC-SPME were performed, exposing the fiber to the HS for 10 min. When using 0.1 g of the sample, also 3-MC-SPME (10 min of extraction) and a single 30-min extraction were tested.

The data obtained from each condition was treated separately to maximize the final results. The manual pre-processing of each batch of samples included: chromatograms alignment and cleaning of the data matrix to remove siloxane. Further reduction was based on a frequency of observation cutoffs of 50% within at least a group (*i.e.*, EVO, VO, or LO). The data matrices pre-processed were then normalized and log-transformed. An additional feature reduction step was applied using the random forest (RF) algorithm. A permutation test evaluates the importance of each feature and an averaged value, called mean decrease accuracy, is returned [21,30]. Features are then ranked according to a decreasing mean decrease accuracy value and the most significative ones are chosen based on the cutoff depicted by the 'elbow' of the graph [31].

Heat maps with hierarchical clustering analysis were used to visualize the pattern and the pair-wise Euclidean distances between groups were calculated (Table 2). Fig. 5 shows the heat-maps obtained after feature selection for all the conditions tested.

Table 2
Pair-wise Euclidean distances with and without features selection at the different conditions tested as for Table 1.

Feature selection	Euclidean distance			Euclidean distance		
	EVO-VO	EVO-L	VO-L	EVO-VO	EVO-L	VO-L
1.5 g						
	1 × 10 min			**6 × 10 min**		
No	17.7	18.7	17.3	21.9	24	20.9
Yes	3.6	4.1	1.5	4.7	6.8	3.1
0.1 g						
	1 × 10 min			**6 × 10 min**		
No	13.5	17.2	16	20.4	20.4	19.9
Yes	3.6	5.1	4.4	2.2	3.1	2.6
	3 × 10 min			**1 × 30 min**		
No	19.3	21.4	19.3	18.1	20.9	20.1
Yes	6	7.8	4.5	0.5	4.2	4

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Although the number of samples is limited, some important outcomes can be discussed. The first relevant comparison is between the saturated and non-saturated HS conditions (alias 1.5 g and 0.1 g of sample volume, respectively) performing a single extraction. Fig. 5A and B shows as the three groups, namely EVO, VO, and LO, are better clustered using 0.1 g of sample volume, also reflected in a higher Euclidean distance reported in Table 2.

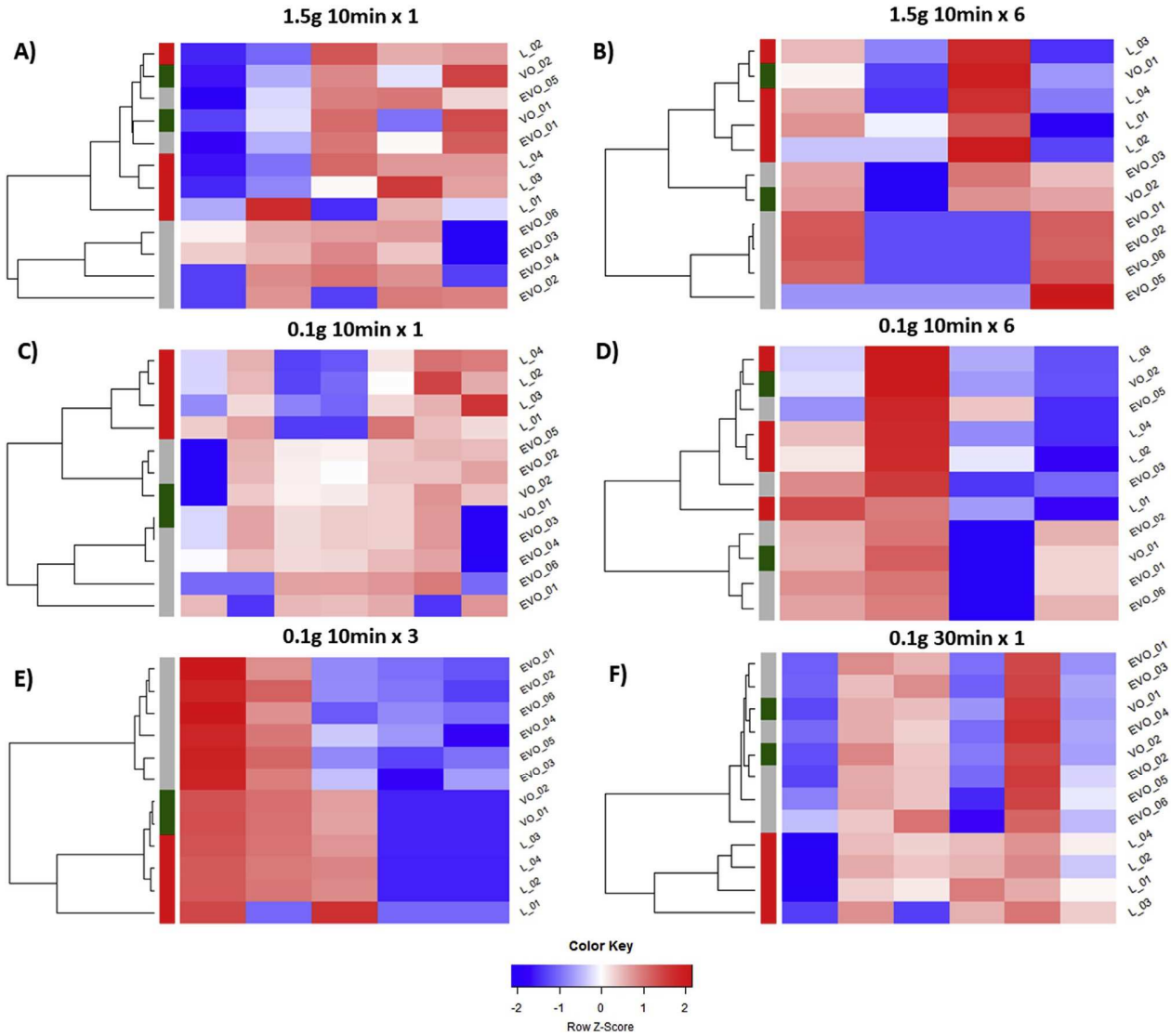

Fig. 5. Heat-maps and hierarchical clustering analysis of olive oil samples using the RF selected features for A) single extraction for 10 min with 1.5 g of sample; B) 6-cumulative 10 min-extractions with 1.5 g of sample; C) single extraction for 10 min with 0.1 g of sample; D) 6-cumulative 10 min-extractions with 0.1 g of sample; E) 3-cumulative 10 minextractions with 0.1 g of sample; F) single extraction for 30 min with 0.1 g of sample. Data were normalized by PQN and log transformed.

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The MC-SPME improved the clustering both using 1.5 g or 0.1 g of samples (Fig. 5B, D, and E), but for different reasons. In the case of 1.5 g, where the HS is saturated, the improvement is related to the amplification of the difference in the volatile profile obtained performing multiple extractions, as discussed in details in Ref. [17]; while in the case of 0.1 g, where the HS linearity is verified, it is most possibly related to an increase of sensitivity, especially for trace compounds. However, it is noteworthy that the trend of improvement when working in the HS linearity range is not proportional. In fact, the Euclidean distance is maximized performing 3-MC-SPME (Table 2, Fig. 5E), both without and with feature selection, although particularly true when data selection was applied. The decrease of the performance performing 6-MC-SPME (Fig. 5D) can be related to an adverse alteration in the actual profiling of the HS of the samples. The sensory perception is correlated to the relative distribution of the overall volatiles rather than to the absolute intensity of specific compounds. Therefore, it can be hypothesized that excessive multiple extractions alter the odorant profile, impacting the samples classification.

Finally, performing the HS-SPME for a longer time, *i.e.*, 30 min, the clustering capability was significantly reduced, especially for the most critical discrimination between EVO and VO (Fig. 5F and Table 2).

It is noteworthy to mention that VOs are the most critical samples to discriminate because, differently from EVO, they present some sensory defects, but not as strong as LOs and still maintaining important positive attributes. The use of 3-MC-SPME for 10 min was the only condition that allowed to clearly discriminate not only EVO and LO, but also VO without any misclassification, which is a highly significant outcome. It can be hypothesized that a major role in this positive result is played by less volatile components, which are enhanced when multiple-short sampling time are performed.

4. Conclusions

In this work, different aspects of the use of HS-SPME were highlighted. The use of short extraction time is highly beneficial when adsorption-type fibers, as DVB/CAR/PDMS, are used. Moreover, the selection of a proper sample volume, avoiding saturation of the HS, provides a more informative HS fingerprinting at a little expense of sensitivity.

In this context, the use of MC-SPME allowed to boost both aspects, *i.e.*, increasing the sensitivity of the less volatile compounds and maximizing the level of information. It has been shown as the use of multiple-short sampling time (3 x 10 min) improved the overall results of the cross-sample comparisons applying pattern recognition algorithms. The efficient discrimination between EVO, VO and LO is of high importance for quality and authenticity studies.

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On the other side, an excessive number of cumulative extractions led to a negative impact on the actual HS profile and thus samples classification performance, probably due to the response enhancment of less informative and confounding components.

Ethical approval

The authors have declared that no ethical issues exist.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Steven Mascrez: Investigation, Formal analysis, Writing - review & editing. **Giorgia Purcaro**: Conceptualization, Methodology, Formal analysis, Visualization, Writing - original draft, Writing review & editing, Resources, Supervision, Project administration, Funding acquisition.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aca.2020.05.007.

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