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Implementing Dynamic Headspace With SPME Sampling Of Virgin Olive Oil Volatiles: Optimization, Quality Analytical Study and Performance Testing

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ABSTRACT

Competition and interaction phenomena among volatiles during their adsorption process by solid phase micro-extraction (SPME) fibers in static headspace sampling procedure (SHS) cast doubt on its ability to quantify virgin olive oil volatiles. SPME fibers being excellent traps, their use was analyzed with a new device allowing the concentration of volatiles in a dynamic headspace sampling procedure (DHS). A central composite experimental design optimized the main variables of the device (4 g sample weight, 40°C temperature, 150 mL/min flow-rate, 50 min adsorption time) while values of the analytical quality control parameters of the method (repeatability, limits of detection and quantification, working range, sensitivity, resolution) were compared with those ones from static headspace. DHS shows better precision results for aldehydes and alcohols than SHS and allowed analyzing higher concentrations with no problem of saturation. In 19 out of 28 compounds analyzed in 50 samples the chromatographic areas were higher when running DHS. The concentration values of volatile compounds in these samples after applying SHS and DHS are discussed together with the ability of the new method for distinguishing virgin olive oil by their categories (extra, virgin and lampante) by the volatiles quantified in commercial oils.

Keywords: virgin olive oil, volatile compounds, solid phase microextraction, dynamic headspace, gas chromatography, internal quality control.

1. INTRODUCTION

The chemical explanation of aroma descriptors detected in virgin olive oil (VOO) by sensory assessors has been a research objective for decades.[1] However, the interest has increased after European Union stated that there is a need for the development and validation of a method for the assessment of the organoleptic characteristics of VOO in its recently funded Framework Programme Horizon 2020.[2]

Volatile compounds are the only chemical compounds responsible for aroma perceived by consumers when their concentrations in VOOs are higher than their odor thresholds. Volatiles have also an important contribution in the retronasal or throat-catching perception[1,3] due to its combination action with phenols.[4] All the volatiles have to be concentrated in traps prior to being determined by GC-FID/GC-MS. Although the initial proposals for the concentration step, mainly based on dynamic headspace (DHS) sampling, produced good results, they have been widely substituted by methods that use static headspace (SHS) sampling with SPME fibers as traps.[5-8] Low cost, simplicity and versatility are the main reasons for this change. However, those powerful reasons seem to have masked problems - even using internal standards - when VOO samples being analyzed have volatiles at high concentrations; for example, VOOs qualified with intense rancid or vinegary defects.[9] Thus, under these circumstances, competition and interaction phenomena among volatiles occur during their adsorption process by the fiber in a static headspace procedure, and these phenomena explain the poor selectivity and low recovery factors for some compounds.[9] In order to compensate this phenomena and reduce their effect on the quantitation, one solution is to use multiple internal standard normalization by selecting the best internal standard for each volatile compound.[10] The quantitation and the procedure of stable isotope

dilution[11-13] have been also developed to avoid these problems and increase accuracy. However, the use of volatile analysis as a routine method requires a major simplicity in quantitation and an actual improvement in the extraction step. Thus, in the search of other solutions, many studies have compared the performance of SPME sampling with other existing alternatives[14-18] or developing a modified procedure.[19]

A SWOT (Strengths, Weaknesses, Opportunities and Threats) analysis of the pre-concentration step based on DHS and SHS procedures[20] results in the opportunity of implementing SPME fiber in DHS by designing a new device adapted to SPME. This new proposal, however, should avoid the complexity and high cost of current DHS instrumental approaches as well as to offer satisfying selectivity, sensitivity, loading capacities and stabilities, and good recovery factors for a higher number of volatiles.

Thus, the aim of this work was to examine the development and optimization of a new sampling unit for volatile analysis that is simple, robust, reliable and solvent-free and permit the use of SPME fiber in a DHS mode. The technique was checked with samples at different concentrations and complexities of VOO aroma profiles, and chromatographic results are scrutinized in terms of the competition and interaction phenomena over quantitation. The work allowed understanding whether DHS-SPME sampling showed better results than SHS-SPME sampling by analyzing their results when determining volatiles in complex VOO samples in terms of their analytical quality parameters as chromatographic capacity, sensitivity and selectivity. The knowledge gained in this study has been checked with commercial VOO samples to determine the usefulness of the method determining differences between the categories of virgin olive oil (extra virgin, virgin and lampante). This new sampling procedure would allow establishing a new usability of SPME to improve its

67 performance in determining VOO flavor compounds, in particular in those cases where
68 sensitivity or recovery pose a problem.
69
70 **2. MATERIALS AND METHODS**
71 **2.1. Reagents**
72 Figure 1 shows two chromatograms in which the volatile compounds identified in this work
73 are indicated with codes. The following compounds (and their codes in the chromatograms)
74 were purchased from Sigma-Aldrich (St. Louis, MO, USA): Octane (1), ethyl acetate (2),
75 ethanol (3), ethyl propanoate (4), pentan-3-one (5), pentanal (6), 4-methylpentan-2-one (7),
76 1-penten-3-one (8), butan-2-ol (9), ethyl butanoate (10), 2-methylpropyl butanoate (11),
77 hexanal (12), butan-1-ol (13), 4-methylpentan-2-ol (14), heptan-2-one (15), heptanal (16), 3-
78 methylbutan-1-ol (17), (2E)-2-hexenal (18), 3-octanone (19), hexyl acetate (20), octanal (21),
79 1-octen-3-one (22), (3Z)-3-Hexenyl acetate (23), (2E)-2-Heptenal (24), heptan-2-ol (25), 6-
80 methyl-5-hepten-2-one (26), hexan-1-ol (27), (3E)-3-hexen-1-ol (28), *(3E)*-3-Hexen-1-ol (29),
81 nonanal (30), 1-octen-3-ol (31), acetic acid (32), propanoic acid (31), butanoic acid (34),
82 pentanoic acid (35), hexanoic acid (36), Z-3-hexenyl acetate (a) and (3Z)-3-hexen-1-ol (b).
83
84 **2.2. Samples**
85 A VOO *var*. Hojiblanca was used for the optimization of the variables affecting the
86 concentration step of volatiles. The determination of quality analytical parameters was,
87 however, carried out with a lampante VOO, qualified as rancid, as the high complexity of the
88 aroma of rancid VOOs (high concentration and numerous types of volatiles) is responsible
89 for incorrect data when the concentration of volatiles in SPME is carried out under static

headspace.[9] An odorless refined olive oil (Aceites del Sur, S.L.) was used for the determination of apparent recovery, linearity and limits of detection and quantification to which different concentrations of volatile standards were added (0.05, 0.10, 0.20, 0.30, 0.50, 1.00, 3.00, 6.00, 10.00, 15.00 mg/kg) from a stock solution of 20 mg/kg.

A total of 50 VOO samples from 7 producer countries (Argentina, Australia, Italy, Portugal, Spain, Turkey, Uruguay) were analyzed using the validated method to compare the concentration of the volatiles quantified by GC after a pre-concentration step using SPME fiber in static and dynamic headspace sampling modes.

Finally, a set of 32 samples - 9 EVOO and 23 VOOs, qualified by the standard procedure for sensory assessment[21] - was used to check the ability of the volatiles to distinguish samples by their categories in the complex classification tasks of distinguishing extra virgin olive oils (with absolute absence of sensory defects, EVOO) and virgin olive oil (with slight sensory defect, VOO), in accordance with sensory assessment. Volatiles were determined by GC after being concentrated in a SPME fiber in dynamic and static headspace sampling modes.

2.3. Concentration step

The design of a sampling unit that allowed the volatile concentration in a SPME fiber through a dynamic headspace (DHS) procedure was based on five conditions: (i) the trap had to be a commercial SMPE fiber coated with polymers with no modification; (ii) volatiles had to be swept from the vial headspace containing the sample by nitrogen inert gas; (iii) vial containing the sample have to be thermostatized by an automatic control system; (iv) sample inside the vial had to be shaken in order to facilitate the release of volatiles; and (v) the design of the new instrument had to guarantee that all the volatiles swept with the inert

gas would be in contact with the coated polymer of SPME fiber during enough time to facilitate their adsorption by the fiber. The last condition requires a special care in the design of the outlet of the inert gas passing through the SPME fiber. Figure 2 shows the simple design that allows converting a static in a dynamic headspace instrument.

Controlled variables of the design were: (i) the vial volume (20 mL); (ii) the temperature of the vial (40ºC); and (iii) the speed of the magnetic shake (100 rpm). The values of all these variables were already proved to be successful in several previous studies.[5,22]

The fiber is placed inside input A (Figure 2), a capillary tube with an internal diameter of 0.75 mm, while the inert gas (N_2), which sweeps the sample headspace, is introducing in the vial through input B (Figure 2) that has an internal diameter of 3.4 mm. Nitrogen flow is controlled by a needle wrench and its flow is measured by a pressure gauge. Nitrogen flow was a variable to be optimized since it greatly depends on the dimensions of system and the required speed by which volatile are in contact with the SPME polymer. The vial was inserted into an aluminium block thermostatized at 40ºC by means of two resistances and a temperature sensor PT100 controlled through a digital temperature controller (Electemp, J.P. Selecta S.A., Barcelona, Spain) and coupled with a magnetic stirrer (KMO2 Basic, IKA, Staufen,Germany).

The SPME fiber was purchased from Supelco (Bellefonte PA, USA). It was of 1 cm length and 50/30 µm film thickness and it was endowed with the stable flex stationary phase of divinylbenzene/carboxen/polydimethylsiloxane. The fiber was previously conditioned following the instructions of the supplier.

2.4. Determination of volatiles

136 The internal standard (4-methylpentan-2-ol) was successively diluted in each VOO sample up
137 to reach a concentration of 2.6 mg/kg, and then placed in a 20 mL glass vial. The volatiles
138 adsorbed by the fiber were thermally desorbed in the hot injection port of a GC for 5 min at
139 260°C with the purge valve off (splitless mode) and deposited onto a TR-WAX capillary
140 column (60 m × 0.25 mm i.d., 0.25 µm coating; Teknokroma, Barcelona, Spain) of a Varian
141 3900 gas chromatograph with a flame ionization detector (FID). The carrier gas was
142 hydrogen, at a flow rate of 1.5 mL/min. The oven temperature was held at 40°C for 10 min
143 and then programmed to rise 3°C/min to a final temperature of 200°C.[3] The signal was
144 recorded and processed with the WorkStation (v6.41) software. Each sample was analyzed in
145 duplicate.
146 The identification of the volatiles was carried out by GC-MS (7820A Agilent Technologies gas
147 chromatography coupled to a Series MSD 5975 Agilent Technologies mass spectrometry)
148 and verified with chemical standards of all the quantified volatiles (35), which were
149 purchased from Sigma Aldrich (St. Louis, MO, USA). The strategy followed to identify the
150 compounds was that described by Molyneux and Schieberle (2007).[23] Thus, the identification
151 was carried out by using two columns with different polarities, TR-WAX capillary column,
152 describe above, and BPX5 capillary column (30 m × 0.25 mm i.d. × 0.25 µm coating; SGE
153 International, Ringwood, Australia). Mass spectra, comparison with standards and linear
154 retention index were considered for a full identification. Although they are however well
155 known compounds in VOO, comparison with previous works of the group[1-2,5] and other
156 authors[10,24] in terms of identification, retention time, linear retention index and mass spectra
157 were also taken into account. The identification was verified by comparing odor qualities
158 determined by GC-olfactometry as reported in previous works[25]. Once all the compounds

were identified with this strategy, for a routine analysis, the TR-WAX capillary column described above was used. Quantification was done with internal standard (4-methylpentan-2-ol), although, the analytical quality parameters were studied on the chromatographic areas to evaluate the efficiency in the volatile extraction in the worst case without the correction effect of the internal standard.[26] The determination of volatiles by GC-FID after a concentration step in a SPME fiber in a static headspace process was the same of that just described above. The concentration step was carried out by placing 2 g of the sample in a 20 mL glass vial, tightly capped with polytetrafluoroethylene (PTFE) septum, and left for 10 min at 40°C to allow for the equilibration of the volatiles in the headspace. After the equilibration time, the septum covering each vial was pierced with a SPME needle and SPME fiber was exposed to the headspace for 40 min.[27] The SPME fiber was the same already described above. The fiber was also conditioned following the instructions of the supplier.

2.5. Mathematical procedures

2.5.1. Statistical procedures

Linear regression analysis and Student's t-test were used in the analyses of internal quality parameters. Correlation curves were evaluated by adjusted R-squared (R^2_{adj}) that is an indicator of the corrected goodness-of-fit. We have used R^2_{adj} instead of R^2 because the latter tends to optimistically estimate the fit of the linear regression while the former attempts to correct for this overestimation. Principal Component Analysis (PCA) was applied to evaluate the ability of volatiles distinguishing between extra-virgin and virgin olive oil samples.

182 *2.5.2. Experimental design*

183 Experimental design is widely used to show the statistical significance of an effect that a
184 particular factor (e.g. flow-rate or temperature) exerts on the dependent variable of interest
185 (e.g. number of chromatographic peaks). The general rule for planning the experiments to
186 be carried out is that the more orthogonal the columns are, the better the design is. That is,
187 the more independent information can be extracted from the design regarding the
188 respective effects of interest.

189 Experiences were planned from a 2-by-2 factorial design to which there were added star-
190 points to produce a rotatable design. Thus, Table 1 shows that runs 5 through 8 are the so-
191 called star points or axial points, and runs 9 and 10 are center points. The information
192 function for this design for the second-order model is rotatable, that is, it is constant on the
193 circles around the origin.

194 The evaluation of the results of the central composite was carried out by considering three
195 criteria:

196 - Total area of the chromatogram.
197 - Number of peaks in the chromatogram.
198 - Chromatographic area of two volatiles, (2*E)*-2-hexenal and hexanoic acid, which are
199 markers of high and low quality VOOs respectively.

200

201 *2.5.3. Internal quality control*

202 Internal quality control (IQC) is the set of the operational techniques used for continuous
203 assessment of the quality of the results of analytical methods, also so-called, analytical
204 quality control. Thus, it is essential that IQC has to be properly validated before any

205 analytical method is put into routine use. The analytical quality parameters - accuracy,
206 precision, working range, limits of detection and quantification, selectivity and sensitivity, for
207 example - should be implemented as a minimum program of validation.[28-29] The first three
208 are known as primary quality parameters[30] and they have major incidence in the quality
209 control. Accuracy is a parameter that refers the total error (systematic and random) while
210 the precision of the method[31-32] is given in terms of repeatability and results are expressed
211 as relative standard deviation (RSD%). The working range of the entire method is
212 determined between the limit of quantification (minimum value) and the highest
213 concentration tested with good linearity[31] (maximum value). Limit of quantification (LOQ) is
214 the lowest amount or concentration of the analyte that can be determined with an
215 acceptable level of precision and accuracy.[29] Limit of detection (LOD) is the minimum
216 amount or concentration of an analyte that can be reliably detected by a given analytical
217 method.[29] LOD and LOQ were calculated as three and ten times the value of the relationship
218 between the standard deviation of the regression and the slope of the calibration curve.[32]
219 The determination of selectivity was based on the calculation of the resolution[32] between
220 two consecutives peaks of the chromatogram (previous and next to the reference peak).
221 Sensitivity results from the ratio between the volatile concentration of volatiles and the
222 concentration of volatile added to the matrix.[32]

223

224 **3. RESULTS AND DISCUSSIONS**

225 The first objective was the optimization study of those analytical variables affecting the best
226 determination and quantification of VOO volatiles with concentration step in solid phase
227 micro-extraction (SPME) under a dynamic headspace (DHS) sampling mode. Experience

228 dictated that sample quantity, sample temperature, adsorption time and flow-rate of inert
229 gas were the four main variables to be controlled.[1,5,22] From the beginning, sample
230 temperature was decided to be 40ºC because it has shown to be the optimum value when
231 the objective is to release only those volatiles perceived by assessors when evaluating
232 VOOs,[1,22] so avoiding sensory perceptions resulting from a thermo-degradation process
233 whether temperature is high enough.[33] Thus, the optimization process was focused on the
234 optimization of three variables: (i) VOO sample quantity; (ii) flow-rate of nitrogen gas used
235 to sweep the sample headspace; (iii) adsorption time or time that SPME fiber is exposed to
236 volatiles released from VOO sample.
237 Optimization of the variables was first carried out by means of independent studies of each
238 variable, followed by an experimental design that allowed perfecting the optimal values of
239 variables already reached from the previous studies. The first study allowed reducing the
240 number of experiments so avoiding a large number of experiments when implementing the
241 experimental design.
242 The flow-rate of the inert gas (N_2) was the first variable to be independently evaluated in
243 duplicate. The experiments, with 5 g of sample and 15 min of exposing SPME fiber to
244 volatiles (adsorption time), were carried out with 10 different flow-rates of inert-gas (12, 25,
245 50, 100, 150, 200, 250, 300, 350 and 400 mL/min). Adsorption time (15 min) was selected
246 because it is short enough to allow multiple experiments and it allows enough amount of
247 volatiles trapped in DHS.[22,34] The information evaluated (dependent variable) for selecting
248 the optimal flow-rate was the total number of peaks and the total area of those peaks for
249 each experiment as in previous studies.[22,34]

250 Table 2 shows that the total area of all the peaks registered in the chromatogram is higher at
251 low flow-rate while there is lower number of peaks. Thus, the number of peaks (20-101) is
252 low enough at low flow-rates (0-25 mL/min) and they do not represent the complex VOO
253 aroma. It is consequence of the fact that the fiber (SPME) adsorbs volatiles with higher vapor
254 pressure when the flow-rate of the inert gas sweeping the sample headspace is low. On the
255 contrary, volatiles with lower vapor pressure need of higher flow-rate to be adsorbed by the
256 fiber polymers. Table 2 also shows the results for a selected set of volatiles. The compounds
257 corresponded to those already identified in previous works and described as sensory
258 relevant and some of them being considered as markers of the most common VOO sensory
259 defects.[1,5,10,24] Thus, chromatographic areas of volatiles with high volatility (e.g., ethanol and
260 ethyl acetate) are larger at low flow-rate, and volatiles with low vapor pressure show larger
261 chromatographic areas at high flow-rate (e.g. acids but with the exception of acetic acid).
262 Some compounds such as butan-2-ol and hexanoic acid showed more than a single
263 maximum, probably due to the effect of the flow rate on the precision of the measurements,
264 which are also shown in Table 2 as relative standard deviation (RSD%). Thus, the optimal
265 values of flow-rate for the central composite experimental design were 50, 100 and 150
266 mL/min on the basis of the percentage (42%) of volatiles that reached their maximum values
267 at one of these flow-rates, their lower values of RSD% in comparison with other
268 experiments, and besides the authors' experience analyzing the volatiles of hundreds of VOO
269 samples with SHS-SPME and DHS-Tenax as pre-concentration step.[1]
270 The next study was performed with 12 different adsorption times (5, 10, 15, 20, 25, 30, 35,
271 40, 80, 120, 180 and 240 min) and keeping a sample amount of 5 g and a flow-rate of 100
272 mL/min as it produced better results (Table 2). Figure 3 highlights that the time of sweeping

promotes the adsorption of volatiles by polymers because nitrogen sweeps them from the sample headspace so favoring the release of new volatile compounds. Thus, the largest number of adsorbed compounds (249) corresponded to 240 min. However, only 80 min were enough to reach a high percentage of the total of the chromatographic areas. Figure 3 also shows saturation in adsorption times over 180 min - in terms of the total of chromatographic areas - while the saturation is reached at 30 min in static headspace[5] although with less amount of volatiles (in number of extracted compounds and their areas). There is, however, a limiting condition, which is the length of the analysis. Thus, the total time for the analysis should be less than 60 min, otherwise the method would be little useful as it would be lengthy enough to be applied in control laboratories.[35]

The explanation of VOO sensory defects by the volatile compounds responsible for them[1,3] is, nowadays, one of the major application for the quantification of volatiles. In this context, 47% of the volatiles responsible for sensory defects were determined after 40 min of adsorption time and the number of chromatographic peaks was high enough (205). The individual analysis of the evolution of the chromatographic areas of each volatile with the adsorption time, however, showed that there was not an agreement about the optimum adsorption time among volatiles because of their different vapor pressures. Thus, Figure 4 shows the evolution of the chromatographic areas of 1-hexanol which maximum was around 40 min of adsorption time in contrast to the sum of all the chromatographic areas (Figure 3). This adsorption time also corresponded to the maximum area of many other volatiles, which was interpreted that the range between 40 and 80 minutes would include the optimum value. Ideally, a short adsorption time is required to avoid a difficult implementation of the method. On the other hand, some volatile compounds have a low odor threshold, so having

296 an important impact on the sensory characteristics of the oils even when they are present at
297 low concentration. These compounds may be undetected or detected with low
298 chromatographic areas with a static headspace system. For that reason, the adsorption time
299 can be considered in a dynamic system as a variable able to modulate the sensitivity of the
300 method and the recovery rates of the volatile compounds considering, firstly, that the
301 compounds have quite different characteristics in their affinity to the fiber and in their
302 sensory impact, and secondly, a short adsorption time has to be sought, as previously
303 mentioned.
304 The third study of variables was focused on the optimal amount of VOO sample. The
305 experiments were carried in duplicate with six amounts of samples (0.5, 1.0, 2.0, 4.0, 5.0,
306 and 6.0 g) and the values for flow-rate and adsorption time were 100 mL/min and 40 min,
307 which were also the values already used for a DHS with Tenax TA traps by authors.[22] The
308 larger number of chromatographic peaks was reached with the sample of 5.0 g, and the
309 lowest with 0.5 g. Figure 5 shows the evolution of the total area of the chromatographic
310 peaks in the six experiments. The last three experiments (4.0-6.0 g) did not show significant
311 differences according to t-test for two independent samples: $p=0.054$ between experiments
312 with 5.0 g and 6.0 g, and $p=0.063$ between experiments with 4.0 g and 5.0 g. Because less
313 amount of sample can allow extracting a larger percentage of the entire content of volatiles
314 in the sample, 4.0 g was selected as the most adequate.
315 A 2-factor factorial design based on central composite rotatable design was carried out for a
316 definitive optimization of the variables of flow rate and adsorption time, considering their
317 interdependence, and fixing the sample amount as 4.0 g and temperature as 40°C. The
318 central values (0,0) for the independent variables were 150 mL/min for nitrogen flow-rate

and 50 min for the adsorption time while the factor levels were ±1.00 and ±1.41 as already said. It was decided that a unit of the central composite (±1.0) would be 50 mL/min for the flow-rate and 20 min for the adsorption time in order to include all the best conditions already pointed out (Table 2 and Figures 3-5). Table 3 shows the ten experiences of the experimental design with the values for the two independent variables.

The dependent variables were the number of peaks, the total area of the chromatographic peaks and chromatographic area of two volatile compounds, (2*E)*-2-hexenal and hexanoic acid. These volatiles were selected because they are markers of sensory defects (hexanoic acid) and cherished attributes (*(2E)*-2-hexenal),[36] and besides they are widely separated in the chromatograms, the retention time for *(2E)*-2-hexenal is 22.34 min and 49.40 min for hexanoic acid. Hexanoic acid was used in the study despite its concentration is usually affected by competition phenomena with other volatiles in SHS concentration step.[9] This phenomenon is not uncommon when the quantification is done with a large number of volatiles that compete among them to be adsorbed by traps (carbon dioxide, Tenax, SPME, cold finger, etc.).

Table 3 shows that the chromatographic areas of E-2-hexenal were higher for medium and high adsorption times (50, 70 and 78 min) while the lowest areas corresponded to the high flow-rate values (150-200 mL/min), overall if it is combined with the lowest adsorption time (22 min). High flow-rate values (≥ 150 mL/min), on the contrary, showed great influence in the registered chromatographic areas of hexanoic acid that were higher excepting when, in general, the adsorption times were low enough (≤ 30 min). It is important to point out that hexanoic acid has lower volatility than *(2E)*-2-hexenal, and it needs of higher flow-rate values to favor its release and adsorption to the fiber. The maximum number of peaks (224)

342 corresponds to 200 mL/min of flow-rate and 70 min of adsorption time. However, the result
343 with 150 mL/min and 50 min is slightly lower (207 -218) but the adsorption time is much
344 better for an analytical method as it does not prolong the entire method too much.
345
346 **3.1. Internal Quality Control Study**
347 As result of the central composite experimental design and additional practical aspects
348 (analysis time), the optimal values of the variables for the DHS-SPME were: 4.0 g for VOO
349 sample, 40 °C for heating the sample during the concentration step of volatiles, a nitrogen
350 flow-rate of 150 mL/min and 50 min for the adsorption time. Figure 1 shows the
351 chromatograms of the volatile compounds of the same VOO sample when concentration of
352 volatiles was carried out with the dynamic headspace (DHS) and a static headspace (SHS)
353 sampling procedures, using the same SPME fiber. The chromatogram from SPME-DHS-GC
354 presents higher concentrations of the volatiles with medium and low vapor pressure than
355 from SPME-SHS-GC. It is important to notice that the sensory differences between EVOO and
356 VOO samples are consequence of the presence of volatiles responsible for sensory defects
357 that mostly are characterized by their medium and low vapor pressures. The fact of using a
358 DHS or a SHS concentration step does not modify the kind of volatile compound quantified
359 by GC though the volatile concentrations are higher after a DHS concentration step.
360 Unfortunately, volatiles with high vapor pressure are, in general, at high concentration in
361 SHS although they also have low odor activity value and hence they scarcely contribute - if
362 they do - to virgin olive oil sensory descriptors.
363 Once the optimal values of the analytical variables of the DHS-SPME procedure were
364 determined, with the help of a central composite experimental design, a validation was

365 carried out according to ISO 17025:2005.[37] The selected quality analytical parameters were:
366 precision, linearity, sensitivity, selectivity, accuracy, and limits of detection and
367 quantification. The most noticeable volatile compounds (36 volatiles shown in Figure 1) were
368 validated with the cited seven quality analytical parameters. Results, however, are shown
369 with five volatiles that comply with two basic requirements (i) to be determined at very
370 different retention times (R_t) of the chromatogram and linear retention index (LRI), and (ii)
371 to be markers or responsible for the perception of the most known sensory defects of virgin
372 olive oils.[36] Thus, ethyl butanoate (R_t: 12.16 min; LRI: 1137) is a marker of fusty sensory
373 defect, 1-octen-3-one (R_t: 26.23 min; LRI: 1360) is a marker of mustiness, nonanal (R_t: 30.87
374 min; LRI: 1492) is a marker of rancidity, 1-octen-3-ol (R_t: 33.31 min; LRI: 1502) is a marker of
375 mouldy defect, and acetic acid (R_t: 33.53 min; LRI:1553) is a marker of vinegary defect.[1,5,33,36]
376 A sixth volatile compound *(2E)*-2-Hexenal (Rt: 21.50 min; LRI: 1318) was selected as
377 representative of desirable VOO sensory attributes. In fact, this volatile is marker of green,
378 bitter almonds and green astringent sensory perceptions.[1]
379 Precision, a parameter that measures the similitude of the values obtained from an
380 adequate number of repeated measurements, was determined with a sample of lampante
381 virgin olive oil (LVOO). DHS-SPME-GC analysis was repeated seven times by the same analyst
382 under identical analytical conditions. The range of values of relative standard deviations
383 (%RSD) for dynamic headspace (DHS: 1.98-16.53%) – for all the quantified volatiles (34) - was
384 similar to those obtained with static headspace (SHS: 2.12-16.02%).[27] Table 4 shows the
385 values of the five selected peaks. Acetic acid showed the lowest RSD (%). In fact, the
386 repeatability of the quantified acids – acetic, propanoic, butanoic, pentanoic and hexanoic -
387 was better in dynamic than static headspace because of their lower volatility and the

388 difficulty of extracting these compounds compared with most of the volatile compounds.
389 With respect to other volatile compounds (Figure 1), DHS sampling procedure shows better
390 results, in terms of %RSD, in aldehydes (ranges of 2.38-9.81 for DHS and 4.82-13.47 for SHS)
391 and alcohols (ranges of 2.30-7.95 for DHS and 3.16-10.35 for SHS) with only two exceptions
392 heptanal (8.28 vs. 4.82) and 3-methylbutan-1-ol (7.01 vs. 3.16).[27]
393 Linearity was the second analytical quality parameter studied because it informs about the
394 ability of the overall analytical method to provide results that are directly proportional to the
395 concentration of each volatile. Seven dilutions were carried out for the linearity study, which
396 covered the whole range of concentrations (min-max) of volatiles for the three VOO
397 categories (extra-virgin, virgin and lampante).[27.38] Table 4 shows the adjusted R-squared
398 (R^2_{adj}) in the range of concentration (C_r), which varies from zero to the maximum
399 concentration where saturation was observed. Although linear regression calculates an
400 equation that minimizes the distance between the fitted line and all of the data points, a
401 high R^2_{adj} does not necessarily indicate that the model has a good fit. The most common
402 validation is the residual plot. Thus, if the points in a residual plot are randomly dispersed
403 around the horizontal axis, a linear regression model is appropriate for the data; otherwise,
404 a non-linear model is more appropriate. In general, volatile compounds showed a linear
405 behavior, and in the case of DHS sampling, this procedure allowed analysing higher
406 concentrations of volatiles with no problem of saturation (e.g. 50 mg/kg nonanal).
407 The next studied analytical quality parameters were the limit of detection (LOD) and the
408 limit of quantification (LOQ). The first informs about the minimum amount of an analyte that
409 can be detected with a reasonable certainty by means of an analytical method, and the
410 second informs about the minimum amount of analyte that can be quantified with an

411 adequate level of precision and accuracy.[29] Another quality parameter is the working range
412 (W_R) that is limited by the concentration for LOQ and the highest concentration checked
413 with good linearity.[27,31] Table 4 also shows the results of these quality parameters in which
414 concern the five selected volatiles determined after the pre-concentration step of DHS
415 sampling procedure. The behavior of the volatiles, however, was not so much different when
416 comparison was carried out taking into account their molecular mass and polarity, or the
417 volatile times of elution in the chromatogram. Values of LOD for SHS are of the same order,
418 slightly lower, compared with DHS sampling. DHS shows, however, higher values for upper
419 working range (W_R) because DHS sampling enable to release much more amount of volatiles
420 than SHS sampling on the basis of their thermodynamic backgrounds.[1] The upper limit of the
421 working ranges (Table 4) is higher for DHS than for SHS up to more than 15 times (i.e. acetic
422 acid)[27] although the lower limit for DHS sampling is higher as well, with the exception of
423 nonanal. It seems that we are facing a shift due to leakages of volatiles, which are not
424 trapped by the SPME fiber when working in this first prototype for DHS sampling procedure.
425 In the case of nonanal and acetic acid the width of the working range was remarkable
426 higher.
427 Sensitivity, expressed as the ratio between changes in the output of an instrument and its
428 corresponding changes in the input, was also studied.[31-32] This ratio was extracted from the
429 slope of the calibration curve for each volatile compound. The sensitivity values should cover
430 a wide range because of the diversity of structures and natures of the volatile compounds
431 responsible for VOO aroma. Table 4 shows that the sensitivity of the volatiles determined
432 using DHS sampling is much higher than using SHS procedure.[27] However, this higher
433 sensitivity does not change the fact that acid compounds have the lowest sensitivity values,

434 the intermediate values correspond to alcohols and the highest values are associated to
435 ethers (e.g. ethyl butanoate), whichever the kind of concentration step because the SPME
436 trap (DVB/CAR/PDMS) is the same for DHS and SHS sampling. In absolute terms, the higher
437 sensitivity to volatiles, as consequence of the DHS concentration step, is improving the use
438 of this technique for particular applications where sensitivity is relevant, such as explaining
439 sensory defects (e.g. rancidity) from volatiles with low recovery factors (e.g., nonanal).
440 VOO aroma is a complex mixture of more than 100 volatiles which chromatograms are not
441 exempted of potential interferences between peaks, which can be evaluated by the
442 selectivity or ratio of the retention factors of two successive peaks (also visualized as the
443 distance between the apices of the two peaks).[32] The highest concentrations of many
444 volatiles after a DHS concentration step complicate even more a good chromatographic
445 resolution. Table 4 also shows the resolution of the five selected volatiles with respect to
446 their previous and next peaks. All the values are higher than 1.5, which means good
447 resolution.[39]
448 An estimate of the accuracy is the percentage of the theoretical amount present in the
449 matrix (natural or added) *versus* the amount measured by the instrument.[31-32] This
450 percentage is related with the apparent recovery (C_{ap} in Table 4). The apparent recovery was
451 calculated by adding a reference concentration (C_{ref} in Table 4) that was located within the
452 working range to a fully refined olive oil, the equation is $C_{ap}=(C/C_{ref})x100$; where c is the
453 concentration determined with the method to be validated. It is a habitual procedure when the
454 concentration range is large and diverse between volatiles, which is the case of the volatiles
455 responsible for the aroma of VOO categories. Acids, for example, are at low concentrations
456 in extra virgin olive oils but can reach very high concentrations in lampante virgin olive

457 oils,[1,36] which are not fit-for-consumption and do have to be refined prior to being
458 consumed. Table 4 shows the recovery, expressed in percentage, obtained in the five
459 selected volatiles after the chromatographic determination of the added reference
460 concentration to the refined olive oil (experiment repeated for six times).
461
462 **3.2. Validation of DHS-SPME-GC with commercial samples.**
463 Once the variables with major influence on the design were optimized and the analytical
464 quality parameters were also determined, the next steps were focused on (i) comparing the
465 volatile areas (34 volatiles) of 50 commercial VOOs concentrated in SPME fibers with SHS
466 and DHS, and (ii) evaluating the ability of DHS-SPME distinguishing between VOO categories
467 (extra-virgin, virgin and lampante) of commercial oils.
468 The set of 50 VOO samples from 8 countries of the 5 continents (Argentina, Australia, Italy,
469 Portugal, Spain, Tunisia, Turkey, Uruguay) was qualified with 34 volatiles compounds. The 34
470 volatiles were selected according to their relevance explaining sensory attributes (mainly
471 defects) of VOO.[1] The ratios between the areas of the volatiles after the concentration steps
472 with SHS and DHS were determined to highlight the differences between the two sampling
473 modes. These ratios and their mean and standard deviation, are shown in Table 5. A major
474 number of volatiles (24) increases their chromatographic areas (ratio < 1.00) when
475 concentration is carried out under DHS sampling, while only 10 volatiles showed higher
476 chromatographic areas (ratio >1.00) working with SHS sampling.
477 In an idealized scenario of DHS sampling, both solution and headspace are depleted of
478 volatiles because they are transferred to the sorption trap (SPME). The presented
479 instrument is a prototype and it was expected that volatiles with high volatility would

present more difficult to be adhered to the SPME fiber because leakages with the carrier gas. Thus, ethyl acetate and ethanol showed the greatest differences between SHS and DHS, in favor of the first option. Table 5 shows the values of correlations (R in the table) between the chromatographic areas obtained by SHS and DHS sampling procedures for those volatiles with significant differences in the areas ($p<0.05$) between these two procedures. Eleven volatiles show correlation values lower than 0.60 so pointing out different behaviors of the volatiles when the concentration step was implemented by SHS and DHS. Other 14 volatiles, however, showed high correlation ($R>0.80$), which means the behavior of the volatiles with the two concentration methods was similar enough. The comparison of results between different compounds do not reveal a clear effect of the chemical series. Thus, the aldehydes *(2E)*-2-hexenal and *(2E)*-2-heptenal show similar ratios (0.44 and 0.36 respectively) but the correlations (R) are dissimilar (0.94 and 0.30 respectively) because of dispersion of data is higher in *(2E)*-2-heptenal than *(2E)*-2-hexenal displayed by their standard deviations (SD 0.05 and 0.10 respectively). The pentanoic and butanoic acids have a dissimilar behavior in terms of ratio for the SHS and DHS procedures (0.94 *vs*. 0.49), but the dispersion of data is much higher in pentanoic acid (SD: 0.25) than butanoic acid (SD: 0.04) and hence the correlation value when comparing the results from both extraction procedures was much lower for pentanoic acid (0.30) than that for butanoic acid (0.81). The reason of the dispersion might be related with the affinity of SPME coated polymers to different compounds, which is diverse even among compounds of the same chemical series. The uncontrollable leak of these volatiles with the carrier-gas can also have an influence in these differences.

Acetic acid provides lower chromatographic areas with DHS than SHS though there is a good correlation (R) between them. It is supposed that there is not an erratic behavior but a less

503 affinity of acetic acid to SPME fiber, which has been underlined in previous works.[5-9] On the
504 opposite side, it is 1-octen-3-one, which was poorly quantified or simply not detected when
505 worked with SHS but was fairly quantified with DHS.
506 The last step was the analysis of the volatiles of 32 samples qualified as extra-virgin (9) and
507 virgin (23) olive oil. Twenty-two volatiles compounds – numbered 1-3, 5, 8-10, 12, 15-19 24-
508 28, 30-33 in Table 5 – were quantified with the internal standard in all the 32 samples. The
509 statistical procedure of principal component analysis (PCA) was applied to the volatiles
510 quantified in those samples as this is an unsupervised statistical procedure oriented toward
511 modelling the variance/covariance data matrix and allows checking the similarity of the
512 samples in terms of their volatile composition. Figure 6 shows the place of EVOO and VOO
513 samples qualified by volatiles concentrated in a SPME with a DHS in the PCA plot. The EVOO
514 samples are in Q2 or in its vicinity with the exception of sample E8. Three VOO samples (V2,
515 V9, V20) are classified together with EVOOs. The position of EVOO samples is explained by
516 higher concentration of ethyl butanoate (PCA1) and lower concentration of nonanal, octane
517 and acetic acid (PCA2) than VOO samples. Samples V5 and E8 were multivariate outliers for
518 their categories though they were not removed when applied PCA. The separation of
519 samples is slightly better when volatiles were obtained by DHS sampling (Figure 6).
520 Considering the results from quality analytical parameters, a device using SPME-DHS shows
521 similar if not better results than the current SPME-SHS. The differences between static and
522 dynamic enrichment methods in some compounds are not so relevant in terms of the values
523 of limit of detection and RSD in repeatability.[40] Thus, the RSD values in repeatability of two
524 compared methods were sufficiently good for all the analytes but somehow better for
525 SPME-DHS. The latter method shows better extraction yield of analytes with medium to low

vapor pressure that is not exhibited with high vapor pressure compounds, probably due to some leakage that could be improved in a second prototype by optimizing the capillary tube section where the SPME fiber is inserted. Thus, SPME-DHS would show better performance in terms of extraction yield in compounds with low recovery factors, such as nonanal. Additionally, SPME-DHS allows to avoid the problems of competence between volatiles in the static enrichment method; particularly when the sample is a lampante virgin olive oil.[9] That explains the higher maximum concentration in the linear working range. Another advantage is that the method based SPME-DHS can be also completely automated, like SPME-SHS. In fact, there are some automated instruments based on different traps (e.g. Tenax), but they are not based on SPME. Particularly, SPME-DHS can be used for routine headspace analysis for quantifying volatiles responsible for virgin olive oil with sensory defects that have low volatility but a remarkable sensory relevance even at low concentrations.[36] Future work in this area could aim at an inter-laboratory comparison for similar methods based on SPME-DHS.

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666

FIGURE CAPTIONS:

Figure 1. Chromatograms of the volatile compounds of a virgin olive oil sample analyzed by SPME-GC-FID with dynamic headspace (DHS) and static headspace (SHS) sampling procedures for concentrating its volatiles in a SPME fiber. Code numbers are displayed in 2.1 Reagents.

Figure 2. Desing of the system for using SPME fiber in a Dynamic HeadSpace (DHS). Note: A, SPME fiber capilar and inert gas outlet; B, intert gas inlet.

Figure 3. Total area of all the chromatographic peaks of a VOO sample (5 g) that was swept with a nitrogen flow-rate of 100 mL/min for 12 different adsorption times (5-240 min) and volatiles were trapped in a SPME fiber (DVB/CAR/PDMS). Adjusted curve was done with distance weighted least squares. All the samples were analyzed in duplicate and the error bars are shown.

Figure 4. Chromatographic areas of 1-hexanol from a VOO sample (5 g) that was swept with a Nitrogen flow-rate of 100 mL/min in 12 different adsorption times (5-240 min) for a triple (DVB/CAR/PDMS) SPME fiber. Adjusted curve was done with distance weighted least squares. All the samples were analyzed in duplicate and the error bars are shown.

689 Figure 5. Total area of chromatographic peaks of the experimental design with six amounts
690 of VOO samples (0.5-6.0 g) that was swept with Nitrogen at 100 mL/min flow-rate during an
691 adsorption time of 40 min. Volatiles were adsorbed in a triple (DVB/CAR/PDMS) SPME fiber.
692 Adjusted curve was done with distance weighted least squares. All the samples were
693 analyzed in duplicate and the error bars are shown.
694
695 Figure 6. Principal component analysis of 32 commercial samples of extra-virgin olive oils
696 (coded E) and virgin olive oils (coded V). The concentration of the volatiles was carried out in
697 a SPME fiber placed inside the instrument of Figure 1. Inserted figure corresponds to
698 another PCA with the same samples but with information from SPME-SHS procedure;
699 triangles (green) correspond to VOO and squares (blues) to EVOO.

Table 1. Central composite experimental design.

Run	Factor 1	Factor 2
1	1	-1
2	1	1
3	-1	1
4	-1	-1
5	0	1.41
6	0	-1.41
7	-1.41	0
8	1.41	0
9	0	0
10	0	0

Table 2. Number of chromatographic peaks, total and individual chromatographic areas of the volatile compounds in the experiments with different flow-rate (mL/min) of inert gas (N_2) sweeping the headspace of a sample of virgin olive oil. Values are the mean of experiments carried out in duplicate. Note: RSD%, percentage of Relative Standard Deviation. The maximum values are highlighted in bold.

Code	Flow rate (mL/min)	0	12	25	50	100	150	200	250	300	350	400
	Peaks	20	85	101	112	**153**	146	123	126	135	148	151
	Total area of volatiles	468850	4815932	4514476	3003129	**3125368**	2607632	3086233	1814679	2065095	1929794	1921622
1	Octane	0	134339	**151240**	116977	104486	70867	70167	47010	45966	46257	38554
2	Ethyl acetate	4236	**86471**	75531	37190	38784	30286	28801	20060	16312	15093	16926
3	Ethanol	295439	1488432	1148811	**426298**	342572	163977	215563	120738	116226	108554	75434
5	Pentan-3-one	0	**46865**	43827	27808	23099	13639	14947	9286	8812	8842	8271
6	Pentanal	0	**63729**	49436	35996	28527	19097	17584	11470	11397	12985	10053
8	1-Penten-3-one	0	2642	4067	2602	2047	3250	**7499**	2302	3691	2552	0
9	Butan-2-ol	0	3313	6218	5130	**6252**	2454	3308	1860	1785	1612	868
12	Hexanal	0	43566	**54159**	53918	53134	44189	37304	30574	28184	31623	11942
15	Heptan-2-one	0	2809	4571	4072	**4930**	4146	3531	2844	3822	3074	1861
16	Heptanal	0	881	1185	3190	3964	**5156**	2843	3305	1704	2693	3644
17	3-Methylbutan-1-ol	0	**71368**	69956	52189	50170	32879	28490	24133	23273	23934	30264
18	*(2E)*-2-Hexenal	0	1685	2977	2669	**3421**	1797	1939	1521	2636	1559	5160
19	3-Octanone	0	37990	**41092**	37199	33647	26538	24905	19679	19753	19558	10942
20	Hexyl acetate	0	40649	69359	83923	108093	**131342**	120479	94223	95350	108256	119774
21	Octanal	0	1216	1812	1555	**3003**	2469	2881	1801	2996	2372	2888
23	*(2E)*-2-Heptenal	0	8329	17837	18981	24661	24536	25550	17867	**39368**	22552	24794
24	Heptan-2-ol	0	883	3429	3469	**3976**	1157	1272	865	1461	901	1010
25	6-Methyl-5-hepten-2-one	0	2485	4769	6564	6304	**7428**	6596	4465	6611	5361	5284
26	1-Hexanol	0	348460	476074	502299	528698	**534268**	510018	399822	398140	425904	297447
27	*(3E)*-3-Hexen-1-ol	0	13028	36978	30947	**37696**	20113	19801	13209	17651	14135	26683
28	Nonanal	0	701	1380	1777	1206	2391	2116	1891	2135	2049	**3076**
30	Acetic acid	6930	**413266**	406186	261911	239398	241884	252281	109183	111491	107026	88931
31	Propanoic acid	0	3580	**4102**	3439	3963	3224	3685	2342	1527	2021	1963
32	Butanoic acid	1433	4034	5562	5044	7213	5634	**7347**	4655	5575	5467	6678
33	Pentanoic acid	0	1694	2564	2525	3972	3382	**5112**	2815	4200	2975	2559
34	Hexanoic acid	0	1768	2889	2221	5720	5145	7972	4423	6032	4866	**8335**
--	Area of these volatiles	308038	**2824183**	2686011	1729893	1668936	1401248	1421991	952343	976098	985221	803341
	RSD%	15.6%	9.9%	11.3%	9.9%	7.1%	5.7%	12.7%	18.4%	19.8%	24.0%	28.3%

Table 3. Experiences of the central composite experimental design for two factors (flow-rate and adsorption time). Standard run indicates the random ordering of the experimental analyses. Factor A corresponds to nitrogen flow-rate and Factor B to adsorption Time. Dependent variables are the areas of *E*-2-hexenal and hexanoic acid, total chromatographic areas and number of peaks. Note: [1], mL/min; [2], minutes; [3], values are rounded; [4], chromatographic area.

Standard Run	Order	Factor A	Flow-rate[1,3]	Factor B	Adsorption time[2,3]	*(2E)*-2-Hexenal[3]	Hexanoic acid[3]	Total area[3]	Peak numbers
2	1	1	200	-1	30	31280	2905	2999813	158
1	2	0	150	1,41	78	64913	9470	5377926	203
9	3	0	150	0	50	52621	7302	5429856	218
4	4	1	200	1	70	49327	11145	5670002	224
8	5	0	150	-1,41	22	22495	1305	2308953	118
3	6	-1.41	81	0	50	68132	1302	6275378	124
5	7	1.41	221	0	50	36929	5693	4045949	179
10	8	-1	100	1	70	63799	5554	6816197	175
6	9	-1	100	-1	30	52179	1641	4476133	137
7	10	0	150	0	50	59538	5294	5185238	207

Tabla 4. Values of the analytical quality parameters for volatiles analyzed with the optimized dynamic headspace (DHS) concentration step. Quality parameters for volatiles analyzed with static headspace (SHS) have been taken from Aparicio-Ruiz et al.[27] Information from calibration straight-line equation are: adjusted R-squared coefficient (R^2_{adj}), concentration range of volatiles (C_r), limits of detection and quantification and working range (W_R), the last four in mg/kg. Repeatability is given in percentage. Reference concentration (C_{ref}) and apparent recovery (C_{ap}) are expressed in mg/kg and percentage respectively. R_{pp} and R_{pn} are the chromatographic resolution respect to the previous and next peak respectively.

		Ethyl butanoate		1-Octen-3-one		Nonanal		1-Octen-3-ol		Acetic acid		(*2E*)-2-Hexenal	
		DHS	SHS	DHS	SHS	DHS	SHS	DHS	SHS	DHS	SHS	DHS	SHS
Repeatability		9.72	26.74	8.93	16.67	9.57	8.98	7.95	6.57	1.98	2.21	2.38	5.30
R^2_{adj}		0.995	0.992	0.998	0.997	0.999	0.991	0.977	0.998	0.998	0.997	0.930	0.996
C_r		0-5	0-1	0-5	0-3	0-50	0-15	0-10	0-10	0-100	0-6	0-25	0-6
Limit of Detection		0.46	0.09	0.74	0.30	1.57	2.41	1.02	0.74	1.43	0.56	0.11	0.54
Limit of Quantification		1.54	0.30	2.47	0.98	5.23	8.05	3.40	2.46	4.28	1.77	0.36	1.81
Working range (W_R)		1.54-5.0	0.30-1.0	2.47-5.0	0.98-3.0	5.23-50.0	8.05-15.0	3.40-10.0	2.46-10.0	4.28-90.0	1.77-6.0	0.23-17	1.81-6.0
Sensitivity ($\times 10^4$)		27.80	1.24	5.00	0.52	9.90	0.26	10.60	0.48	2.00	0.08	5.07	0.48
Resolution	R_{pp}	2.56	1.88	2.01	1.57	4.23	4.25	2.04	22.01	11.07	8.57	1.72	2.00
	R_{pn}	10.09	4.17	3.01	1.12	2.04	0.61	4.51	13.96	4.13	3.94	6.81	20.29
Apparent Recovery	C_{ref}	3.0	-	3.0	-	10.0	-	5.0	-	50.0	-	5.0	-
	C_{ap}	101.1		98.1		95.2		98.5		104.7		94.13	

Table 5. Ratio (mean±SD) between the chromatographic area of volatiles concentrated with static (SHS) and dynamic (DHS) headspace sampling procedures. Correlations (R) between the areas of the volatiles showed significant difference in the values ($p<0.05$) obtained with SHS and DHS procedures. Fifty is the number of samples used in the study. The number of codes corresponds to each peak describes in Figure 1. Note: LRI, empirical linear retention index. The Code number 14 corresponds to the internal standard (IS).

Code	Volatile	Ratio	R	LRI	Code	Volatile	Ratio	R	LRI
1	Octane	0.91±0.05	0.87	800	19	3-Octanone	1.46±0.10	0.47	1328
2	Ethyl acetate	8.01±1.41	0.82	886	20	Hexyl acetate	0.18±0.02	0.97	1377
3	Ethanol	22.87±4.69	0.75	999	22	1-Octen-3-one	0.03±0.02	0.22	1360
4	Ethyl propanoate	1.11±0.25	0.35	1034	23	*(3Z)*-3-Hexenyl acetate	0.11±0.01	0.97	1421
5	Pentan-3-one	1.35±0.11	0.82	1062	24	*(2E)*-2-Heptenal	0.36±0.10	0.30	1429
6	Pentanal	0.95±0.10	0.75	1064	25	Heptan-2-ol	2.06±0.38	0.15	1437
7	4-Methylpentan-2-one	0.46±0.04	0.64	1128	26	6-Methyl-5-hepten-2-one	0.30±0.05	0.88	1444
8	1-Penten-3-one	0.54±0.06	0.33	1118	27	Hexan-1-ol	0.72±0.03	0.92	1463
9	Butan-2-ol	0.62±0.07	0.42	1133	28	*(3E)*-3-Hexen-1-ol	0.48±0.04	0.77	1472
10	Ethyl butanoate	0.26±0.04	0.51	1137	29	*(3Z)*-3-hexen-1-ol	0.31±0.05	0.92	1477
11	2-Methylpropyl butanoate	0.90±0.09	0.65	1163	30	Nonanal	0.25±0.05	0.72	1492
12	Hexanal	0.92±0.04	0.87	1181	31	1-Octen-3-ol	0.29±0.06	0.98	1502
13	Butan-1-ol	0.56±0.10	0.34	1254	32	Acetic acid	1.81±0.10	0.98	1553
15	Heptan-2-one	0.17±0.01	0.89	1282	33	Propanoic acid	1.21±0.26	0.70	1643
16	Heptanal	0.15±0.06	0.44	1286	34	Butanoic acid	0.49±0.04	0.81	1731
17	3-Methylbutan-1-ol	2.69±0.24	0.62	1316	35	Pentanoic acid	0.94±0.25	0.30	1842
18	*(2E)*-2-Hexenal	0.44±0.05	0.94	1318	36	Hexanoic acid	1.92±0.39	0.69	1942

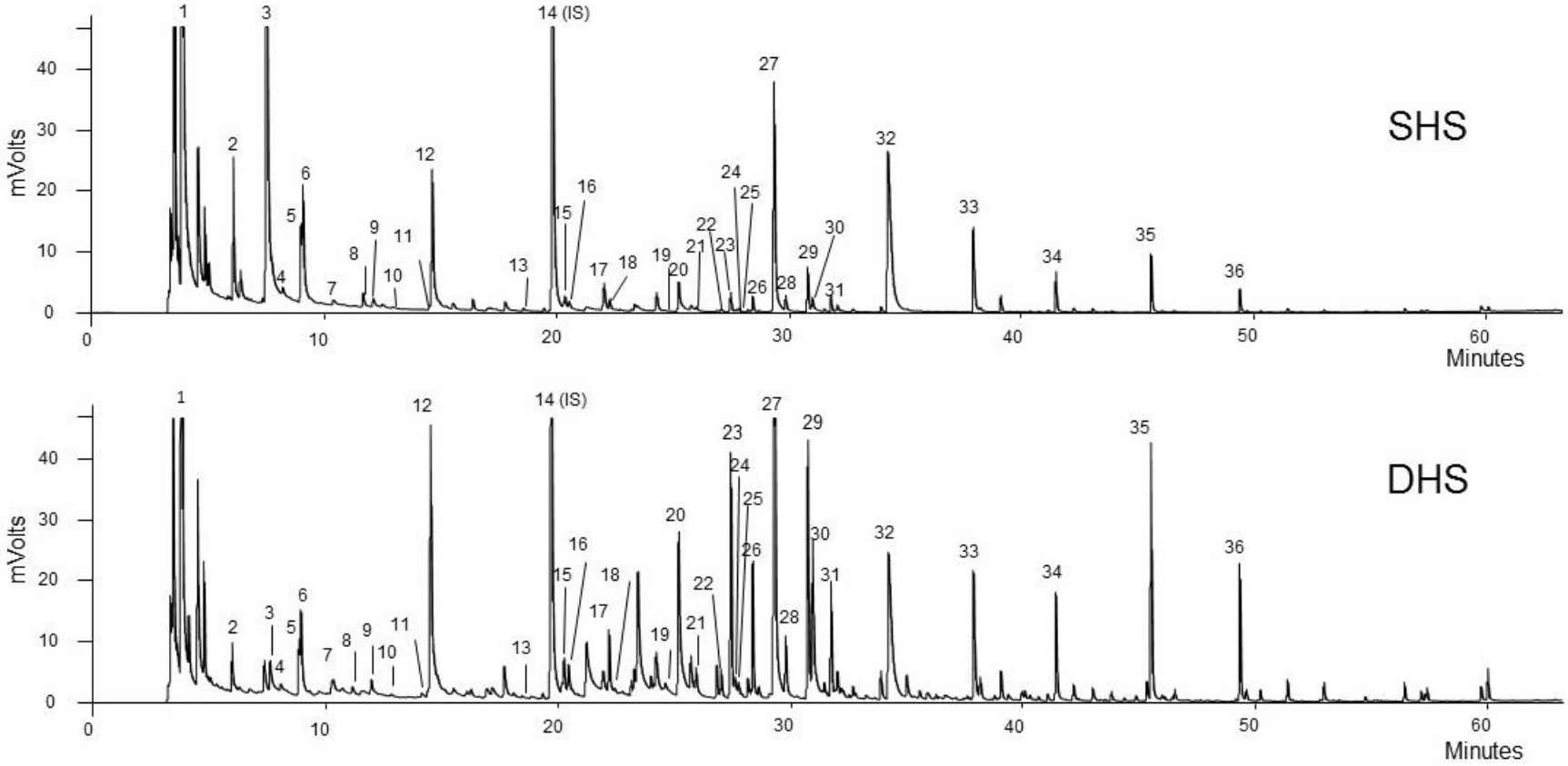
SHS
mVolts
Minutes
1
2
3
4
5
6
7
8
9
10
11
12
13
14 (IS)
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
0
10
20
30
40
50
60
DHS
mVolts
Minutes

FIGURE 1

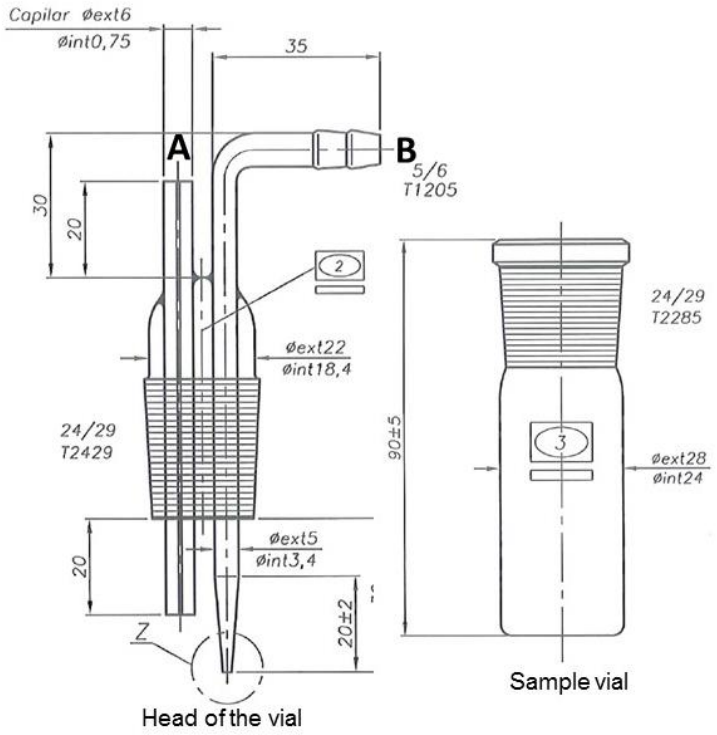
Capilar øext6
øint0,75
35
A
B
5/6
T1205
30
20
2
øext22
øint18,4
24/29
T2429
20
øext5
øint3,4
20±2
Z
Head of the vial
90±5
24/29
T2285
3
øext28
øint24
Sample vial

FIGURE 2

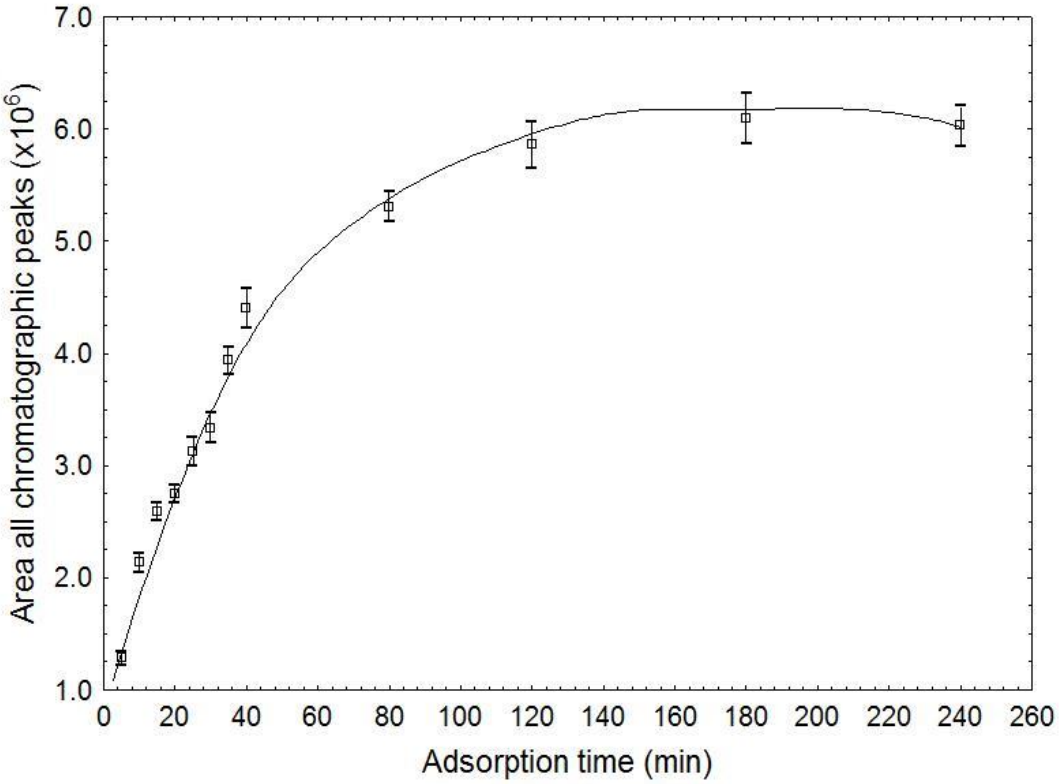
Area all chromatographic peaks (x10^6)
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
20
40
60
80
100
120
140
160
180
200
220
240
260
Adsorption time (min)

FIGURE 3

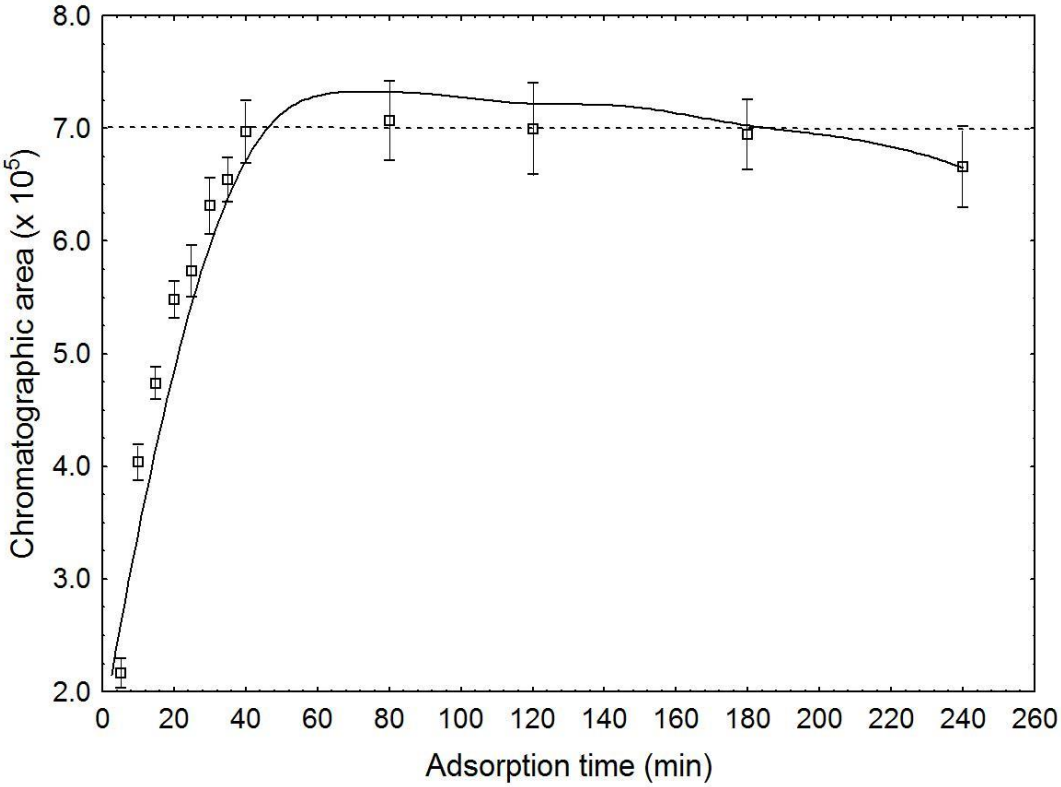
8.0
7.0
6.0
5.0
4.0
3.0
2.0
Chromatographic area (x 10^5)
0
20
40
60
80
100
120
140
160
180
200
220
240
260
Adsorption time (min)

FIGURE 4

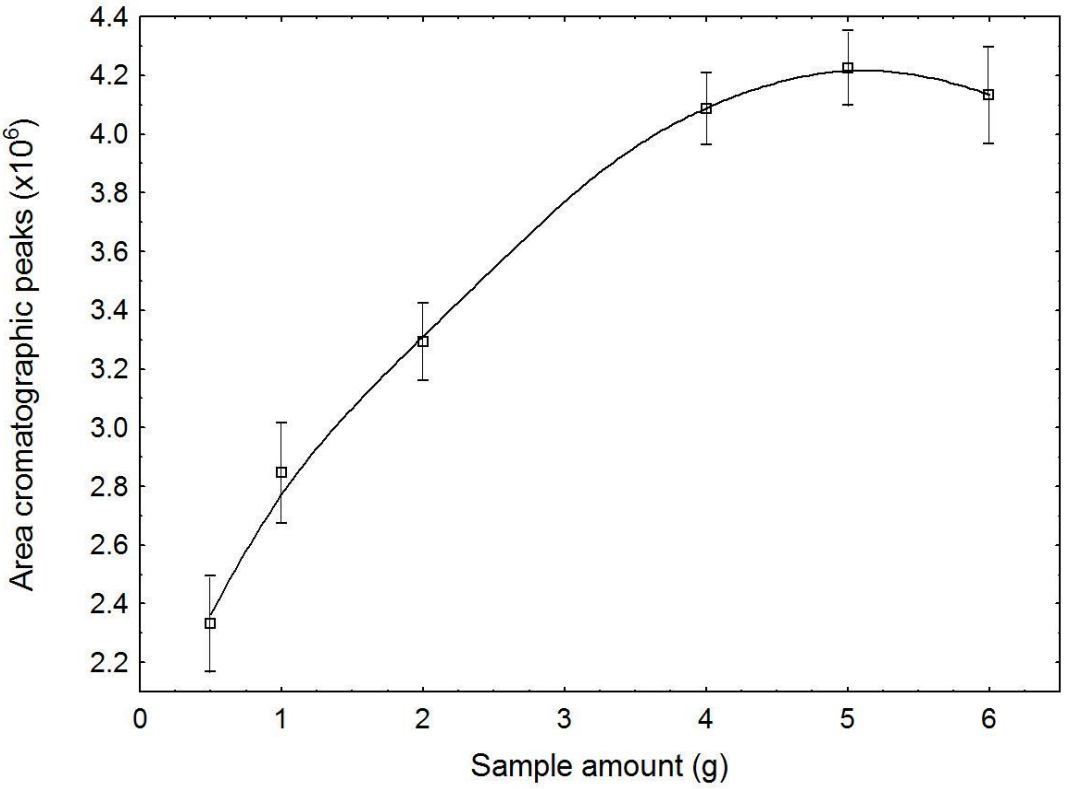
Area cromatographic peaks (x10^6)
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
0
1
2
3
4
5
6
Sample amount (g)

FIGURE 5

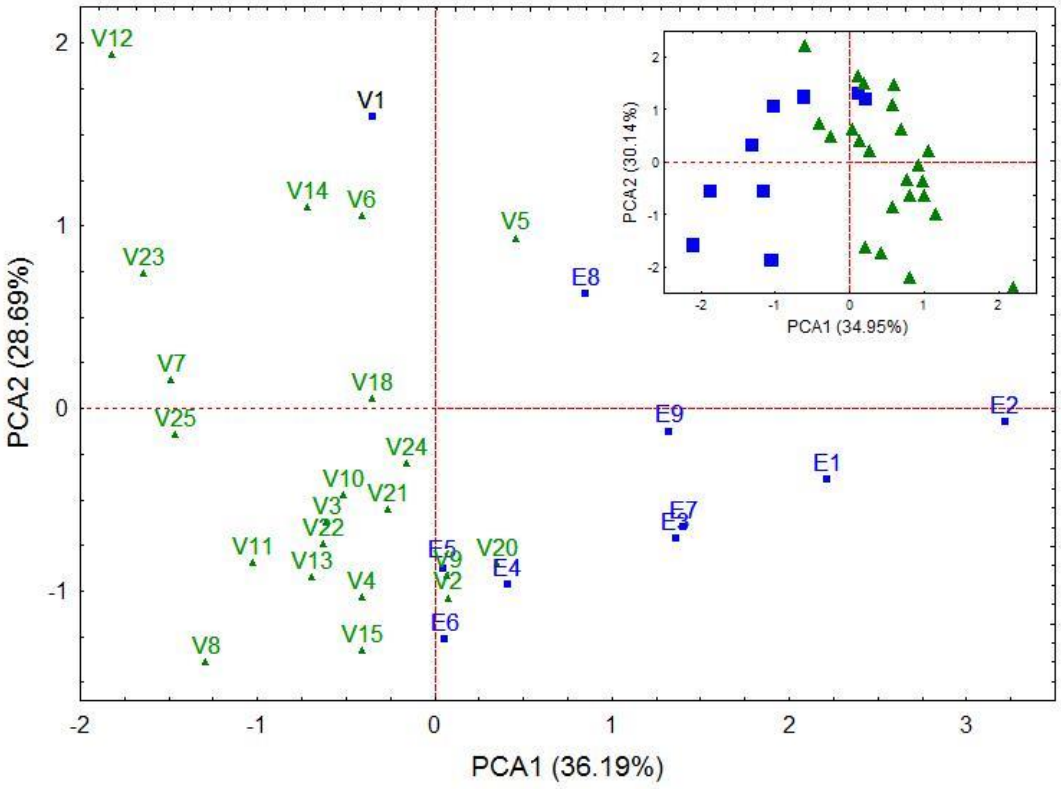
V12
V1
V14
V6
V5
V23
E8
V7
V18
E9
E2
V25
V24
E1
V10
V21
V3
E7
V22
V11
V13
E5
V20
E4
V9
V4
V2
E6
V15
V8
PCA2 (28.69%)
PCA1 (36.19%)
PCA2 (30.14%)
PCA1 (34.95%)

FIGURE 6

Graphic for Table of Contents