Vacuum Assisted Headspace Solid-phase Microextraction: A Powerful Tool for Olive Oil Analysis

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The performance of vacuum-assisted headspace solid-phase microextraction (Vac-HS-SPME) was compared to regular headspace solid-phase microextraction (HS-SPME) for the analysis of extra-virgin olive oil. Vac-HS-SPME proved beneficial in particular for semi-volatile compounds, significantly improving the kinetics of extraction. Moreover, for viscous oil samples combining the effects of heating the sample and vacuum was proven beneficial since it reduced the viscosity of the sample, increased the diffusivity of compounds in the liquid phase and improved volatilisation of less volatile compounds.

1. Introduction

Volatile secondary metabolites are an important class of compounds in many fields of applications such as clinical [1-3], environmental [4-7, and food [4,8,9]. In the latter one, volatiles can provide highly informative hints on botanical and geographical origin and/or on the quality of food in terms of aroma profile, spoilage, or technological impact on the secondary metabolite profile. Among the many high-value food commodities for whom volatile metabolites play an essential role, extra virgin olive oil represents an urgent challenge. The goal is to support the official sensor evaluation with a more objective and robust method.

Headspace (HS) solid-phase microextraction (SPME) is most widely applied for volatile profiling and fingerprinting since it provides easy automation, solvent-free applications, and flexibility due to the different sorbents commercially available [10]. HS-SPME is a technique based on the equilibrium between three-phases, namely sample-headspace-fibre. The equilibrium can be reached in a few minutes or several hours, depending on the physicochemical properties of the analytes and the sample. Therefore, a compromise is needed between sensitivity and throughput.

An exciting possibility to minimise such a compromise is the use of reduced pressure conditions during sampling, a technique termed vacuum-assisted HS–SPME. The theory for water-based and solid samples was effectively clarified in a tutorial published by Psillakis et al. [11]. More recently, the theory was extended to oily samples [12]. From a thermodynamic viewpoint, the equilibrium concentration is not affected by reduced pressure conditions [11,13], while the kinetics is mostly dependent on medium, temperature, and pressure. This means that an increase in the mass transfer is recorded when increasing the temperature and/or decreasing the pressure [11,13].

The mass transfer in the HS towards the SPME fibre is not considered a limiting process [14,16], while the mass transfer from the liquid to the HS, although highly analyte-matrix dependent, is usually the limiting step [11,16]. This behaviour is explained by considering the concentration gradient located in the stagnant film layers at the liquid/HS interface, assuming that the bulk of the two phases is well mixed. Such a theory proved successful in describing the Vac-HS-SPME process in water-based samples [11,14]. Recently [12], it was clarified that the overall resistence to transfer from the liquid to the HS (1/k_O) is due to two diffusional resistances in series, namely the sum of the gas-phase resistance (1/(K_{GL} k_G)) and the liquid-phase resistance (1/k_L), in particular for viscous liquid as olive oil, according to the following equation:

$$\frac{1}{k_0} = \frac{1}{k_L} + \frac{1}{K_{GL} k_G}$$

where, k_G and k_L are the mass transfer coefficients for the gas and olive oil boundary layers and K_{GL} is the gas phase-olive oil partition coefficient representing the ratio of the equilibrium concentrations in the gas phase over that in the liquid sample. Moreover, the diffusivity should be taken into account in viscous liquid samples, leading to additional resistance in the liquid-film compared to an aqueous phase [17]. On the other hand, the diffusion coefficient in the gas-phase shows an inverse proportionality to the total pressure in the system, regardless of the model chosen for describing it [14]. Therefore, sampling by Vac-HS-SPME is beneficial for analytes where gas-phase resistance controls their volatilisation rate, improving their extraction kinetics, while for compounds where the limiting process is the liquid diffusion, the temperature will play a beneficial role.

This work aimed to investigate the Vac-HS-SPME sampling on the extra-virgin olive oil profile, comparing the extraction temperature and time profile under reduced and normal pressure conditions.

2. Materials and Methods

2.1. Chemicals and reagents

A mixture of normal alkanes (C_7-C_{30}) (Supelco, USA) dissolved in hexane (HPLC grade, MilliporeSigma®, USA) was used for calculating the linear retention index (LRI) for confirming peak identity. A divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) d_f 50/30 µm 1 cm length fibre was used (offered by Millipore Sigma, Bellefonte, PA, USA).

Extra-virgin olive was purchased in a local supermarket (Gembloux, Belgium).

2.2. HS-SPME procedure for regular and reduced pressure conditions

The fibre was conditioned as suggested by the manufacturer before the first use. Blanks were run periodically to verify the absence of carryover.

1.5 g of oil sample was weighed into a 20 mL screw top vial (Restek, Bellefonte, USA). The sample was allowed to equilibrate with the headspace for 5 min at the temperature set for extraction (30°C and 43°C). Then, the SPME fibre was exposed to headspace and sampling was performed under agitation (250 rpm) at the selected sampling time (10, 20, 30, 40 min).

The fibre was desorbed at 250°C for 2 min (split 1:5) into the GC-MS inlet equipped with an SPME glass liner. All experiments were run in triplicate.

The difference between regular and Vac-HS-SPME is in the vial cap used. For the former, metallic caps with a central hole and a polytetrafluoroethylene (PTFE)/silicone septa (Restek, Bellefonte, USA) were used. For Vac-HS-SPME a custom-made closure design was implemented (provided by the Laboratory of Aquatic Chemistry, School of Environmental Engineering, Technical University of Crete [18]). The air inside the vial was evacuated for 1 min using a MD 4C diaphragm vacuum pump (7 mbar = 0.007 atm ultimate vacuum without gas ballast) (Vacuubrand GmbH & Co KZ, Wertheim, Germany). Then a 5 mL gastight syringe (SGE, Australia) was used to introduce 1.5 g of oil samples in the vial (Figure 1).

2.3. Central Composite Design

Central composite experimental design (CCD) was used to optimise extraction temperature and time, both for regular and Vac-HS-SPME. The extraction temperature was tested between 30°C and 55°C, and the exposition time from 10 to 30 minutes, based on conditions reported in previous works [19,20].

2.4. GC-MS analysis

An Agilent 7890B GC coupled to a 5977 MSD was used for all analyses. Carrier gas: helium at 1 mL/min flow rate. GC column: a 30 m x 0.25 mm i.d. x 0.5 µm df SLB-5ms capillary column [(silphenylene polymer, practically equivalent in polarity to poly(5%diphenyl/95% methylsiloxane)] kindly obtained from MilliporeSigma (Bellefonte, PA, USA). GC oven temperature program: 35°C (hold 2 min) to 250°C at 3°C/min and to 300°C at 25°C/min.
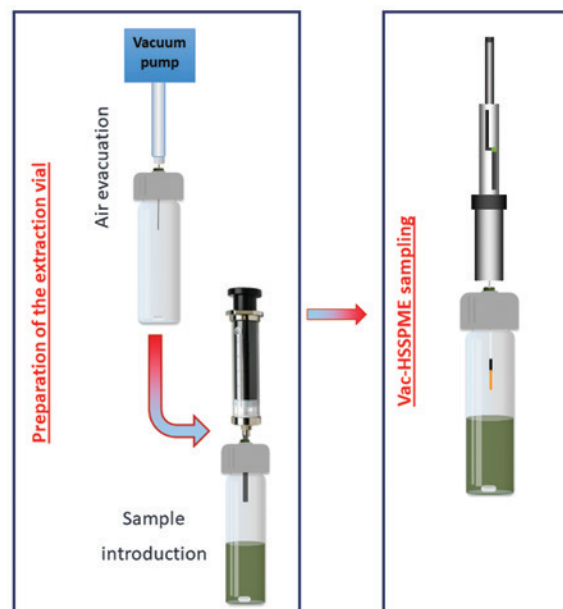

Figure 1: Schematic of the Vac-HSSPME

MS: in EI mode at 70 eV; source temperature: 230°C; quadrupole temperature: 150°C; full scan mode in the 35-500 m/z range.

2.5. Data elaboration and statistical analysis

Twelve compounds were selected over the entire chromatogram (Table 1). All the chemical-physical properties of the 12 compounds selected (reported in and obtained from the ChemSpider website (http://www.chemspider.com/).

All statistical analyses were performed using R v3.3.2 (R Foundation for Statistical Computing, Vienna, Austria) and Minitab 19 (https://www.minitab.com/en-us/) and https://software.broadinstitute.org/morpheus/.

Table 1: List of the 12 selected compounds together with their Chemical Abstracts Service (CAS) number, boiling point (B_P), Henry's constant (K_H, atm m³ mol⁻¹), octanol-air partition constant (K_{oa}, atm m³ mol⁻¹), octanol-water partition constant (K_{ow}, atm m³ mol⁻¹), Vapour pressure (V_p, mmHg at 25°C), Molecular weight (M_W, g mol⁻¹) and Molecular volume (V_M, cm³) , and linear retention index (IT) experimentally calculated and reported in the literature.

| #	Compound Name	CAS	M_W (g mol⁻¹)	V_M (cm³)	B_P (°C)	Log K_H (atm m³ mol⁻¹)	log K_{oa} (atm m³ mol⁻¹)	log K_{ow} (atm m³ mol⁻¹)	V_p (mmHg at 25°C)	m/z	IT_{ex}	IT_{Lib}
v1	Acetic acid	64-19-7	60.1	56.2	117.1	-5.54	5.218	0.09	13.9	60	634	641
v2	Penten-3-one	1629-58-9	84.1	103.3	104.3	-3.71	3.748	0.90	31.1	55	691	683
v3	Hexanal	66-25-1	100.2	124.9	127.0	-3.45	3.84	1.80	10.9	56	801	801
v4	1-Hexanol	111-27-3	102.2	125.0	158.2	-4.76	5.185	1.82	0.9	56	872	867
v5	2(E)-Heptenal	18829-55-5	112.1	135.0	166.0	-3.81	4.341	2.07	1.8	83	960	956
v6	Benzaldehyde	100-52-7	106.1	101.1	178.7	-4.64	4.442	1.71	1.0	106	965	960
v7	Octanal	124-13-0	128.2	157.9	163.4	-3.20	4.457	2.78	2.1	84	1004	1006
v8	Hex-(3Z)-enyl acet-ate	3681-71-8	142.2	157.7	175.2	-3.35	4.195	2.61	1.4	67	1005	1008
v9	ß-Ocimene,(E)	13877-91-3	136.2	175.5	175.2	-0.62	3.398	4.80	1.6	93	1047	1046
v10	Nonanal	124.19-6	142.2	174.4	190.8	-3.10	4.793	3.27	0.5	57	1105	1107
v11	Methyl salicylate	119-36-8	152.1	125.8	222.0	-5.24	4.947	2.60	0.1	120	1197	1192
v12	α-Farnesene, (E,E)	502-61-4	204.3	251.5	279.6	-0.19	5.067	7.10	0.0	93	1506	1504
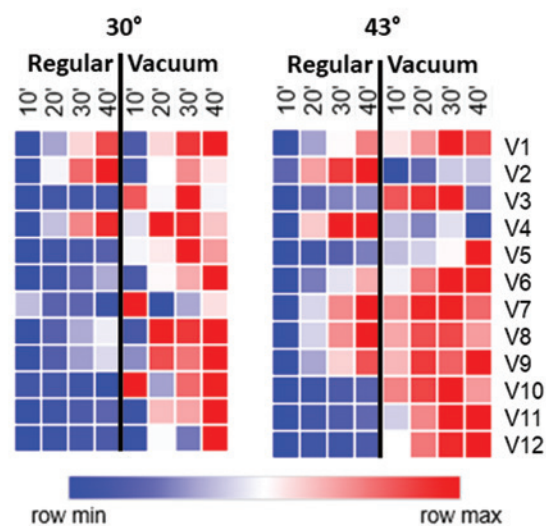
Figure 2: Heat-maps showing the extraction response obtained using regular and Vac-HS-SPME at 30 and 43 °C for different extraction time, namely 10, 20, 30 and 40 min. Compounds identification as reported in Table 1.
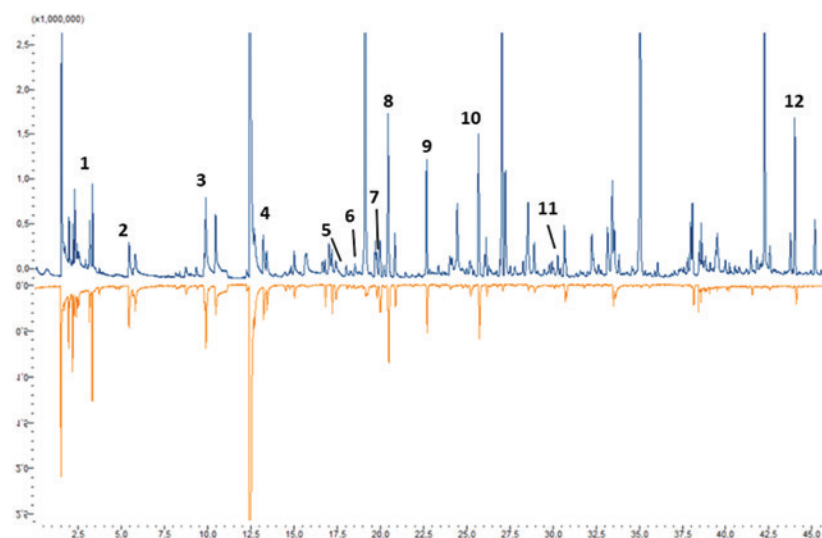

Figure 3: Total ion chromatogram obtained using regular (yellow, lower chromatogram) and Vac-HS-SPME (blue, upper chromatogram). Compound identification as for Table 1.

3. Results and Discussion

The heat maps reported in Figure 2 illustrate the overall change in the profile of the 12 targeted compounds when sampled using Regular and Vac-HS-SPME at both 30 and 43°C for different extraction times (10, 20, 30, and 40 min). The two heatmaps are normalised separately to emphasise the change in the response within a single temperature tested.

It can be observed as the Vac-HS-SPME sampling increased the general profile (colour turning toward red). However, an important distinction needs to be made between the compounds with the highest and lowest volatilities. For the former, generally, the same performance can be observed between regular and Vac-HS-SPME at 30 and 43°C, with even a slightly lower extraction yield at 43°C. The kinetics of these compounds is usually rapid; thus the effect of using reduced pressure conditions is limited or even non existent as these analytes have reached 'equilibrium'. The slight reduction of the signal at 43°C using Vac-HS-SPME indicates that the effect of temperature for the earlier eluted compounds is comparable to the gain in extraction yield obtained using vacuum. This behaviour can be related to both an acceleration of the extraction kinetic (not assessable in the range of extraction time tested) and competition effect. It is interesting to notice that for the rest of the compounds the effect of vacuum and temperature is instead synergic, significantly improving the extraction efficiencies at an earlier sampling time, in fact for the latest

compounds (V6-V12) almost the same intensity of response is obtained at 43°C after 20 min using Vac-HS-SPME, meaning that the equilibrium is almost reached; while under regular conditions, the response is much lower compared to Vac-HS-SPME and in a clear ascending trend moving from 10 to 40 min.

Noteworthy is the improvement obtained for α-farnesene (V12), reported as an important marker for discriminating the geographical origin of extra-virgin olive oil [21,22]. An almost 10-fold increment was observed when Vac-HS-SPME sampling and higher temperatures are applied. Figure 3 shows the comparison of the chromatographic traces between regular- and Vac-HS-SPME when sampling at 43°C for 10 min.

It is important to highlight that the effect of increasing the temperature in Vac-HS-SPME of water-based solutions was not always successful especially for absorbent type SPME fibres [11]. In fact, the increased humidity in the headspace increased the pressure in the vial, thus reducing its benefits. In edible oil samples, like olive oil, water is not present (or is in trace amounts); therefore, heating can be exploited with beneficial effect, although care must be paid to avoid artifact formation and thermal degradation products. Moreover, in high viscous samples like olive oil, the increase in temperature decreases the viscosity of the fluid. The high viscosity value of olive oil (49 mPa at 30°C, 60-times larger than water) increased the liquid-phase resistance, 'delaying' the analyte diffusion through the liquid boundary layer of the

olive oil matrix. This phenomenom is of high importance when targeting analytes with a small affinity for the headspace, regardless of the pressure conditions used [16]. In fact, for these analytes, a multi-stage process occurs: analyte molecules are transferred from the liquid sample to the gas phase every time the headspace concentrations fall below equilibrium levels [14,16]. This 'replenishment' process depends on the resistance in the liquid phase (related to the viscosity). It was shown here, that heating the sample from 30°C to 43°C led to a 40% decrease in viscosity of the sample (i.e., ~30 mPa s) improving the liquid-phase diffusivity and thus the overall extraction yield. Since Vac-HS-SPME sampling impact significantly on the kinetics of extraction, this process of 'replenishment' becomes even more limiting than in regular HS-SPME. However, the use of still milder temperature (43°C) in combination with reduced pressure provide a synergic improvement on the overall extraction yield..

Two-variable CCD: a study of the response surfaces

To better characterise the gain in performance obtained using Vac-HS-SPME over regular, a full factorial central composite design (CCD) was used to optimise at the same time and temperature ranges both the regular and Vac-HS-SPME. Based on previous findings that highlighted that time and temperature were the main significant variables [19,20], a two-variable (k=2; temperature and time) CCD was applied. The extraction temperature

was tested over a range of 30 (minimum temperature settable in the autosampler) and 55°C, and the extraction time was set between 10 and 30 min.

In fingerprinting and profiling studies, the goal is to maximise the overall response, maximising the number of peaks detected and their intensities. The response surface (Figure 4) was built considering the cumulative area intensity of all the peaks detected. It is evident from Figure 4 that the maximum is reached at a milder temperature and shorter time using Vac-HS-SPME, while using regular-HS-SPME, a maximum is not reached even after 55°C and 30 min of extraction. The total number of peaks detected increased from ~150 to ~180 using Vac-HS-SPME.
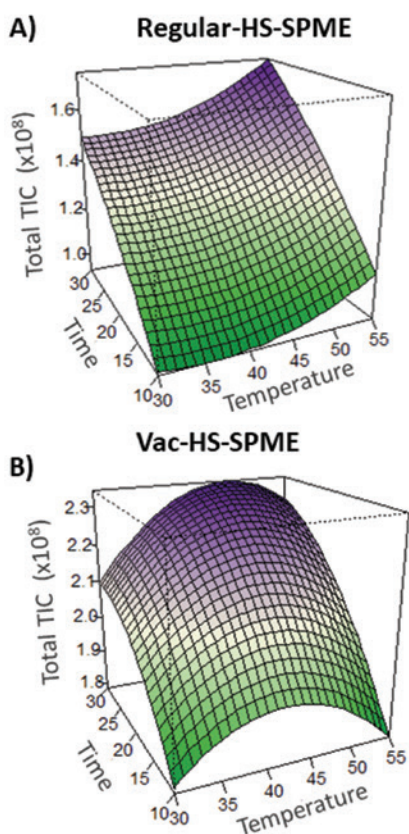

Figure 4: Surface responses are obtaining applying the two-variable inscribed rotatable CCD. A) Regular HS-SPME and B) Vac-HS-SPME. Temeprature range: 30-55°C; time range: 10-30 min.

Conclusions

The advantages of using Vac-HS-SPME compared to regular-HS-SPME were shown and discussed. Heating the sample (43°C compared to 30°C) further enhanced the benefit of vacuum. Moreover, for viscous liquid samples, the temperature remained an important parameter since it increased the diffusivity through the liquid phase facilitating mass transfer at the interface.

The use of Vac-HS-SPME for untargeted studies of olive oil can importantly increase the level of information obtainable and the effectiveness of cross-sample comparison applying pattern recognition algorithms, allowing more effective markers identification for quality and authenticity studies.

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