The use of headspace solid phase microextraction for the characterization of volatile compounds in olive oil matrices

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ABSTRACT

Two different fibre coatings, for solid phase microextraction (SPME) sampling, poly(dimethylsiloxane) (PDMS) and poly(acrylate) (PA), were studied in order to test, for olive oil matrixes, two mathematical models that relate the directly proportional relationship between the amount of analyte absorbed by a SPME fibre and its initial concentration in the sample matrices. Although the PA fibre was able to absorb higher amounts of compounds from the olive oil sample, the equilibrium was reached later then with the PDMS fibre. In both cases, the amount of analyte present affected the time profile or the equilibrium time in two of the concentrations studied, 0.256 μL/kg, 2.56 μL/kg and for 2-ethylfuran, pentan-3-one, pent-1-en-3-one, hexanal, *trans,trans*-non-2,4-dienal and in the four concentrations studied, 0.256 μL/kg, 2.56 μL/kg, 6.25 μL/kg and 400 μL/kg, for 4-methyl-pent-3-en-2-one, 2-methylbutan-1-ol, methoxybenzene, hexan-1-ol, *cis*-hex-3-en-1-ol, *trans*-hex-2-en-1-ol, 2-ethyl-hexan-1-ol and *trans,trans*-dec-2,4-dienal. Comparing the mathematical models of both fibres, the PA-coated fibre showed direct proportionality between the initial concentration and amount extracted, that allows the possibility of relative quantification in a non-equilibrium state in non-aqueous media. The same was not observed for the PDMS fibre.

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1. Introduction

In the overall quality of olive oil, the aroma plays an important role in directing consumer preference. Some components present in low concentration, contribute to the pleasant aroma note in olive oils, but when they are present in higher concentrations their contribution seems to be negative [1]. So it is important to determine, at least, the relative amounts of the aroma components of olive oil.

Compared to several techniques of sample preparation for gas chromatographic (GC) analysis of aroma compounds, headspace solid phase microextraction (HS-SPME) shows some advantages such as solvent-free extraction, low sample volumes and low cost. SPME is an equilibrium method, which does not require exhaustive extraction of a volume of sample [2].

It is known that olive oil is a complex matrix, with a high content of natural volatile compounds that have differences, among other physicochemical properties, in volatility and polarity. These different compounds are expected to present different equilibrium times. As HS-SPME is a multiphase equilibrium process [3], the maximum sensibility is obtained by allowing the analyte(s) to reach equilibrium. It is not practical to do so when equilibration times are excessively long. Ai [4] presented a mathematical model (Eq. (1)) to demonstrate that SPME quantification, in aqueous solution, using fibres coated with a polymeric liquid, is feasible before absorption equilibrium is reached, if the amount of analyte absorbed (n) is proportional to the initial concentration in the sample matrix (C_0):

$$\frac{n}{n^\infty} = [1 - \exp(-a_h t)] \tag{1}$$

$n\infty$ represents the amount extracted at equilibrium and the parameter a_h is a measure of how fast partition equilibrium can be reached in the HS-SPME process. The parameter a_h is controlled by mass transfer coefficients, equilibrium constants and the physical dimensions of the sample matrix, headspace and the polymeric film. It has different magnitudes depending on the rate-determining step of the mass transfer process: matrix/headspace and headspace/polymer, diffusion in the polymeric film and evaporation from the matrix phase as described by Ai [4,5].

However, in Eq. (1) the initial period of extraction is neglected. In fact, during the sampling process, when the equilibrium between sample and headspace is reached, it is disturbed by the introduction of the fibre needle into the headspace. This disturbance is not considered in the model described by Eq. (1). Moreover, in complex

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Table 1
Typical concentrations and odour characteristics of compounds identified as odorants in olive oil

No.	Compound	Concentration in olive oil (μg/kg)	Odour quality [6]
1	2-Ethylfuran	–	Powerful, sweet, ethereal, burnt odour, coffee taste on dilution
2	Pentan-3-one	153–1053 [7]	–
3	Pent-1-en-3-one	634 [7] 40–300 [8] 26 [9]	Pungent, mustard
4	Hexanal	169–6367 [7]	Fatty, green, grassy, powerful, penetrating
5	4-Methyl-pent-3-en-2-one	–	Unpleasant, pungent, vegetable, acrylic
6	2-Methylbutan-1-ol	2809 [7] 230–10,260 [8]	–
7	Methoxybenzene	–	Phenolic, gasoline, ethereal, anise
8	Hexan-1-ol	685–50,200 [7] 680–10,260 [8]	Herbaceous, woody, fragrant, mild, sweet, green
9	*cis*-Hex-3-en-1-ol	252–8587 [7] 460–870 [8] 684 [9]	Fresh, green grass
10	*trans*-Hex-2-en-1-ol	1118–89,100 [7]	Powerful, leafy, green, wine-like, fruity
11	2-Ethyl-hexan-1-ol	–	Mild, oily, sweet, slight rose
12	*trans,trans*-Non-2,4-dienal	49 [9]	Strong, fatty, floral
13	*trans,trans*-Dec-2,4-dienal	127–918 [9]	Powerful, fatty, citrus

matrices, such as olive oil, this disturbance is even more important, since more equilibria are now being influenced by fibre needle introduction. Ai [5] proposed a biexponential equation (Eq. (2)) that accounts for this fact:

$$\frac{n}{n^{\infty}} = \alpha[1 - \exp(-ct)] + \beta[1 - \exp(-c_1 t)] \tag{2}$$

Hybrid parameters α, c, β and c_1 are defined as

$$\alpha = \frac{b_1}{c - c_1}; \quad \beta = \frac{a_1}{c_1} - \frac{b_1}{c - c_1}; \quad c = k + \frac{A_f m_f K_{fh}}{V_h}; \quad c_1 \frac{2A_f m_f}{V_f};$$

$$a_1 = A_f m_f K_{fh} a; \quad b_1 = 2A_f m_f K_{fh} b; \quad a = \frac{k K_{hs} V_h}{k V_h + A_f m_f K_{fh}} C_0;$$

$$b = \frac{A_f m_f K_{fh} K_{hs}}{k V_h + A_f m_f k_{fh}} C_0$$

where A_f is the surface area of the SPME polymer film; m_f is the mass transfer coefficient of the analyte in polymer film phase; k is the evaporation rate constant; V_h and V_f are the volumes of the headspace and the coating, respectively; K_{fh} is the polymer/headspace distribution constant; K_{hs} is the headspace/matrix distribution constant. Since α and β (in mol, accounting for the variation of the quantity of a particular compound present in the headspace when the equilibrium is disturbed) are directly proportional to C_0 then n is directly proportional to C_0. This fact implies that quantitative determination can be performed in a non-equilibrium state.

Since both mathematical models (Eqs. (1) and (2)) describe an absorption process, poly(dimethylsiloxane) (PDMS) and poly(acrylate) (PA) fibres were chosen for the present study. The methyl groups of PDMS make this film relatively apolar, whereas PA is a more polar fibre due to the presence of carbonyl groups.

This work aims the application of the mathematical models to a non-aqueous matrix, in this case an oily matrix, such as olive oil. This type of matrices are a better solvent for the majority of the volatile components of olive oil. It is intended to demonstrate that an important and complex matrix such as olive oil can also be studied considering the estimation parameters calculated according to one or to both mathematical models studied. A SPME sampling method was developed procedure for reproducible qualitative and relative quantitative determination of aroma compounds

using non-equilibrium extraction conditions. We intended to determine not only which model describes better the experimental data obtained with each of the fibres studied, but also which fibre is more efficient for non-equilibrium state extraction in a non-aqueous medium.

2. Experimental

2.1. Standards

The standard compounds, 2-ethylfuran, pentan-3-one, 4-methyl-pent-3-en-2-one, pent-1-en-3-one, 2-methylbutan-1-ol, hexanal, methoxybenzene, hexan-1-ol, *cis*-hex-3-en-1-ol, *trans*-hex-2-en-1-ol, 2-ethyl-hexan-1-ol, *trans,trans*-non-2,4-dienal and *trans,trans*-dec-2,4-dienal were purchased from Aldrich (Deisenhofen, Germany). The internal standard (IS) used was nonan-2-ol also from Aldrich (Deisenhofen, Germany).

2.2. Sample preparation and analysis

Standard compounds were dissolved directly in refined olive oil. Four concentrations, 0.256 μL/kg, 2.56 μL/kg, 6.25 μL/kg and 400 μL/kg were used. Fifteen grams of olive oil with the dissolved standards were placed in a 22 mL vial and kept in a water-thermostatized bath at 37 °C for 30 min before the HS-SPME extraction. The septum covering the vial was pierced with a SPME needle and the fibre was exposed to the olive oil headspace for 1 min, 5 min, 15 min, 30 min, 60 min, 90 min and 120 min. During sampling, the oil phase was stirred with a magnetic stirrer at a constant and defined stirring rate. After sampling, the fibre was inserted manually into the GC injection port for 5 min and desorbed at 260 °C.

2.3. SPME fibres and conditioning

SPME device and fused silica fibres were purchased from Supelco Inc. (Bellefonte, Pennsylvania, USA). Poly(acrylate) (PA) and poly(dimethylsiloxane) (PDMS) SPME fibres with coating thicknesses of 85 μm and 100 μm, respectively were used. Prior to use, the fibres were conditioned according to manufacturer instructions.
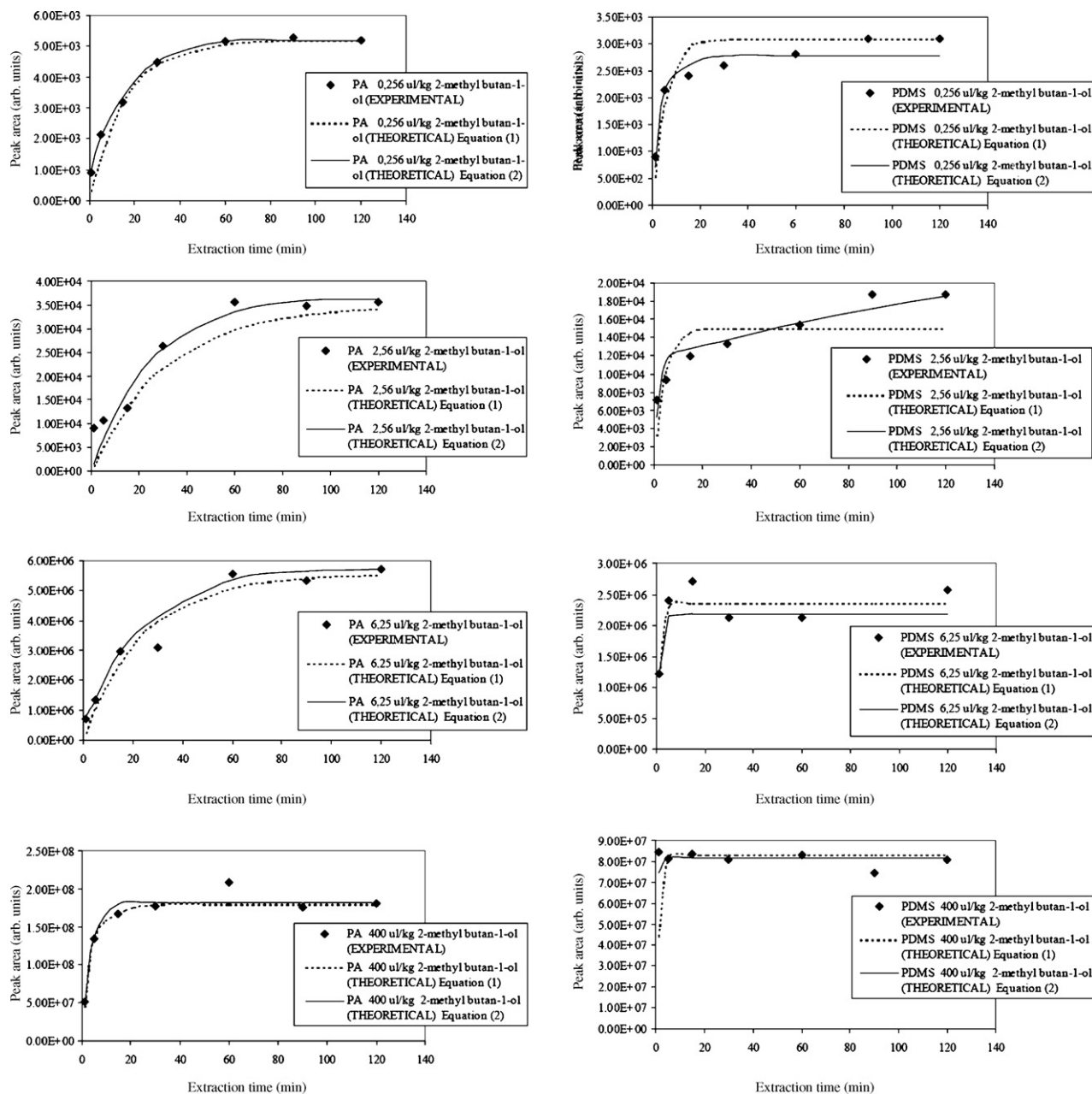
Fig. 1. Absorption kinetic curves. Comparison of experimental data *vs.* theoretical model for 2-methylbutan-1-ol in four different concentrations (0.256 μL/kg, 2.56 μL/kg, 6.25 μL/kg and 400 μL/kg) at 37 °C for the PA and the PDMS fibres.

2.4. Equipment

GC analyses were performed using a GC-Trace Thermo Quest Instruments. For GC analyses, a fused silica DB-Wax column (J&W Scientific, Folsom, USA), 60 m × 0.25 mm i.d. and 1.0 μm film thickness was used. The oven temperature program was as follow: 50 °C (5 min), 2 °C min^{-1} to 210 °C (10 min). Hydrogen was used as the carrier gas at 100 kPa; the injector temperature and the split flow were set at 260 °C and 30 mL min^{-1}, respectively, after a splitless time of 1 min.

2.5. Data processing

The relative area data of each compound were obtained from the collected chromatograms. The adjustment of the extraction data (for the HS-SPME studies) to the theoretical models described above as Eqs. (1) and (2), was performed through an estimation method by non-linear regression method.

Data were analysed using STATISTICA 6.0 software (StatSoft Inc., Version 6.0, Tulsa, USA). Non-linear estimation user-specified regression was used. The estimation method was the quasi-Newton with a maximum number of iterations of 100. The convergence criterion was set to 0.0001 and the loss function was equal to (observed data – predicted values)^2.

3. Results and discussion

The volatile compounds chosen for method evaluation were studied with two different SPME fibre coatings and with four different initial concentrations that are comparable to the con-

Table 2
Parameter a_h values calculated according to Eq. (1) for selected compounds in PA fibre at 37 °C using different initial concentrations

Compound	C_0 (μL/kg)	Parameter, a_h (estimated)	Correlation coefficient	Explained variance (%)	Final loss
PA Eq. (1) model: $n/n^\infty = 1 - \exp(-a_h t)$					
2-Ethylfuran	0.256	**0.2003**	0.9587	90.679	0.01718
	2.56	**0.1046**	0.9259	85.729	0.05610
Pent-3-one	0.256	**0.0213**	0.9687	93.819	0.01632
	2.56	**0.0748**	0.9944	99.548	0.00683
Pent-1-en-3-one	0.256	**0.0276**	0.9574	90.817	0.01521
	2.56	**0.0504**	0.9908	98.171	0.00635
Hexanal	0.256	**0.0947**	0.9511	90.459	0.01633
	2.56	**0.1201**	0.9685	93.927	0.02447
4-Methyl-pent-3-en-2-one	0.256	**0.0610**	0.9118	83.134	0.03176
	2.56	**0.0613**	0.9794	94.726	0.02241
	6.25	**0.0665**	0.9601	93.717	0.05509
	400	**0.1573**	0.9746	94.958	0.02166
2-Methylbutan-1-ol	0.256	**0.0620**	0.9685	93.797	0.01431
	2.56	**0.0317**	0.9221	85.027	0.05140
	6.25	**0.0414**	0.9706	94.801	0.04762
	400	**0.2853**	0.9681	93.877	0.03018
Methoxybenzene	0.256	**0.0094**	0.9537	90.956	0.01864
	2.56	**0.0411**	0.9726	94.882	0.02659
	6.25	**0.0912**	0.9656	93.997	0.02575
	400	**0.1900**	0.9505	90.418	0.05919
Hexan-1-ol	0.256	**0.0639**	0.9790	95.852	0.00991
	2.56	**0.0689**	0.9712	94.791	0.02348
	6.25	**0.0638**	0.9683	93.918	0.01927
	400	**0.5292**	0.9679	93.874	0.01956
cis-Hex-3-en-1-ol	0.256	**0.0519**	0.9616	92.461	0.01810
	2.56	**0.0721**	0.9668	93.889	0.01992
	6.25	**0.0754**	0.9999	99.999	0.19901
	400	**0.3774**	0.9621	93.357	0.05625
trans-Hex-2-en-1-ol	0.256	**0.0348**	0.9575	91.682	0.04388
	2.56	**0.0710**	0.9698	93.969	0.01981
	6.25	**0.0789**	0.9729	94.816	0.02480
	400	**0.3495**	0.9957	99.655	0.00842
2-Ethyl-hexan-1-ol	0.256	**0.0262**	0.9736	94.797	0.02069
	2.56	**0.0399**	0.9821	96.613	0.00227
	6.25	**0.0415**	0.9973	99.677	0.00445
	400	**0.2235**	0.9912	99.204	0.00640
trans,trans-Non-2,4-dienal	0.256	**0.0497**	0.9338	87.206	0.02590
	2.56	**0.0270**	0.9535	90.671	0.01638
trans,trans-Dec-2,4-dienal	0.256	**0.0814**	0.9596	91.727	0.04991
	2.56	**0.0354**	0.9582	91.699	0.05290
	6.25	**0.0377**	0.9758	94.817	0.02538
	400	**0.0682**	0.9649	93.669	0.05166

centrations found in olive oils. Their diversified chemical nature and different odorific notes [6] determined their choice. All of these compounds were previously detected in olive oil [7–10]. Table 1 presents the compounds used in this study and it also indicates the concentrations which have been previously measured in olive oil samples and the respective odour perceptions [6]. The analytes chosen intend to represent the broad chemical functionalities of the compounds present in olive oils. An internal standard was used in order to control the retention time.

Prior to the analyses, a temperature study was performed for a time periods from 1 min to 120 min in order to establish the optimal work temperature of the extraction procedure [11]. Three temperatures were studied: 25 °C, 37 °C and 40 °C. At 37 °C and

40 °C the largest amount of compounds was extracted with both fibres (results not shown) [11]. Another concern was to choose a temperature as close as possible that at which the human olfative systems perceives volatile emissions on one hand, and on the other hand to avoid compound degradation that might produce unwanted artefacts. For these reasons 37 °C was chosen as the sampling temperature.

The standard compounds were extracted at six or seven different periods of time and the results of each area *vs.* extraction time were determined. As an example, the absorption curves for 2-methylbutan-1-ol for the concentrations 0.256 μL/kg, 2.56 μL/kg, 6.25 μL/kg and 400 μL/kg are shown in Fig. 1. A sigmoidal-shape curve can be observed which does not depend on the initial concentration, the nature of the coating fibres or the mathematical model

Table 3
Parameter a_h values calculated according to Eq. (1) for selected compounds in PDMS at 37 °C using different initial concentrations

Compound	C_0 (µL/kg)	Parameter, a_h (estimated)	Correlation coefficient	Explained variance (%)	Final loss
PDMS Eq. (1) model: $n/n^\infty = 1 - \exp(-a_h t)$					
2-Ethylfuran	0.256	**0.3127**	0.9981	99.778	0.00501
	2.56	**0.0337**	0.9698	94.051	0.02642
Pent-3-one	0.256	**0.0427**	0.9912	99.101	0.00958
	2.56	**0.0411**	0.9833	96.687	0.00184
Pent-1-en-3-one	0.256	**0.0376**	0.9823	96.489	0.01829
	2.56	**0.2242**	0.9962	99.607	0.00221
Hexanal	0.256	**0.0857**	0.9841	96.915	0.00231
	2.56	**0.0835**	0.9944	99.366	0.00992
4-Methyl-pent-3-en-2-one	0.256	**0.0245**	0.9833	96.687	0.00180
	2.56	**0.2629**	0.9951	99.566	0.00734
	6.25	**0.9898**	0.9764	94.856	0.02873
	400	**0.5240**	0.9993	99.877	0.00157
2-Methylbutan-1-ol	0.256	**0.1886**	0.9983	99.772	0.00441
	2.56	**0.2419**	0.9961	99.714	0.00710
	6.25	**0.7468**	0.9997	99.897	0.00122
	400	**0.7667**	0.9999	99.999	0.01144
Methoxybenzene	0.256	**0.0315**	0.9861	96.799	0.00312
	2.56	**0.2799**	0.9945	99.423	0.00980
	6.25	**0.3732**	0.9994	99.818	0.00411
	400	**0.3483**	0.9798	94.901	0.02391
Hexan-1-ol	0.256	**0.0289**	0.9891	96.988	0.00673
	2.56	**0.3106**	0.9559	91.667	0.04535
	6.25	**0.7631**	0.9845	96.918	0.00522
	400	**0.7316**	0.9584	91.701	0.03990
cis-Hex-3-en-1-ol	0.256	**0.0260**	0.9978	99.267	0.00371
	2.56	**0.5509**	0.9999	99.999	0.05426
	6.25	**0.9401**	0.9992	99.879	0.00248
	400	**0.5509**	0.9751	94.841	0.02866
trans-Hex-2-en-1-ol	0.256	**0.0322**	0.9833	96.855	0.00719
	2.56	**0.9185**	0.9989	99.998	0.00422
	6.25	**0.9193**	0.9996	99.879	0.00310
	400	**0.7284**	0.9581	91.655	0.04901
2-Ethyl-hexan-1-ol	0.256	**0.0971**	0.9959	99.864	0.00371
	2.56	**0.3625**	0.9999	99.999	0.06117
	6.25	**0.3906**	0.9776	94.966	0.01136
	400	**0.4728**	0.9859	96.997	0.00281
trans,trans-Non-2,4-dienal	0.256	**0.0647**	0.9590	91.675	0.04547
	2.56	**0.3603**	0.9999	99.999	0.00131
trans,trans-Dec-2,4-dienal	0.256	**0.0542**	0.9813	96.289	0.01512
	2.56	**0.1133**	0.9966	99.274	0.00282
	6.25	**0.4030**	0.9755	94.899	0.02711
	400	**0.2586**	0.9991	99.884	0.00109

applied. The adjusted curves according to Eqs. (1) and (2) of the model of Ai [4] are shown in the graphs of Fig. 1, for 2-methylbutan-1-ol for both fibres at the concentrations studied. Here the points are raw experimental data and the lines represent the result of the application of the two equations to the experimental data.

The parameters a_h from Eq. (1) and α, c, β, and c_1 from Eq. (2) for all the compounds studied, in PDMS and PA coating fibres, are obtained through exponential regression. Tables 2 and 3 show the values for the a_h parameter, correlation coefficient, explained variance and the loss function for the curves fitted with Eq. (1). The parameters of Eq. (2), α, c, β, and c_1 are listed in Tables 4 and 5, as well as the correlation coefficients, explained variance and the loss function for all fitted curves.

For the selected compounds, the experimental data appears to fit poorly, as expected, to the theoretical model given by Eq. (1).

All the curves could however be satisfactorily described by Eq. (2) and confirm the relevance of the initial period of extraction for the absorption of the olive oil volatiles mainly in PA fibre. Similar results were reported previously by Matich et al. [12] who observed that a two-exponential equation, rather a one-exponential equation, described the experimental data for apple volatiles extracted by SPME.

The values for the a_h parameter for the four different initial concentrations studied are slightly dependent on the initial concentration, as expected [13], especially for the PA fibre.

In Fig. 1 it can be easily perceived how fast the partition equilibrium (between the headspace/polymer interfaces) can be reached,

Table 4
Parameters α, c, β, c_1 values calculated according to Eq. (2) for selected compounds in PA fibre at 37 °C using different initial

Compound	C_0 (μL/kg)	Estimated parameters				Correlation coefficient	Explained variance (%)	Final loss
		α	c	β	c_1			
PA Eq. (2) model: $n/n^\infty = \alpha[1 - \exp(-ct)] + \beta[1 - \exp(-c_1 t)]$								
2-Ethylfuran	0.256	**0.8998**	**0.0085**	**0.5753**	**0.4331**	0.9996	99.95	0.00092
	2.56	**0.5346**	**0.0894**	**0.5346**	**0.0894**	0.9948	99.835	0.00441
Pent-3-one	0.256	**0.6162**	**0.0232**	**0.6163**	**0.0232**	0.9473	89.745	0.06236
	2.56	**0.8777**	**0.0180**	**0.4186**	**0.9755**	0.9987	97.861	0.00111
Pent-1-en-3-one	0.256	**0.9766**	**0.0102**	**0.8245**	**0.0100**	0.9913	99.114	0.00779
	2.56	**0.8133**	**0.0988**	**0.9798**	**0.0054**	0.9999	99.997	0.00011
Hexanal	0.256	**0.6407**	**0.9699**	**0.9778**	**0.0052**	0.9733	95.644	0.02711
	2.56	**0.7769**	**0.0953**	**0.8766**	**0.0091**	0.9998	99.960	0.00014
4-Methyl-pent-3-en-2-one	0.256	**0.9888**	**0.0091**	**0.4657**	**0.9589**	0.9981	99.650	0.00208
	2.56	**0.6044**	**0.0237**	**0.6044**	**0.0237**	0.9857	97.168	0.01673
	6.25	**0.6573**	**0.0332**	**0.37822**	**0.2617**	0.97509	95.081	0.03474
	400	**0.7798**	**0.0081**	**0.8712**	**0.3866**	0.9878	97.921	0.02067
2-Methylbutan-1-ol	0.256	**0.9068**	**0.0401**	**0.2331**	**0.9918**	0.9988	99.630	0.00148
	2.56	**0.5297**	**0.0396**	**0.5297**	**0.0396**	0.9693	93.944	0.02704
	6.25	**0.2437**	**0.8876**	**0.9552**	**0.0476**	0.9982	99.236	0.00283
	400	**0.5064**	**0.2760**	**0.5064**	**0.2760**	0.9689	93.883	0.02942
Methoxybenzene	0.256	**0.3101**	**0.7898**	**0.7748**	**0.0122**	0.9997	99.944	0.00023
	2.56	**0.5842**	**0.0307**	**0.5842**	**0.0307**	0.9439	89.094	0.06708
	6.25	**0.4158**	**0.9661**	**0.8803**	**0.0730**	0.9794	95.918	0.03102
	400	**0.4758**	**0.2163**	**0.4758**	**0.2163**	0.9932	98.653	0.00604
Hexan-1-ol	0.256	**0.4623**	**0.0748**	**0.4623**	**0.0748**	0.9606	92.284	0.01966
	2.56	**0.3484**	**0.5082**	**0.8578**	**0.0296**	0.9995	99.907	0.00033
	6.25	**0.2716**	**0.9908**	**0.8527**	**0.0677**	0.9962	99.559	0.00142
	400	**0.4922**	**0.5519**	**0.4922**	**0.5519**	0.9955	99.738	0.00221
cis-Hex-3-en-1-ol	0.256	**0.8938**	**0.0289**	**0.2878**	**0.7679**	0.9913	98.266	0.00614
	2.56	**0.2259**	**0.9986**	**0.8984**	**0.0311**	0.9766	94.078	0.01948
	6.25	**0.5018**	**0.0747**	**0.5018**	**0.0747**	0.8730	76.216	0.19902
	400	**0.5020**	**0.3736**	**0.5020**	**0.3736**	0.9317	86.800	0.05527
trans-Hex-2-en-1-ol	0.256	**0.5822**	**0.0257**	**0.5822**	**0.0257**	0.9700	94.089	0.03119
	2.56	**0.1267**	**0.7637**	**0.9931**	**0.0310**	0.9709	94.256	0.02623
	6.25	**0.5034**	**0.0776**	**0.5034**	**0.0776**	0.9001	81.002	0.16214
	400	**0.4911**	**0.3662**	**0.4911**	**0.3662**	0.9135	83.445	0.06991
2-Ethyl-hexan-1-ol	0.256	**0.6386**	**0.0195**	**0.6389**	**0.0195**	0.9736	94.788	0.03154
	2.56	**0.8587**	**0.5337**	**0.8779**	**0.0034**	0.9997	99.913	0.00023
	6.25	**0.1956**	**0.9898**	**0.8761**	**0.0397**	0.9475	89.775	0.06889
	400	**0.4985**	**0.2256**	**0.4985**	**0.2256**	0.9328	87.001	0.07414
trans,trans-Non-2,4-dienal	0.256	**0.7998**	**0.0209**	**0.2311**	**0.8667**	0.9953	98.45	0.00563
	2.56	**0.3798**	**0.0244**	**0.3798**	**0.0244**	0.9994	99.880	0.00032
trans,trans-Dec-2,4-dienal	0.256	**0.5858**	**0.0129**	**0.6200**	**0.2060**	0.9621	92.566	0.01750
	2.56	**0.6417**	**0.0220**	**0.6417**	**0.0220**	0.9847	96.954	0.01949
	6.25	**0.6997**	**0.0344**	**0.6997**	**0.0344**	0.9961	98.54	0.00314
	400	**0.7733**	**0.0586**	**0.1872**	**0.3585**	0.9736	94.794	0.03901

for a given compound. In this figure, the ordinate represents the fibre uptake normalized to the equilibrium value. Compared to olive oil headspace/PA interface partition, the parameter a_h at the olive oil headspace/PDMS interface is higher, which means that in the PDMS fibre the partition equilibrium is reached sooner. This is in agreement with the fact that on the PA fibre diffusion coefficients are lower in comparison with the PDMS fibre [14]. Longer extraction times with the PA coating fibre for volatile analytes in the headspace is an expected result. The selection of the fibre that offers the best reproducible results for quantitative purposes, should depend not only on the amount extracted, but also on some other physical/chemical properties such as mass transfer coefficients, equilibrium constants, among others, in order to achieve a linear range behaviour in a non-equilibrium state [4,15]. This work demonstrates that PDMS fibres present for all the compounds studied, lower equilibration times when compared to PA fibres, although PA fibres present a better absorption capacity for compounds with a wide range of functionalities. The initial concentration is crucial for the time profile and the achievement of equilibrium in both fibres. Generally the a_h parameter is higher when the initial concentration is higher, indicating that equilibrium is reached quicker in that situation for both fibres. Since Eq. (1) does not fit as well to the experimental data when compared to Eq. (2), one cannot have any information about the proportionality between the initial concentration and amount extracted. So the biexponential equation (2) was applied to the data.

Table 5
Parameters α, c, β, c_1 values calculated according to Eq. (2) for selected compounds in PDMS fibre at 37 °C using different initial concentrations

Compound	C_0 (µL/kg)	Estimated parameters				Correlation coefficient	Explained variance (%)	Final loss
		α	c	β	c_1			
PDMS Eq. (2) model: $n/n^\infty = \alpha[1 - \exp(-ct)] + \beta[1 - \exp(-c_1 t)]$								
2-Ethylfuran	0.256	**0.6107**	**0.3265**	**0.6107**	**0.3265**	0.9520	91.752	0.00567
	2.56	**0.4215**	**0.7108**	**0.4215**	**0.7108**	0.9894	97.883	0.00941
Pent-3-one	0.256	**0.8939**	**0.0078**	**0.5624**	**0.9778**	0.9654	93.204	0.01904
	2.56	**0.5384**	**0.0353**	**0.5384**	**0.0353**	0.9879	97.597	0.01333
Pent-1-en-3-one	0.256	**0.5780**	**0.0279**	**0.5780**	**0.0279**	0.9950	99.003	0.00523
	2.56	**0.4510**	**0.3209**	**0.4510**	**0.3209**	0.9677	93.312	0.02235
Hexanal	0.256	**0.5975**	**0.0239**	**0.5092**	**0.3247**	0.9992	99.839	0.00032
	2.56	**0.4759**	**0.6125**	**0.5894**	**0.0246**	0.9999	99.991	0.00001
4-Methyl-pent-3-en-2-one	0.256	**0.3514**	**0.9886**	**0.9599**	**0.0011**	0.8919	79.558	0.07828
	2.56	**0.4410**	**0.3978**	**0.4410**	**0.3978**	0.9816	97.427	0.01521
	6.25	**0.6602**	**0.9898**	**0.6602**	**0.9898**	0.9368	87.762	0.00970
	400	**0.4958**	**0.5474**	**0.4958**	**0.5474**	0.7958	63.324	0.00191
2-Methylbutan-1-ol	0.256	**0.4486**	**0.2906**	**0.4486**	**0.2906**	0.9861	97.548	0.01414
	2.56	**0.9998**	**0.0051**	**0.7756**	**0.5926**	0.9916	98.088	0.00619
	6.25	**0.4651**	**0.8052**	**0.4651**	**0.8052**	0.8776	77.019	0.04126
	400	**0.4929**	**0.9798**	**0.4929**	**0.9798**	0.9265	85.836	0.00101
Methoxybenzene	0.256	**0.6215**	**0.9869**	**0.9994**	**0.0013**	0.9793	95.907	0.01221
	2.56	**0.4605**	**0.3680**	**0.4605**	**0.3680**	0.9877	97.568	0.00977
	6.25	**0.4930**	**0.3893**	**0.4930**	**0.3894**	0.9216	84.931	0.05279
	400	**0.4825**	**0.3943**	**0.4825**	**0.3943**	0.9868	97.614	0.01247
Hexan-1-ol	0.256	**0.9899**	**0.0021**	**0.3315**	**0.92357**	0.9756	95.046	0.03832
	2.56	**0.4984**	**0.3250**	**0.4984**	**0.3250**	0.9884	97.721	0.00872
	6.25	**0.5294**	**0.6566**	**0.5294**	**0.6566**	0.9155	83.819	0.04517
	400	**0.5813**	**0.9788**	**0.5813**	**0.9788**	0.7363	54.212	0.02446
cis-Hex-3-en-1-ol	0.256	**0.8898**	**0.0024**	**0.4353**	**0.7689**	0.9231	85.664	0.06423
	2.56	**0.5245**	**0.4418**	**0.5245**	**0.4418**	0.9927	98.058	0.00781
	6.25	**0.4945**	**0.9355**	**0.4945**	**0.9355**	0.9236	85.311	0.02124
	400	**0.4779**	**0.7546**	**0.4779**	**0.7546**	0.9832	97.578	0.01247
trans-Hex-2-en-1-ol	0.256	**0.9899**	**0.0032**	**0.3973**	**0.3177**	0.9616	92.461	0.02358
	2.56	**0.5734**	**0.3859**	**0.5734**	**0.3859**	0.9931	98.107	0.00577
	6.25	**0.4736**	**0.9656**	**0.4736**	**0.9656**	0.8242	67.924	0.04853
	400	**0.4904**	**0.7967**	**0.4904**	**0.7967**	0.9865	97.547	0.01351
2-Ethyl-hexan-1-ol	0.256	**0.4784**	**0.1109**	**0.4784**	**0.1109**	0.9446	89.233	0.01924
	2.56	**0.5413**	**0.2835**	**0.5413**	**0.2835**	0.9922	98.098	0.00712
	6.25	**0.4818**	**0.4189**	**0.4818**	**0.4189**	0.95464	91.133	0.03867
	400	**0.5934**	**0.4728**	**0.5934**	**0.4728**	0.9921	98.203	0.00439
trans,trans-Non-2,4-dienal	0.256	**0.6485**	**0.3395**	**0.8898**	**0.0041**	0.9668	92.742	0.01942
	2.56	**0.5277**	**0.3203**	**0.5277**	**0.3203**	0.9918	98.179	0.00455
trans,trans-Dec-2,4-dienal	0.256	**0.5156**	**0.0508**	**0.5156**	**0.0508**	0.9864	97.311	0.01391
	2.56	**0.4643**	**0.1482**	**0.4643**	**0.1482**	0.9878	97.598	0.01033
	6.25	**0.5219**	**0.3641**	**0.5219**	**0.3641**	0.9557	91.329	0.04179
	400	**0.4911**	**0.3165**	**0.4911**	**0.3165**	0.9869	97.687	0.00733

We found that absorption of olive oil volatiles onto PDMS and PA SPME fibres follow, in a good approximation, the general kinetic equation (2), especially in the case of the PA fibre. In the PDMS fibre the experimental data does not fit well to Eq. (2), suggesting that linearity between initial concentration and extracted amount is not achieved. Thus relative quantification is not possible with the PDMS fibre in a non-equilibrium state. With the PA fibre, a good fit was achieved with Eq. (2), which indicates that there is a linear relationship between the amount of analyte extracted (n), from olive oil headspace, and its initial concentration in the sample matrix (C_0) as a function of sampling time (t). This equation considers the equilibrium disturbance due to fibre introduction into the headspace of the sample, the equilibrium reposition and the variation of the compound concentrations in the sample headspace, which occurs between the disturbance and reposition time periods. For compounds that present c values equal to c_1 values (presenting both adjustment parameters in Eq. (2), which measure how fast the equilibrium is reached in HS-SPME) $(c - c_1) = 0$, meaning that the equilibrium is not disturbed, or the equilibrium reposition takes place immediately. For these compounds it is also verified that for α and β, which measure the variation of the concentration of a particular compound presented in the headspace, due to the equilibrium disturbance, $|\alpha| = |\beta|$. Hence the $a_h = 0$ and the parameter c in Eq. (2) is equal to a_h in Eq. (1).

The PA fibre allows, for the compounds studied, a successful relative quantification at a non-equilibrium state in non-aqueous media. Consequently, in non-aqueous media studies must be conducted to determine which compounds have similar behaviour and only those compounds can be quantified in a non-equilibrium situation with the PA fibre. For all the others, and for quantitative purposes, equilibrium should be reached.

4. Conclusion

The HS-SPME sample preparation technique coupled with GC analysis is well suited for qualitative and relative quantitative analysis of aroma compounds in olive oil. The amount (n) of analyte extracted, from olive oil headspace, might have a linear proportional relation with its initial concentration in the sample matrix (C_0) as a function of sampling time (t), which can be an advantage in the case of routine use. When a given compound in a given sample matrix exhibits this property, quantification can be achieved at non-equilibrium state, which reduces analysis time considerably. In all the other situations equilibrium should be reached for quantitative purposes.

Although it was observed that apolar PDMS-coated fibre is capable of extracting faster the olive oil aroma compounds for routine purposes in comparison with the PA fibre, since equilibrium is reached more quickly, the experimental data obtained with the PDMS fibre did not adjust to both mathematical models used. This indicates that linear proportionality between the initial concentration and amount extracted was not achieved, which does not allow relative quantification in non-equilibrium situations. On the other hand the PA fibre was found to be the adequate fibre to perform relative quantification of aroma compounds in a non-equilibrium state in a non-aqueous sample like olive oil.

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