Supporting Information

Headspace versus direct immersion solid phase microextraction in complex matrices: investigation of analyte behavior in multicomponent mixtures

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Section 1.1 – Chemicals and Materials

Analyte standards, of purity > 97 %, were obtained from Sigma-Aldrich (Oakville, Canada). HPLC grade methanol was obtained from Supelco (Oakville, Canada). Starch gels at different water percentages were prepared using unmodified starch purchased from Sigma-Aldrich (Oakville, Canada). Ultra-pure water was used in the preparation of all samples and starch gels.

Section 1.2 – Standards and Samples Preparation

Individual stock solutions (2.5 mg/mL) of each analyte were prepared in methanol and acetone. A stock mixture standard solution and subsequent working dilutions were prepared in methanol, and stored in a freezer at -30°C.

Section 1.3 - Rationale behind the selection of model analytes

In order to guarantee the relevance of this work to food applications, model analytes were carefully selected among several homologous groups of metabolites frequently encountered in food and environmental sample matrices, such as primary alcohols, 2-ketones, ethyl esters, terpene hydrocarbons, oxygenated terpenes, and aromatic compounds (Table S1). The compound selected to induce saturation of the coatings was alpha-pinene, a terpene hydrocarbon forming part of the secondary metabolism of most plant-derived foodstuffs, as well as a major contributing constituent of most essential oils[1]. This compound is characterized by high hydrophobicity (log P ≈ 4.4) and high affinity for the tested coatings, and is easily deductible from the fiber constants calculated in studies recently carried out.[2] Moreover, the high Henry's Law constant for this compound induces its rapid enrichment in the headspace above the sample, even at very mild extraction conditions and short extraction times.

Most common sample preparation protocols for food matrices prior to SPME sampling consist of crushing the biological tissue constituting the matrix and moving an aliquot of it in glass vials (10-

or 20-ml), where the extraction is performed in direct immersion or headspace mode. This procedure provokes the disruption of well-regulated biological compartments of the tissue and the consequent release of high concentrations of hydrophobic compounds that readily enrich the sample headspace. For this reason, saturation of the SPME coating and consequent displacement phenomena are most like to occur in these sampling conditions.

In light of all of these considerations, the created model system consists of three classes of compounds:

Class A: the saturating compound, alpha-pinene, that for the above described reasons, is well suitable to mimic the release of hydrophobic compounds when biological food tissue are disrupted prior the sampling.

Class B: easily displaceable compounds bearing low to medium polarity ($1 < \log P < 3$) and various functionalities: 1-pentanol, 2-hexanone, ethyl butanoate, benzaldehyde, acetophenone, benzene, and eucalyptol.

Class C: other hydrophobic compounds that have similar hydrophobicity to alpha-pinene and should be not be displaced: 1-undecanol, 2-undecanone, and ethyl nonanoate.

Section 1.4-Preparation of Starch Dispersions and Gels

Preparation of gelatinized systems at different concentrations (2.5, 5, 10 *w/w*) was carried out according to the procedure described by Lopes Da Silva et al.[3] Sample preparation for HS and DI dynamic extractions was accomplished by blending the gels systems and moving aliquots into individual vials. The samples for DI static extractions were prepared carrying out the gelatinization of the starch dispersion directly in-vial, directly followed by analysis of the starch gel.

Section 1.5-Instrumentation

A Hewlett Packard 6890/5973 gaschromatograph/mass spectrometer (GC-MS) equipped with a split/splitless injector and a CTC Combipal autosampler for automated SPME (CTC Analytics AG) was used.

The capillary column used for chromatographic separation was a J&W DB5-MS UI (30 m, 0.25 mm i.d., 0.25 μm film thickness). The column temperature program was initially set at 35 °C for 6 min, ramped at 20 °C/min to 140 °C, then ramped at 40 ºC/min to 260ºC , where it was held for 2 min, giving a total run time of 16.25 min. Helium was used as a carrier gas with its flow set at 1.2 ml/min. The mass spectrometer working conditions were as follows: electron ionization (EI) 70eV; mass range 50-350 m/z; ion source temperature 230 °C; quadrupole temperature 150 °C; transfer line temperature 280°C.

Section 1.6- Competitive adsorption: further considerations for complex matrices

Since SPME is an equilibrium based technique able to extract analytes in free form, every alteration in the binding equilibria between any compound and the matrix components will be reflected in the amount of analytes extracted onto the SPME coating. It is also important to mention that binding equilibria may involve not only the matrix itself, but also labware used to process the sample, inducing attachment of the most hydrophobic analytes and leading to false negative determinations. When multi-analytes systems, in particular complex matrices, need to be analyzed, particular attention must be paid to the formation of coating-related artefacts associated with swelling (liquid coatings) and saturation (solid coatings). In particular, saturations effects are quite challenging, as they can result in displacement effects when analytes and/or interference concentrations exceed the coating capacity. Conversely, investigation of saturation phenomena also provides insights over the interaction of analytes with the solid components of the matrix.

Competitive adsorption effects are also associated with solid SPME sorbents, which consist of porous particles suspended into a liquid polymer, such as PDMS. For these coatings, the number of sorption sites available for extraction determines the capacity of the coating and limits the amount of analytes that can be extracted. If sorption sites are substantially occupied, competition between analytes for the active coating surface occurs, where analytes with higher affinity for the coating will displace analytes bearing lower Kfs values.[4] A mathematical description of the phenomenon for a two compound system is presented below (Eq. 1), where B is the competing analyte towards the adsorption of compound A:[5]

$$C_{fA}^{\infty} = \frac{C_{fmax} K_A C_{sA}^{\infty}}{1 + K_A C_{sA}^{\infty} + K_B C_{sB}^{\infty}} \quad \text{(Eq. 1)}$$

Where C_{fA}^{∞} , C_{sA}^{∞} and C_{sB}^{∞} represent the equilibrium concentrations on the fiber and in the sample of the analytes A and B, respectively. K_A and K_B are the adsorption equilibrium constants for the analytes A and B, and C_{fmax} represents the maximum concentration of active sites on the coating.[5] From the equation above, the concentration of compound A is inversely dependent on the amount of compound B in the sample matrix. The influence of the competitive compound B becomes more prominent as the $K_B C_{sB}^{\infty}$ term increases. This implies that even in the presence of a low concentration of the competitive compound (B), adsorption of other compounds that have low affinity for the coating (low K_A) can be easily affected at equilibrium, provided that $K_A C_{sA}^{\infty} << K_B C_{sB}^{\infty}$.

An applicable example of such a system could be visualized when a plant-based tissue is crushed during the initial steps of sample preparation. For example, crushing of plant tissues may result in some native and well-regulated biological compartments being disrupted; in turn, this may lead to the release of high concentrations of compounds characterized by high affinities for the coating.

In other cases, the high amount of hydrophobic compounds released may induce phase separation when their free concentration exceeds their solubility in the water-based matrix. Under such circumstances, the HS is readily enriched with hydrophobic compounds, leading to saturation of the active sites of the solid porous coatings. Thus, compounds having a relatively higher solubility in water, lower Henry's law constants, and affinity for the coating (smaller $\boldsymbol{K_A}$) will be easily displaced from the surface of the coating. Relating to Eq. 1, displacement will thus be observed when the product of $\boldsymbol{K_A C_{sA}^{\infty}}$ is much lower than the summation ($\sum_1^n \boldsymbol{K_i C_{si}^{\infty}}$) of contribution of each potential competitive interfering analyte (*i*) present in the matrix; accordingly, in very complex mixtures, the extracted amount of the compound being displaced will vary with the concentration of the displacing compound, whether this is a matrix interference, another targeted analyte in the mixture, or a summation of these effects. Artefacts related to coating saturation and competitive adsorption may affect calibration procedures and also alter the extraction time profiles of the displaced analytes in a multicomponent system.[5,6]

However, in biological matrices in which the natural system has not been disrupted, the likelihood of competitive adsorption is negligible because of the high degree of binding to matrix components that characterize hydrophobic compounds[7]. Release of high concentrations of hydrophobic compounds during disruption into living tissue will likely cause disturbance of living processes, particularly when saturation of the matrix components occur.

Section 1.7- Evaluation of the composition and pH of different matrices

Other parameters related to matrix composition, such as water content percentage and pH were also evaluated in this work as potential factors affecting the occurrence of displacement phenomena, due to their influence on the amount of analyte extracted by the SPME coating[8].

For this purpose, three different starch/water ratios (w/w) were evaluated, taking into account the different water contents found in fruits and vegetables, ranging from 98 to 80%. Three different starch gels were prepared at 2.5, 5, and 10% of native starch powder in water. Extractions were performed for 30 minutes. Different amounts of starch in the system were shown to affect the diffusivity of the analytes in the media, especially for higher molecular weight compounds, which suggests a different kinetic of uptake on the coating that favors the adsorption of small polar molecules over larger hydrophobic ones. As presented in Table S4, such effect is especially remarkable for the matrix model containing a higher amount of starch. Regarding the experiments at pH 3.8 and 6.8, the obtained results differ in a range of 0.3 to 10%. Therefore, within the range tested, it can be assumed that pH has no remarkable effect on the inter-analyte displacement phenomenon. These results can be explained based on the chemical propreties of the analytes tested; as they are not easily ionizable compounds, pH changes are not expected to influence their uptake on the SPME coating.

Moreover, the obtained results point out that particularly for *in vivo* simulated conditions, the phenomenon is not critically affected by changes in matrix composition. This factor leads to undisputed advantages for the analyst, as significant physiological variability is often encountered in the composition of food matrices due to differences such as cultivation style and ripening grade, among other factors.

Section 1.8- Evaluation of matrix saturation

Generally, saturation of a sorbent by a compound or a group having high affinity for the coating manifests in non-linear adsorption of other analytes extracted. In order to verify if the large amount of α-pinene spiked in the starch gel saturates the matrix itself, a series of extractions at a short extraction time (2 minutes) were performed increasing the amount of α-pinene spiked (166, 332,

1663, 16628 μg L^{-1}), in a similar fashion that was used to induce SPME coating saturation. This strategy allows for the prevention of saturation of the SPME coating, thus monitoring the actual distribution of the analytes in the matrix at varying concentrations of α-pinene. Results shown in Figure S4 reveal that non-linear adsorption was encountered even at such short extraction times, implying that the spiked amounts of α-pinene saturated the matrix. It is interesting to notice that for the most hydrophobic compounds, namely ethyl nonanoate, 2-undecanone, and 1-undecanol, the obtained response can be considered statistically equal at different amounts of spiked α-pinene.; this behavior, interestingly, resembles what was already discovered in previous coating saturation experiments. This finding reaffirms the applicability of solid phase microextraction as a tool for monitoring living systems, since the behavior of the matrix is reflected on the coating. Similar trends were observed when performing the same set of experiment using a liquid coating (PDMS 100μm), thus confirming that deviation from linearity is exclusively related to matrix saturation (Fig. S5).

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Table S1: Target metabolites and their physicochemical properties: molecular weight[9], log Kow[9], boiling point[9], EI quantification ion, molecular structures[9].

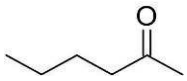
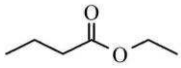
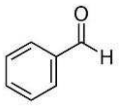
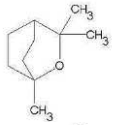
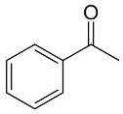
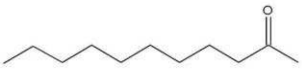
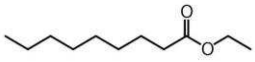
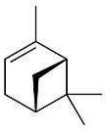
Analyte	MW (g/mol)	log K_{ow}	B.P. (°C)	Henry's Law constant ($atm*m^3/mol$)	Quantitation ion (m/z)	Molecular structure
benzene	78.11	2.0	78.8	$5.39*10^{-3}$	78	
1-pentanol	88.15	1.3	136.9	$1.33*10^{-5}$	55	
2-hexanone	100.16	1.3	118.7	$9.30*10^{-5}$	58	
ethyl butanoate	116.16	1.8	125.7	$4.10*10^{-4}$	88	
benzaldehyde	106.12	1.5	178.1	$1.34*10^{-5}$	105	
eucalyptol	154.25	2.8	174.0	$2.04*10^{-4}$	154	
acetophenon	202.00	1.6	201.9	$9.81*10^{-6}$	105	
2-undecanone	170.30	4.1	224.1	$4.78*10^{-4}$	58	
ethyl nonanoate	186.30	4.3	229.7	$1.69*10^{-3}$	88	
1-undecanol	172.31	4.2	256.2	$7.26*10^{-5}$	55	
alpha-pinene	136.24	4.4	157.3	$1.07*10^{-1}$	93	

Table S2: Comparison between the tested solid coatings in terms of relative amounts displaced for HS extraction, carried out at the same working conditions (60 minutes extraction, 35°C, 500 rpm). Extractions were performed from an aqueous solution containing :α-pinene, 11069 µg L^{-1} benzene 14.9 µg L^{-1}, 1-pentanol 664 µg L^{-1} , 2-hexanone and ethyl butanoate 66.5 µg L^{-1}, eucalyptol 83.1 µg L^{-1}, acetophenone and benzaldehyde 133 µg L^{-1}, 2-undecanone, ethyl nonanoate and 1-undecanol 6.6 µg L^{-1}. Relative amount displaced was calculated considering amounts extracted at the lowest amount of spiked α-pinene (8.3 µg L^{-1}).

analyte name	HS extractions		
	PDMS/DVB*	DVB/Car/PDMS*	Car/PDMS*
benzene	42.4	36.1	3.0
1-pentanol	58.5	48.0	9.4
2-hexanone	63.5	47.0	7.5
ethyl butanoate	62.9	49.5	3.1
benzaldehyde	34.3	5.8	4.1
eucalyptol	84.3	81.8	81.8
acetophenon	21.3	6.3	4.9
2-undecanone	n.s.d**	n.s.d**	n.s.d**
ethyl nonanoate	n.s.d**	n.s.d**	n.s.d**
1-undecanol	n.s.d**	n.s.d**	n.s.d**

***: results expressed as relative percentage of compound displaced, calculated from the nanograms extracted**

****: no statistical differences were noted in a t-test analysis of the extracted amount results**

Table S3: Differences in displacement occurrence in direct immersion (DI) and headspace (HS) mode for DVB/Car/PDMS and Car/PDMS coatings. Extractions were performed from an aqueous solution containing :α-pinene, 11069 μg L^{-1} benzene 14.9 μg L^{-1}, 1-pentanol 664 μg L^{-1} , 2-hexanone and ethyl butanoate 66.5 μg L^{-1}, eucalyptol 83.1 μg L^{-1}, acetophenone and benzaldehyde 133 μg L^{-1}, 2-undecanone, ethyl nonanoate and 1-undecanol 6.6 μg L^{-1}. Relative amount displaced was calculated considering amounts extracted at the lowest amount of spiked α-pinene (8.3 μg L^{-1}).

	DVB/Car/PDMS*		Car/PDMS*	
Analytes	DI	HS	DI	HS
benzene	n.s.d**	13.13	n.s.d**	n.s.d**
1-pentanol	n.s.d**	18.15	n.s.d**	10.12
2-hexanone	n.s.d**	11.47	n.s.d**	7.15
ethyl butanoate	n.s.d**	9.67	n.s.d**	9.37
benzaldehyde	n.s.d**	8.68	n.s.d**	n.s.d**
eucalyptol	n.s.d**	10.52	n.s.d**	37.54
acetophenon	n.s.d**	9.15	n.s.d**	n.s.d**
2-undecanone	n.s.d**	n.s.d**	n.s.d**	n.s.d**
ethyl nonanoate	n.s.d**	n.s.d**	n.s.d**	n.s.d**
1-undecanol	n.s.d**	n.s.d**	n.s.d**	n.s.d**

***: results expressed as relative percentage of compound displaced, calculated from the nanograms extracted after 15 minutes of extraction**

****: no statistical differences were noted in a t-test analysis of the extracted amount results**

Table S4: Evaluation of different extraction modes for different starch gel systems (extraction time 30 minutes). Individual concentrations of analytes were properly adjusted to guarantee adequate sensitivity (benzene 99.6 μg kg^{-1},1-pentanol 1329 μg kg^{-1} , 2-hexanone and ethyl butanoate 79.8 μg kg^{-1}, eucalyptol 99.7 μg kg^{-1}, acetophenon and benzaldehyde 199 μg kg^{-1} 2-undecanone, ethyl nonanoate and 1-undecanol 166 μg kg^{-1}, α-pinene 16628 μg kg^{-1}. Relative amount displaced was calculated considering amounts extracted at the lowest amount of spiked α-pinene (8.3 μg L^{-1}).

Analyte	2.5% (*w/w*)			5% (*w/w*)			10% (*w/w*)		
	DI-static	DI-dynamic	HS	DI-static	DI-dynamic	HS	DI-static	DI-dynamic	HS
benzene	12.40	39.29	49.20	n.s.d**	26.3	48.0	n.s.d**	17.1	36.8
1-pentanol	24.00	27.59	44.20	n.s.d**	24.1	57.4	n.s.d**	13.1	52.4
2-hexanone	n.s.d**	33.37	50.00	n.s.d**	25.5	48.5	n.s.d**	23.7	47.0
ethyl butanoate	n.s.d**	33.84	55.60	n.s.d**	20.9	49.9	n.s.d**	20.1	42.8
benzaldehyde	n.s.d**	24.05	34.40	n.s.d**	13.0	29.6	n.s.d**	10.3	29.1
eucalyptol	n.s.d**	38.30	76.20	n.s.d**	32.7	70.2	n.s.d**	26.0	66.1
acetophenon	n.s.d**	24.29	31.40	n.s.d**	10.7	22.7	n.s.d**	8.8	17.7
2-undecanone	n.s.d**	n.s.d**	n.s.d**	n.s.d**	n.s.d**	n.s.d**	n.s.d**	n.s.d**	n.s.d**
ethyl nonanoate	n.s.d**	n.s.d**	n.s.d**	n.s.d**	n.s.d**	n.s.d**	n.s.d**	n.s.d**	n.s.d**
1-undecanol	n.s.d**	n.s.d**	n.s.d**	n.s.d**	n.s.d**	n.s.d**	n.s.d**	n.s.d**	n.s.d**

***: results expressed as relative percentage of compound displaced, calculated from the nanograms extracted after 15 minutes of extraction**

****: no statistical differences were noted in a t-test analysis of the extracted amount results**

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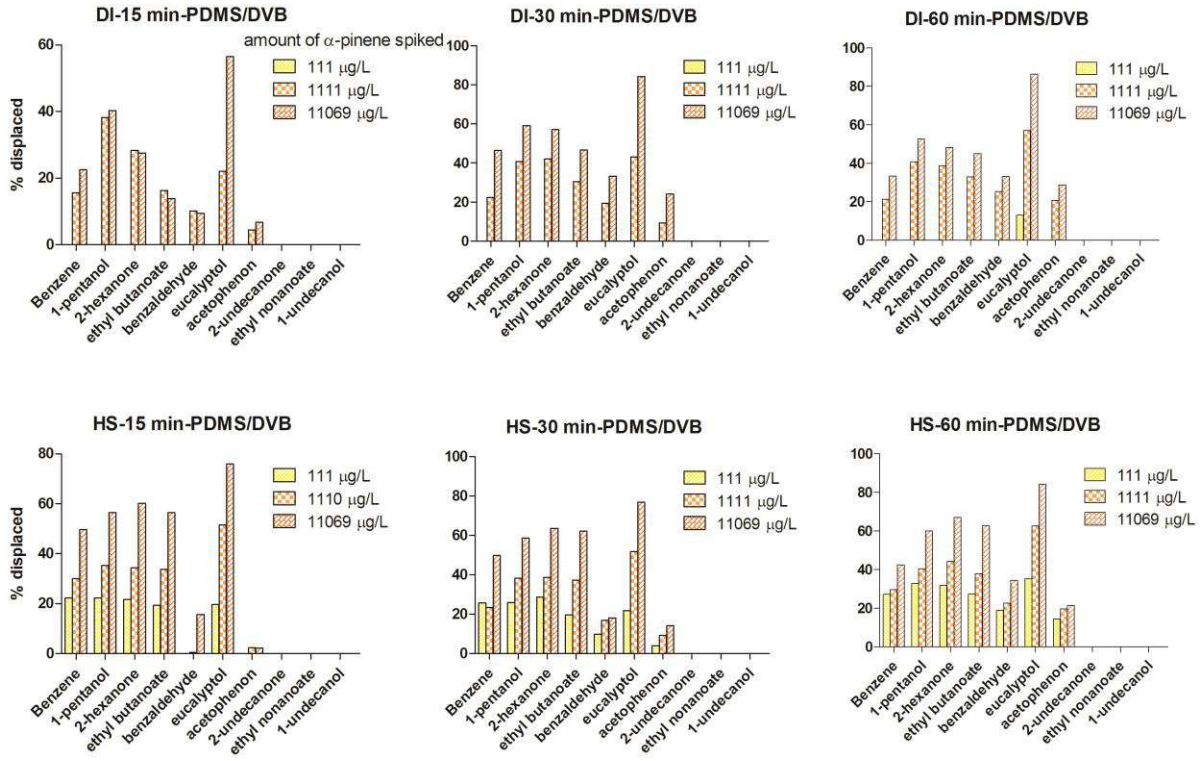

Figure S1: Comparison of trends of inter-analyte displacement at different extraction times (15, 30, 60 minutes) for PDMS/DVB coating in DI and HS modes. Extractions were performed at 35°C and 500 rpm from an aqueous solution containing varying concentrations of α-pinene (111, 1111, 11069 µg L^{-1}) and constant concentrations of analytes (benzene 14.9 µg L^{-1}, 1-pentanol 664 µg L^{-1}, 2-hexanone and ethyl butanoate 66.5 µg L^{-1}, eucalyptol 83.1 µg L^{-1}, acetophenone and benzaldehyde 133 µg L^{-1}, 2-undecanone, ethyl nonanoate and 1-undecanol 6.6 µg L^{-1}). Error bars represent ± standard deviation obtained for three replicates.

The results are expressed as a percentage of analyte displaced relative to extractions where α-pinene was spiked at 8.3 µg L^{-1}.

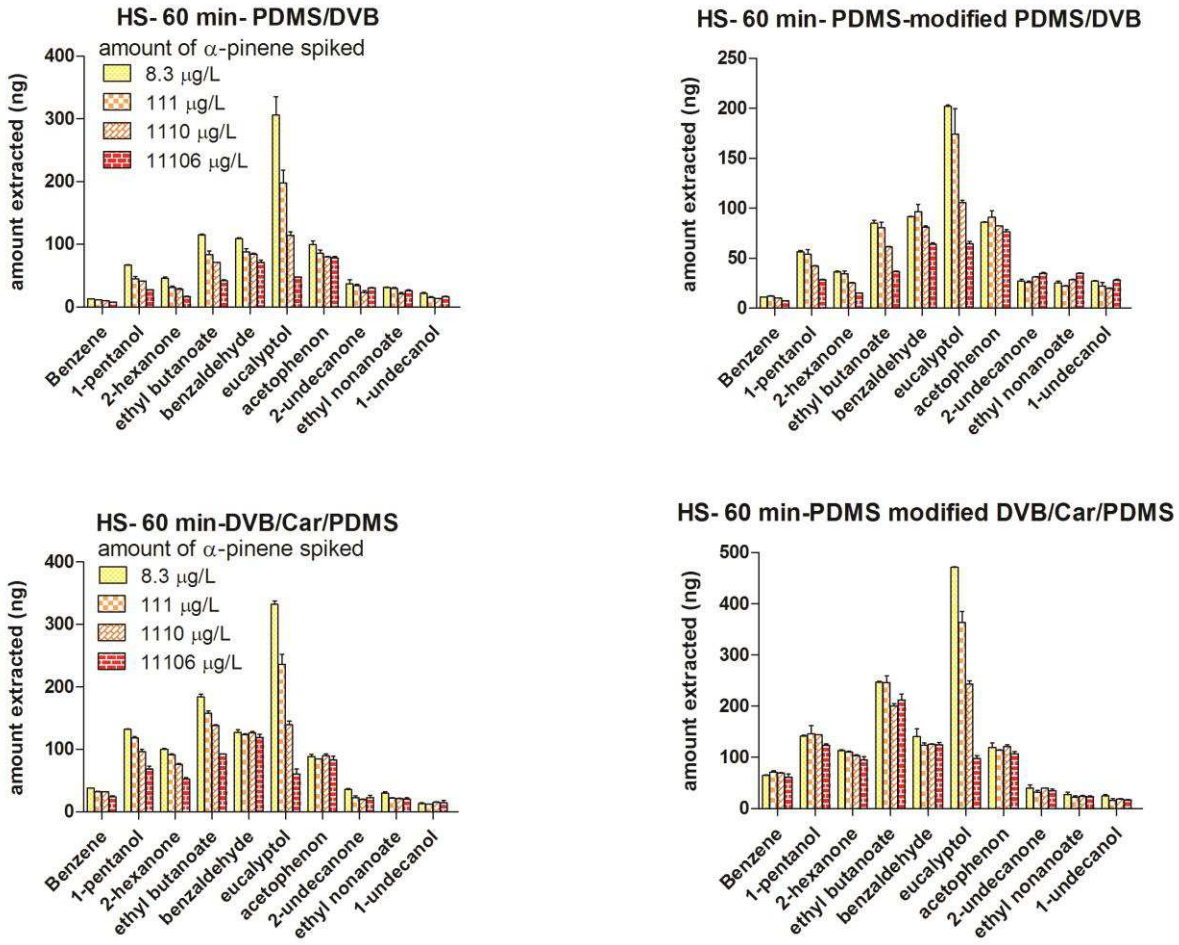

Figure S2: Comparison of commercial coatings and PDMS-modified coatings for displacement occurrence in HS mode. Extractions were performed at 35°C and 500 rpm from an aqueous solution containing varying concentrations of α-pinene (8.3, 111, 1111, 11069 µg L^{-1}) and constant concentrations of analytes (benzene 14.9 µg L^{-1}, 1-pentanol 664 µg L^{-1} , 2-hexanone and ethyl butanoate 66.5 µg L^{-1}, eucalyptol 83.1 µg L^{-1}, acetophenone and benzaldehyde 133 µg L^{-1}, 2-undecanone, ethyl nonanoate and 1-undecanol 6.6 µg L^{-1}). Error bars represent ± standard deviation obtained for three replicates.

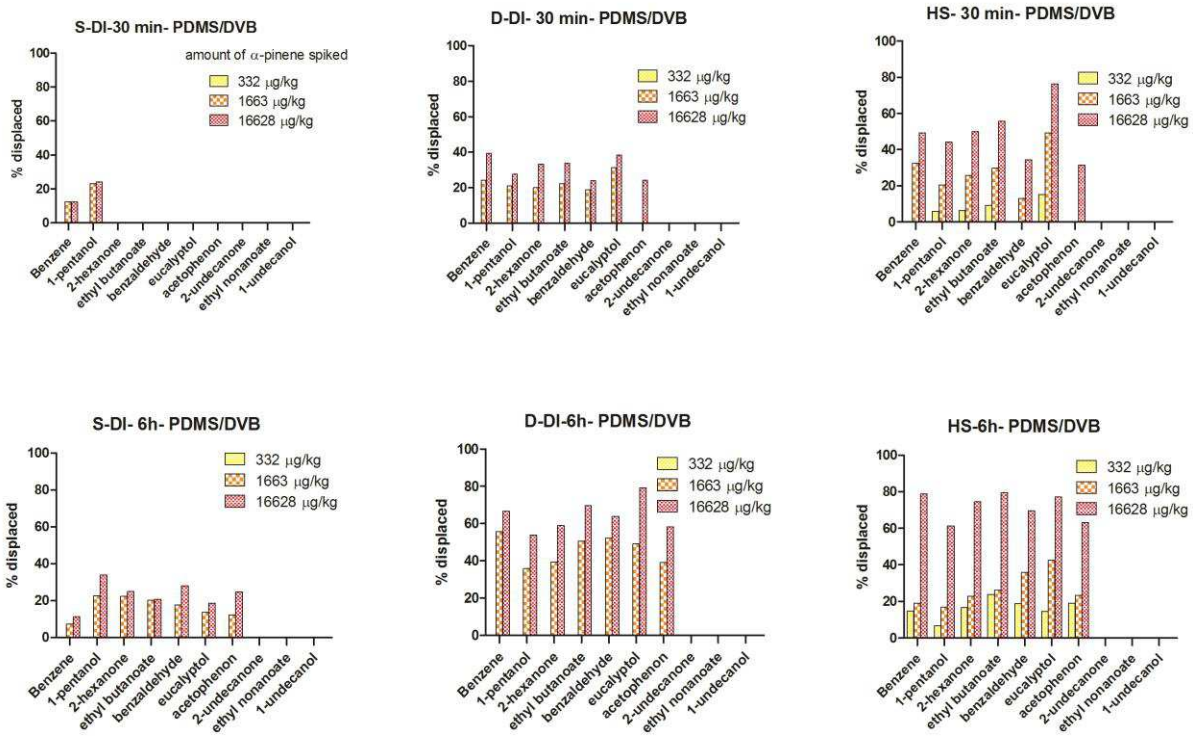

Figure S3. Comparison of relative amounts displaced in different extraction modes at pre-equilibrium (30 minutes) and equilibrium (6 hours) conditions for static starch gel system 2.5% (w/w). Starch gel was spiked at the following concentrations: benzene 33.2 µg kg^{-1},1-pentanol 665 µg kg^{-1} , 2-hexanone and ethyl butanoate 66.5 µg kg^{-1}, eucalyptol 83.1 µg kg^{-1}, acetophenon and benzaldehyde 133 µg kg^{-1} 2-undecanone, ethyl nonanoate and 1-undecanol 166 µg kg^{-1}, α-pinene 332, 1663 and 16628 µg kg^{-1}. The results are expressed as a percentage of analyte displaced relative to extractions where α-pinene was spiked at 166 µg L^{-1}.

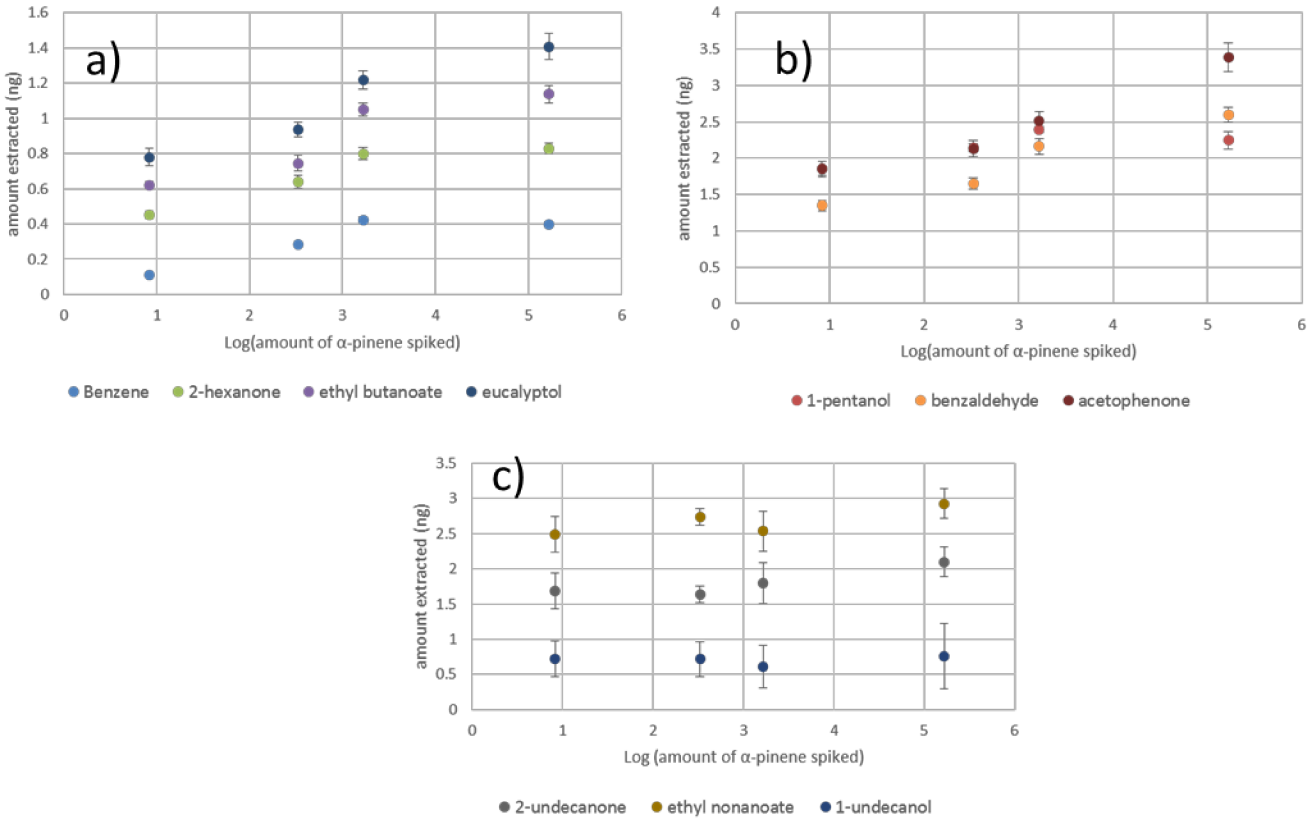

Figure S4 Extraction profiles for a) benzene, 2-hexanone, ethyl butanoate, and eucalyptol, b) 1-pentanol, benzaldehyde, and acetophenone, c) 2-undecanone, ethyl nonanoate, and 1-undecanol acquired in starch gel by PDMS/DVB coating at increasing amounts of spiked α-pinene (166, 332, 1663, 16628 µg kg^{-1}). Extractions were carried out at 35°C and 500 rpm for 2 minutes in direct immersion from starch gel enriched with the following amounts of analytes: benzene 33.2 µg kg^{-1}-,1-pentanol 665 µg kg^{-1} , 2-hexanone and ethyl butanoate 66.5 µg kg^{-1}, eucalyptol 83.1 µg kg^{-1}, acetophenon and benzaldehyde 133 µg kg^{-1} 2-undecanone, ethyl nonanoate and 1-undecanol 166 µg kg^{-1}

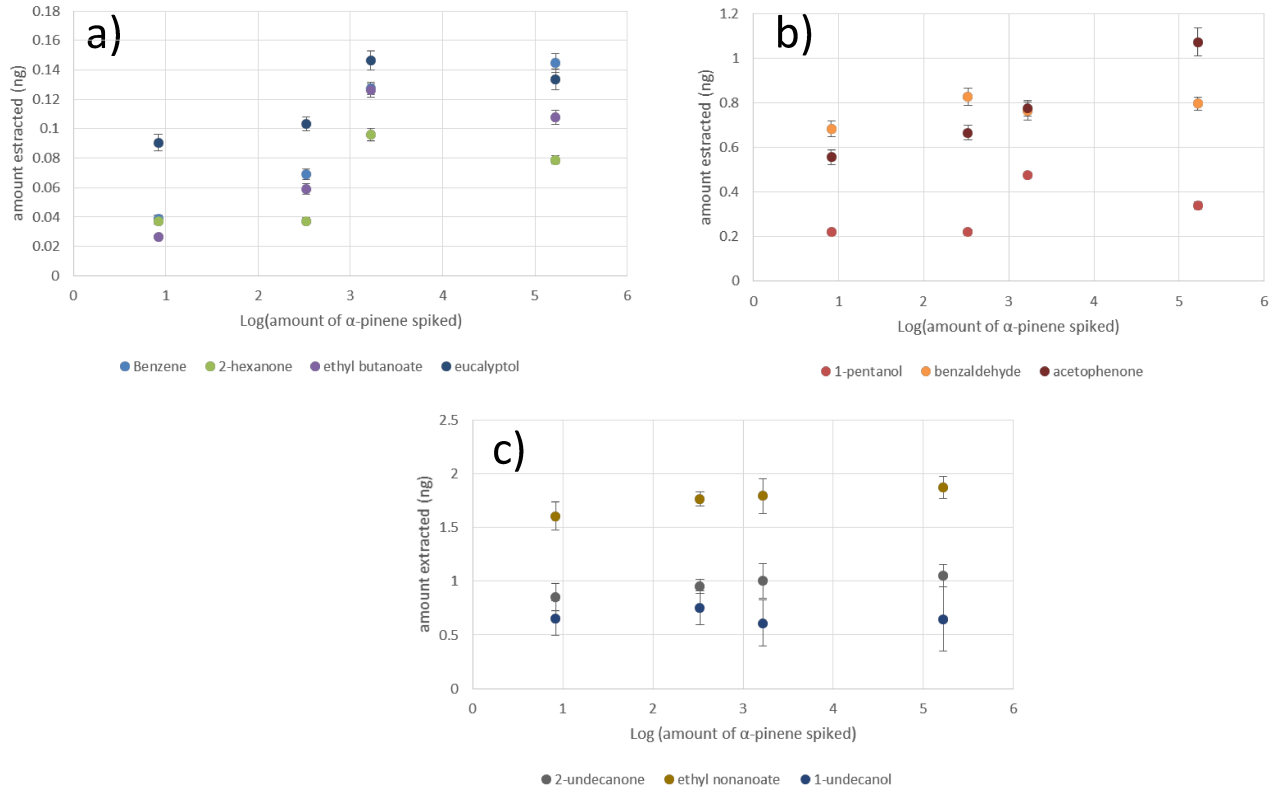

Figure S5: Extraction profiles acquired for a) benzene, 2-hexanone, ethyl butanoate and eucalyptol, b) 1-pentanol, benzaldehyde and acetophenone, c) 2-undecanone, ethyl nonanoate and 1-undecanol in starch gel by PDMS(100µm) coating at increasing amounts of spiked α-pinene (166, 332, 1663, 16628 µg kg^{-1}). Extractions were carried out at 35°C and 500 rpm for 2 minutes in direct immersion from starch gel enriched with the following amounts of analytes: benzene 33.2 µg kg^{-1}-,1-pentanol 665 µg kg^{-1} , 2-hexanone and ethyl butanoate 66.5 µg kg^{-1}, eucalyptol 83.1 µg kg^{-1}, acetophenon and benzaldehyde 133µg kg^{-1} 2-undecanone, ethyl nonanoate and 1-undecanol 166 µg kg^{-1}

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