Fractionated dynamic headspace sampling in the analysis of matrices of vegetable origin in the food field

FRACTIONATED DYNAMIC HEADSPACE SAMPLING IN THE ANALYSIS OF MATRICES OF VEGETABLE ORIGIN IN THE FOOD FIELD.

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Abstract

Recent technological advances in dynamic headspace sampling (D-HS) and the possibility to automate this sampling method have lead to a marked improvement in its the performance, a strong renewal of interest in it, and have extended its fields of application. The introduction of in-parallel and in-series automatic multi-sampling and of new trapping materials, plus the possibility to design an effective sampling process by correctly applying the breakthrough volume theory, have make profiling more representative, and have enhanced selectivity, and flexibility, also offering the possibility of fractionated enrichment in particular for high-volatility compounds. This study deals with fractionated D-HS ability to produce a sample representative of the volatile fraction of solid or liquid matrices. Experiments were carried out on a model equimolar (0.5 mM) EtOH/water solution, comprising 16 compounds with different polarities and volatilities, structures ranging from C5 to C15 and vapor pressures from 4.15 KPa (2,3-pentandione) to 0.004 KPa (t-β-caryophyllene), and on an Arabica roasted coffee powder. Three trapping materials were considered: Tenax TA^{TM} (TX), Polydimethylsiloxane foam (PDMS), and a three-carbon cartridge Carbopack B/Carbopack C/Carbosieve S-III^{TM} (CBS).

The influence of several parameters on the design of successful fractionated D-HS sampling. including the physical and chemical characteristics of analytes and matrix, trapping material, analyte breakthrough, purge gas volumes, and sampling temperature, were investigated. The results show that, by appropriately choosing sampling conditions, fractionated D-HS sampling, based on component volatility, can produce a fast and representative profile of the matrix volatile fraction, with total recoveries comparable to those obtained by full evaporation D-HS for liquid samples, and very high concentration factors for solid samples.

Keywords: Dynamic headspace, volatile fraction fractionation, representative profile, volatility and vapor pressure, GC-MS, vegetable matrices

1. Introduction

Modern strategies of analysis are increasingly based on the Total Analysis System (TAS) approach introduced by Manz et al. in 1990. These are strategies in which the three main steps of an analytical procedure (sample preparation-analysis-data processing) are combined on-line and merged into a single step. [1-2]. The increasing popularity of this approach, together with the introduction of integrated analysis systems, has also contributed to the development of new techniques and/or to the renewed interest in others. The latter is true of headspace sampling in cases in which the volatile fractions of matrices from different fields (flavor, fragrance, food, environment etc.) must be analyzed. There are two main approaches to headspace sampling: static and dynamic. [3] Static headspace (S-HS) is simple, easy to automate, and reliable, but limited by relatively low analyte concentration factors; vice versa, dynamic headspace (D-HS) is characterized by high flexibility and analyte concentration factors, but it requires more complex and expensive technologies and is less easily applied to quantitative analysis. The limits in concentration rate of static headspace were almost completely overcome in the early 1990s, with the development of high concentration capacity static headspace techniques (HS-SPME, HSSE, HS-STE, HS-SMSE, HS-SDME, etc.); these are techniques in which the analytes in the vapor phase are accumulated onto a stationary phase by (ab)sorption or adsorption [4-8]. Advances in S-HS have restricted the use of D-HS to well-established applications and analysis, in spite of its wide possibilities.

Dynamic headspace sampling (D-HS), also known as *purge and-trap,* is a non-equilibrium *continuous gas extraction* technique [3] first introduced by Wahlroos in 1963 [9]. A number of approaches are available for D-HS sampling, including: a) the best known and currently used is *breakthrough sampling,* in which analytes of interest are transferred to the trapping material until the first analyte of interest starts to elute from the trap; b) *Full Evaporation D-HS (FED-HS),* in which the volatiles of a liquid matrix are fully evaporated into the vial to obtain a representative sample of the volatile fraction (i.e. without matrix effect) and transferred to the trapping material [10-11], c) *Equilibrium D-HS sampling* or *Equilbrium Gum Phase Extraction (EGPE)* with trapping material operating by sorption, in which all compounds are continuously extracted until they achieve equilibrium with the sorptive extractant, and are independently partitioned into it without displacement effects [12]; d) the very recent *Multi-volatile method (MVM),* in which the headspace components are sequentially sampled with a number of traps filled with different trapping materials and/or under different conditions. The traps are then sequentially desorbed in the same cryotrap and the collected analytes are transferred, all together and on-line, to a GC-MS system [13].

In addition to the improvement of conventional applications, there are other and equally stimulating fields in which modern D-HS can successfully be applied. One is the extension of the MVM-D-HS

method combined with the full evaporation method for the injection of milliliter volumes, proposed by Ochiai et al. in 2014; they applied the system to green tea [14]. MVM-D-HS has successfully also been used to achieve exhaustive sampling of the volatile fraction of a liquid matrix (coffee brew), minimizing component discrimination due to their different volatilities [13]. When sampling matrices whose volatile fraction consists of components having a wide range of volatilities and polarities (e.g. coffee, cocoa, tea, etc.), compromise sampling conditions (trapping material, temperature and gas volumes) are in general adopted. However, this produces some discrimination due either to breakthrough of the most volatile compounds, because of an excessive volume of transfer gas used, or to the incomplete transfer of the less volatile components to the trapping material, because of an insufficient volume of transfer gas. The latter case is particularly true of solid matrices, because release is also conditioned by the texture of the matrix. A possible solution would be to fractionate D-HS sampling by carrying it out stepwise with trapping material and under conditions tuned for the optimal recovery of components in function of their volatility and polarity. This approach can lead to an on-line sequential sampling method, in which compounds with different volatilities are sampled in separate steps.

This study reports the results of a series of experiments aimed at achieving fractionated D-HS sampling, exploiting a modern automatic sampling assembly.

2. Experimental

2.1. Materials

Three different trapping materials were tested: Tenax TA™ (TX), Polydimethylsiloxane foam (PDMS), and a three-carbon cartridge Carbopack B/Carbopack C/Carbosieve S-III™ (CBS) supplied by Gerstel (Germany). Carboxen/divinylbenzene/PDMS (CAR/DVB/PDMS) SPME fiber was from Supelco Co. (Bellafonte, PA, USA). Before use, the fiber was conditioned as recommended by the manufacturer.

Pure standard samples of 2,3 pentandione, 2-methtylbutanal, 2-methylbutanol, 2,5-dimethyl pyrazine, thymol, α terpinene, eucalyptol, octanal, limonene, 2-pentylfuran, hexanal, *E*-2-octenal, linalyl acetate, linalool, *E*-2-decenal, *trans*-β-caryophyllene were from Sigma Aldrich (Milan, Italy). Solvents (ethanol 96%) were all HPLC-grade from Sigma Aldrich (Milan, Italy). Experiments were carried out on an equimolar 0.5 mM solution in 9:1 EtOH/water (v:v) of the above model components.

Coffee samples 100% Arabica (*Coffea arabica* L.) consisting of roasted ground coffee suitable for a coffee-filter machine, were kindly supplied by Lavazza Srl (Turin, Italy).

2.2.Sampling conditions

Optimized sampling conditions adopted to evaluate breakthroughs, and to analyze the standard solution and coffee powder, are reported in **Table 1**. Measurements were taken on the three traps used, with sample agitation at 500 rpm for 10s; transfer heater was set to 150°C.

Breakthrough ranges were determined by applying an increasing volume of purge gas (20, 100, 500, 1000, 2500 and 5000 mL) to different 20 mL vials containing 50 µL of the model solution. The analyte concentration capability of trapping materials was measured by consecutive extractions of the same vial with increasing purge gas volumes.

2.3. Analysis conditions

Analyses were carried out with a MPS-2 multipurpose sampler equipped with a DHS module (Gerstel, Mülheim a/d Ruhr, Germany) installed on an Agilent 7890A GC unit coupled to an Agilent 5975C MSD (Agilent, Little Falls, DE, USA). This configuration can run either conventional HS-SPME or Dynamic headspace analysis. In particular, the dual-needle design of the DHS system provides dynamic transfer of the headspace to a suitable adsorbent/sorbent. The trapped analytes were subsequently thermally desorbed using a TDU thermal desorption unit equipped with a CIS 4 programmed temperature vaporizing (PTV) inlet.

CIS temperature program: from -50°C to 250°C at 12°C/s; hold time at final temperature: 5 min; equilibration time: 0.10 min; initial time: 0 min.

TDU temperature program: from 30°C to 250°C at 60°C/min; hold time at final temperature: 5 min; delay time: 0 min; initial time: 0.10 min, splitless modalities.

GC conditions: injector temperature: 250°C, injection mode: split, ratio: 1/20; carrier gas: helium, flow rate: 1 mL/min; column: Mega 8-10%-phenyl –aryl polysiloxane column 60 m×0.25 mm d_c ×2.0 µm d_f, from MEGA (Milan, Italy). Temperature program: from 50°C (1 min) to 250°C (5 min) at 5°C min^{-1}.

MSD conditions: MS operated in EI mode (70 eV), scan range: 35 to 350 amu; target ions selected for quantitation are reported in **Table 2**; ion source temperature: 230°C; quadrupole temperature: 150°C; transfer line temperature: 280°C. Analytes were identified by comparing their mass spectra and linear retention indices to those of authentic standards.

3. Results and discussion

These results are the first part of a study to determine the parameters that condition the success of fractionated D-HS sampling. Several factors, which are very often closely inter-related, condition the success of fractionated D-HS sampling, besides, of course, the physical and chemical characteristics of matrix and analytes. They include trapping material, analyte breakthrough, purge

gas volumes, and sampling temperature. In particular, the temperature strongly influences headspace composition, while the chemical nature of the trapping material conditions analyte recoveries and affects their breakthrough volume, thus also affecting the purge-gas volume and flow to be applied. Analyte breakthrough volume is very critical in D-HS sampling when a headspace profile that is truly representative of the investigated matrix in quali-quantitative terms must be obtained. Therefore, having selected the trapping material(s), the fractionation chiefly depends on a) the sampling temperature, which conditions the analyte's release from matrix to headspace (i.e. the HS composition), and b) the purge gas volume that produces the highest recovery, although it must not be so abundant as to induce breakthrough from the trap(s).

The experiments were carried out on a model sample consisting of an equimolar EtOH/water solution (0.5 mM) of 16 compounds ranging from C5 to C15, with different volatilities and polarities. The 16 components are listed in **Table 2**, together with their vapor pressures and boiling points [15]. Three trapping materials were tested, i.e. Tenax TA (TX), Polydimethylsiloxane (PDMS), and a three-carbon cartridge (Carbopack B/Carbopack C/Carbosieve S-III (CBS)). All experiments on the EtOH/water model solution with CBS cartridge required a cartridge dry purge step with nitrogen, to avoid water interference with analyte recoveries because of the water/carbon interaction; experimental conditions derived from those reported by Ochiai et al. were applied [12-14]. GC-MS analyses were carried out on an 8-10%-phenyl–aryl polysiloxane thick film column (2.0 µm) in order to increase retention of the most volatile components, so as to facilitate their separation and detection.

3.1. Fractionated D-HS and breakthrough volume

The breakthrough volume of each component in the model sample on the trapping materials tested was first investigated. Several approaches are available to determine this [16-18]. In this study, the components of the model sample were grouped within six ranges of purge gas volume (**Table 2**) at which their breakthrough takes place with each trapping material, since the aim of this study was to fractionate the headspace, and not to recover one or more target analytes. The breakthrough range of each analyte was determined assuming that the purge gas volume at which its area begins to decrease after achieving maximum value indicates its breakthrough. These experiments were carried out in D-HS mode at 20 mL/min and 75°C under full evaporation (FE) conditions, to avoid possible discrimination between analytes because of their different volatilities. **Figure 1** reports the trend of peak area of 2,3-pentandione and that of thymol on a Tenax TA trap, *versus* the six gas volumes after FED-HS sampling at 75°C. The results in **Table 2** are the means of three experiments, and clearly show that, as expected, breakthrough volume depends on the trapping

material, but also that a given trapping material cannot provide the simultaneous full recovery of analytes with widely differing volatilities in a single step. Such wide ranges are very common in the volatile fraction compositions of many real-world matrices. The 16 compounds investigated presented breakthrough volumes within six different ranges of purge-gas volume. As expected, the least retentive material was PDMS, with a maximum breakthrough volume of many components in the 500-1000 mL range of purge gas; conversely, the most retentive phase was CBS, which released several components after 5000 mL [13]. The results reported for some compounds are in full agreement with those found in the literature [16]. Moreover, they indicate that PDMS can successfully be used for low-volatility components and CBS for high-volatility components, which are poorly retained by the other materials.

3.2. Fractionated D-HS and recovery

A series of experiments were carried out to evaluate the optimal sampling conditions, offering recoveries capable of providing a representative sample profile. Experiments were carried out by sequentially sampling the model solution at 50°C from the same HS vial with the three trapping materials at two different purge gas speeds (20 and 100 mL/min) and with four gas volumes, i.e. 20, 100, 1000 and 5000 mL. The temperature of 50°C was chosen as being a good compromise, providing a representative HS while minimizing artifact formation with real-world samples; further, this temperature may be adopted when D-HS sampling is used on a solid matrix, where the full evaporation approach cannot be applied. Conversely, a temperature of 75°C is not sufficiently discriminative, since analytes are not selectively vaporized (paragraph 3.4), while 35°C produces severe discrimination of low-volatility components. CBS traps were submitted to dry-purge to eliminate water, with a fixed volume of 300 mL of gas [13].

As a preliminary, the purge gas speed was investigated. The 100 mL/min flow-rate was abandoned, because of the decidedly low recovery achievable with all analytes. This is presumably because this speed is too high, and interferes with correct analyte trapping. All subsequent experiments were therefore carried out at 20 mL/min.

3.2.1 Recovery - Recovery depends on the trapping material and the characteristics of the analyte(s) (i.e. vapor pressure and boiling point, and solubility for liquid samples) and it is closely connected with analyte breakthrough volume. In this study, recovery was determined on the components of the model sample following the method described by Ochiai et al. [13]. 50µL of the EtOH/water model-solution was sampled at 50°C with the three trapping materials, which were maintained at 40°C, with four purge volumes (i.e. 20, 100, 1000 and 5000 mL) taking into account the breakthrough volumes reported in **Table 2**. The recovery was calculated from the peak areas

obtained with D-HS experiments by external calibration [13]. The calibration curves were constructed via direct liquid injection into the TDU of standard solutions of five concentrations of each analyte, in the range 5-35 mM in EtOH. Maximum recovery was with the CBS trap because, under the sampling conditions applied, analyte breakthrough occurred only marginally, if at all (**Table 2**). **Figure 2A** reports recoveries of each analyte on the CBS trap and % distribution achieved with the purge volumes applied. CBS recoveries ranged between 103% for 2,3 pentandione (**1**) and 84% for 2-pentylfuran (**11**). The higher recovery of linalyl acetate (**13**, 90%), *E*-2-decenal (**15**, 86%) and *t*-β-caryophyllene (**16**, 92%) than of linalool (**14**, 85%) is most probably related to their different solubilities in EtOH/water, which influences their release from liquid to vapor phase, and/or to their partial non-reversible interaction with CBS [19-20]**.** For most analytes, the highest recoveries were obtained within the first 1000 mL of purge gas; the contribution of the 5000 mL volume to recovery was thus only significant for *E*-2-decenal (**15**) and *t*-β-caryophyllene (**16**).

Under the same conditions, as expected, Tenax TA showed breakthrough at high purge volumes for some analytes, due to its lower retention. For each analyte, **Figure 2B** reports the percentage recovery referred to CBS, taken as 100%**.** Recoveries with the Tenax TA trap ranged from 41% for *E*-2-decenal (**15**) to 85**%** for 2-methylbutanol (**4**) and α-terpinene (**7**). This reduction was expected because, under the conditions applied, complete transfer of most analytes to the vapor phase requires some time and, as a consequence, adequate purge-gas volumes is delivered and exceed the breakthrough volumes of some analytes on this material. For these reasons, the largest purge volume (5000 mL) did not significantly enhance the recovery of most analytes, because breakthrough occurred at the same time.

Recoveries achieved with PDMS were decidedly lower (**figure 2B**); compared to CBS, values ranged from 21% for 2,3 pentandione (**1**) to 61% for limonene (**13**). PDMS is affected by more serious analyte breakthrough, because sorption takes longer than adsorption to establish analyte/PDMS partition equilibrium, in particular for highly volatile compounds (e.g. 2,3 pentandione (**1**), 2-methtylbutanal (**2**), 2-methylbutanol (**4**)); it is also possible that the D-HS purge gas flow-rate does not afford a suitable equilibration time [12].

3.2.2 Analyte distribution at different purge volumes – The next step involved determining analyte percentage distribution on the trapping material investigated, in function of purge volume, in order to achieve D-HS fractionation.

Figure 2A reports the area percentage distribution of each analyte using CBS as trapping material. The results clearly show that, with all trapping materials, more than 90% of the area of all analytes accumulates within a purge gas volume of 1000 mL. More in detail, thymol (**6**), linalool (**14**), *E*-2-

243 decenal (**15**) and t-β-caryophyllene (**16**)) require a purge gas volume of 1000 mL, since they only
244 achieve percentages below 60% of the total area within the first 100mLs.
245 Tenax TA produces similar results but, as shown above, under the same CBS conditions, the lower
246 Tenax TA adsorption power caused breakthrough, leading to lower recovery and a different
247 percentage distribution of the areas of the analyte investigated. **Figure 3** reports percentage
248 distribution within the total area, calculated versus CBS trapping taken as 100%, at 50°C for each
249 analyte with each trapping material at different volumes of purge gas. Most compounds are mainly
250 recovered within the first 100 mL purge volume (**Figure 3**). Only 2,5-dimethylpyrazine (**5**) thymol
251 (**6**), octanal (**9**), linalyl acetate (**13**), linalool (**14**), and *E*-2-decenal (**15**) showed a significant
252 increase in recovery with the third purge volume (1000 mL). Conversely, breakthrough of some
253 compounds become significant on increasing the purge volume from 100 to 1000 mL (**Table 2**).
254 As has already been said, PDMS showed decidedly lower total areas.
255
256 ***3.3. Fractionated D-HS and temperature***
257 Special attention was paid to sampling temperature, because of its strong influence on HS
258 composition. An increase of temperature increases analyte evaporation in the HS, thus improving
259 transfer to the trapping material at lower volume of purge gas, in particular for low-volatility
260 analytes, thereby reducing sampling time and breakthrough risk. A set of experiments were run to
261 investigate temperature, purging the model sample sequentially; the results show that temperature
262 can re-address the partition of an analyte between first and second fraction, acting on the amount of
263 purge gas necessary for recovery of that analyte. Temperature can thus be exploited to handle
264 headspace composition and, thereby, to reduce the purge gas volume necessary to transfer analytes
265 to the trap(s). In other words, most of an analyte can be redirected to the desired trap. **Figure 4**
266 reports the relative percentage distribution of the total areas of the 16 analytes of the model mixture,
267 sampled at three different temperatures (35, 50 and 75°C) by D-HS on Tenax TA traps, and
268 sequentially purged with 20 and 100 mL gas. A temperature increase from 35°C to 75°C (i.e. from
269 spontaneous evaporation to full evaporation mode) re-directs the transfer of most components (11
270 of 16; in all cases around 80% or more) from the second to the first fraction. A clear example is *t*-β-
271 caryophyllene (**16**) where, with the same purge volume (20 mL), the percentage distribution in the
272 first trap increases from 9% at 35°C to 89% at 75°C. However, for other compounds (5 of 16),
273 increasing the temperature does not suffice to obtain a sufficiently high transfer to the vapor phase,
274 and purge volume must also be increased (to 100 mL) to maximize percent abundance. This is
275 typical of thymol (**6**), whose percentage distribution in the first trap only increases from 3 to 28%
276 with a temperature rise from 35°C to 75°C.

Similar results were obtained with the other two traps (PDMS and CBS), although the PDMS results were less marked, because this material has low trapping power. Sequential sampling at different temperatures thus provides complementary profiles representative of the volatile fraction of the investigated matrix, by maximizing recovery independently of analyte volatility, at the same time reducing purge volume and minimizing breakthrough. High recovery of low volatility components may also be achieved at low temperatures, by stripping them from the matrix with a high volume of purge gas, but this solution inevitably increases both sampling time and the risk of breakthrough of medium-to-high volatility components.

3.4. Fractionated D-HS and repeatability

The reliability of these results is closely conditioned by their repeatability. D-HS repeatability with each trapping material was determined by analyzing 50µL of the model mixture five times; repeatability was calculated on the absolute areas of the 16 model components. D-HS was sampled at 50°C, purge-gas flow-rate 20 mL/min., for 1, 5, 50, 250 minutes (purge volumes: 20, 100, 1000 and 5000 mL); CBS traps were also submitted to dry-purge. The results are in **Table 3**; RSD% ranged from 0.5% for 2,3-pentandione (**1**) with Tenax TA trap (20 mL) to 23.7% for 2,5-dimethyl pyrazine (**5**) with CBS trap (20 mL). Despite some exceptions, repeatability was satisfactory, in particular at low purge gas volumes. In general, CBS repeatability was slightly lower than that of Tenax TA or PDMS, as expected, because of the need for a dry-purge step, and the different strength of the analyte/CBS interaction, which partly depends on the analyte structure [19-20].

3.5. Fractionated D-HS sampling

The above results were used to design a fractionated D-HS sampling experiment using the model mixture. Two D-HS runs with Tenax TA as trapping material were carried out sequentially on the same HS vial, with 20 and with 100 mL purge gas. Two different temperatures were adopted: the first (35°C) compatible with vaporization of the most volatile components, the second (75°C) applied to operate in full evaporation-D-HS mode with all analytes. **Figure 5a** shows the GC patterns of the two fractions, and Figure 5b the percent contribution that each sampling step gives to the total recovery. 2-Methtylbutanal (**2**), 2,3-pentandione (**1**) and (to a lesser extent) 2-methylbutanol (**4**) were mainly recovered in the first fraction; hexanal (**3**), α-terpinene (**7**), eucalyptol (**8**), were partitioned between the two fractions, as was limonene (**10**), although at a different ratio. The other components were mainly recovered in fraction 2 (i.e. 2,5-dimethylpyrazine (**5**), thymol (**6**), octanal (**9**), 2-pentylfuran (**11**), *E*-2-octenal (**12**), linalool (**14**), linalyl acetate (**13**), *E*-2-decenal (**15**), and *t*-β-caryophyllene (**16**)).

These results show that the components of the model solution can be recovered in two fractions, depending principally on their vapor pressure (VP>1 and VP<1), by exploiting an appropriate combination of temperature, trapping material and purge gas volume.

3.6 Fractionated D-HS sampling of solid matrices

Aroma plays a fundamental role in defining coffee characteristics, and its economic value. A fundamental property for consumers is the so-called "supervolatile" fraction, i.e. the smell at first impact when a consumer opens a new package of coffee. Because of the very high volatility of several components, correct sampling is fundamental to obtain a reliable profile of a roasted coffee powder. Significant results have been achieved with HCC-S-HS techniques (e.g. HS-SPME, HSSE etc.); however, their concentration factors [21] and sampling speeds are conditioned by the need to apply sampling conditions that are compatible with the small amount of trapping material and with static headspace equilibration. When a higher enrichment rate is required, D-HS is the approach of choice. However, to obtain a representative coffee aroma profile, trapping material and purge gas volume must be carefully selected, i.e. sampling conditions must be appropriate to avoid loss of very high volatility analytes, because of breakthrough, and of low volatility components, because of only partial vaporization. Appropriate selection should also lead to a reasonable sampling time, this being another fundamental parameter involved in successful TAS development.

Fractionated D-HS sampling with a fully automatic system can be a good approach to obtain a representative profile in a relatively short time. It offers the possibility of applying the most suitable conditions (temperature, purge gas flow and volume, and trapping material) in function of the HS component volatility, i.e. covering the whole range of volatility of the sample components. A sample (200 mg) of Arabica roasted coffee powder from Costa Rica was submitted to fractionated D-HS, using Tenax TA as trapping material at 50°C with a purge gas flow of 20 ml/min for 5 minutes for the first fraction, and PDMS at 75°C with the same flow-rate for another 5 minutes for the second fraction. The resulting total purge volume was 200 mL, with total sampling time 10 minutes. These results were compared to those obtained by HS-SPME-GC-MS analysis using a 2 cm CAR/DVB/PDMS fiber at 50°C for 30 minutes. In both cases, GC-MS analysis was carried out with the same column and conditions. **Figure 6** shows the GC-MS profiles of the sample, after A) HS-SPME sampling, B) D-HS sampling of the first fraction using the Tenax TA trap at 50°C, and C) D-HS sampling of the second fraction using PDMS at 75°C. The abundance of the profiles of the two fractions highlights the concentration capability of D-HS, both of itself and compared to HS-SPME, and particularly with the highly volatile components. Measured by signal intensity, the average increase *versus* HS-SPME is approximately a factor of ten (abundance about 700,000 *vs.*

70,000 counts) for both fractions. The concentration factors of 34 markers of coffee aroma, calculated on their absolute areas, illustrate this difference over HS-SPME even more clearly [21]. It was again possible to determine reliable concentration factors because of the satisfactory repeatability of the areas, evaluated on five experiments under the same conditions; the resulting RSDs% values were in line with those previously measured, and ranged from 3.1 for pyridine to 17.4 for guaiacole in fraction 1 and from 4.6 for furfuryl alcohol and 18.2 for furfurylpyrrole in fraction 2. Table 4 lists the concentration factors of 34 components, calculated from their absolute areas. The concentration capacity with the selected markers in both fractions was of two orders of magnitude, ranging from about 221 for furfural (**9**) to 4299 for 1-methyl pyrrole (**2**) in fraction 1, and from 117 for acetoxyacetone (**12**) to 2898 for 1-methyl pyrrole carboxyaldehyde (**33**) in fraction 2. These results are of particular interest in consideration of the fact that sampling time is reduced by a factor three compared to that of S-HS-SPME (10 min in total for D-HS vs. 30 min for HS-SPME).

4. Conclusions

This study has shown that fractionated D-HS can provide a representative profile of the volatile fraction, with high recoveries of components across an extended range of volatility. Modern automated instrumentation makes possible a D-HS sampling strategy based on the sequential and/or concurrent adoption of different methods, including breakthrough and full evaporation approaches, and of different materials; non-discriminant recovery of all components of the volatile fraction can be achieved, independently of their physico-chemical properties, by suitably tuning sampling conditions (temperature, purge gas volume and trapping material).

Fractionated D-HS may be considered complementary to the Multi Volatile Method (MVM) [13] in profiling a matrix volatile fraction representatively, or as a specific method, when applied to the selective enrichment of a diagnostic sub-fraction of interest, whose components fall within a given range of volatility. The choice of trapping material should thus be optimized in function of the information required; from the above results, Tenax TA appears to provide a good compromise between recovery, breakthrough, and sampling time and purge volume.

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Captions to Figures

Figure 1 Graph of peak areas of 2,3-pentandione and thymol *versus* gas volumes on a Tenax TA trap after FED-HS sampling at 75°C.

Figure 2 (A) Recovery of each analyte on the CBS trap, % distribution with the purge gas volumes applied (20, 100, 1000 and 5000 mL) and vapor pressure. (B) Total recovery of each analyte on Tenax TA and PDMS traps relative to CBS, taken as 100%.

Figure 3 Percent abundance of total areas of the analytes of the model mixture with Tenax TA and PDMS *versus* CBS , taken as 100%, and their % distribution within the total areas recovered with each purge volume (20, 100, 1000 and 5000 mL) at 50°C.

Figure 4 Relative percent distribution of the peak areas of the model mixture components sampled by D-HS, sequentially purged with 20 and 100 mL, and with a Tenax TA trap at three different temperatures (35, 50 and 75°C).

Figure 5 (A) GC-MS patterns of the two fractions resulting from fractionated D-HS-on the model sample; (B) percent of analyte areas recovered in the two sampling steps.

Figure 6 GC-MS profiles of an Arabica roasted coffee powder after a) HS-SPME sampling, b) D-HS sampling of the first fraction using a Tenax TA trap at 50°C, and c) D-HS sampling of the second fraction using PDMS at 75°C, compounds are reported in Table 4

456 Table 1. Optimized sampling conditions adopted to evaluate breakthroughs and to analyze the
457 model solution and coffee powder.

	Sample amount	Sample Incubation		Dry purge conditions for CBS			Sampling conditions		
		Temp °C	Time min	Volume mL	Flow mL/min	Trap Temp °C	Volume mL	Flow mL/min	Trap Temp °C
Breakthrough evaluation on model mixture components	50 μL	50	10	300	100	50	20 100 500 1000 2500 5000	20	50
Model mixture	50 μL	35 50 75		300	100	50	20 100 1000 5000	20 100	50
Coffee powder	200 mg	50 75		-	-	-	100		

458

Table 2. List of the 16 components of the model mixture ordered by decreasing vapor pressure. giving vapor pressure. boiling point. and breakthrough volume ranges. on traps filled with three different carbons (Carbopack B/Carbopack X/Carbosieve S-III (CBS)). Tenax TA. polydimethylsiloxane (PDMS).

#	*Compound*	*Target ion (m/z)*	*Vapore Pressure (KPa)*	T_{ebol}	LogP	*TX Breakthrough Volume*	*PDMS Breakthrough Volume*	*CBS Breakthrough Volume*
1	2.3-Pentandione	43	4.15	140.60	-0.85	20-100mL	20-100mL	100-500mL
2	2-Methtylbutanal	57	1.39	94.52	1.23	20-100mL	20-100mL	100-500mL
3	Hexanal	56	1.51	132.20	1.80	100-500mL	100-500mL	1000-2500mL
4	2-Methylbutanol	57	0.605	123.17	1.26	100-500mL	100-500mL	500-1000mL
5	2.5-Dimethylpyrazine	108	0.424	168.64	1.03	1000-2500mL	500-1000mL	>5000mL
6	Thymol	135	0.293	236.92	3.52	2500-5000mL	500-1000mL	2500-5000mL
7	α-Terpinene	121	0.221	169.36	4.75	100-500mL	100-500mL	1000-2500mL
8	Eucalyptol	43	0.208	174.13	3.13	100-500mL	100-500mL	2500-5000mL
9	Octanal	43	0.199	175.95	2.78	>5000ml	500-1000mL	>5000mL
10	Limonene	68	0.193	137.66	4.83	1000-2500mL	100-500mL	2500-5000mL
11	2-Pentylfuran	81	0.16	175.55	3.87	100-500mL	100-500mL	1000-2500mL
12	E-2-Octenal	55	0.115	182.37	2.57	2500-5000mL	500-1000mL	>5000mL
13	Linalyl acetate	93	0.017	228.95	4.39	2500-5000mL	500-1000mL	>5000mL
14	Linalool	71	0.011	204.05	3.38	1000-2500mL	500-1000mL	>5000mL
15	E-2-Decenal	55	0.01	221.95	3.55	2500-5000mL	1000-2500mL	>5000mL
16	t-β-Caryophyllene	93	0.004	256.80	6.30	2500-5000mL	1000-2500mL	>5000mL

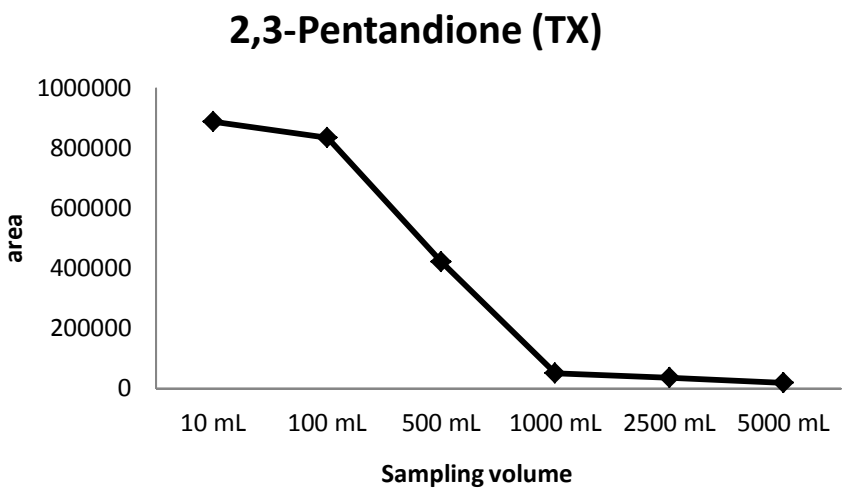
Table 3. D-HS repeatability of the 16 components of the EtOH/water model sample with each trapping material measured at 50°C with a purge-gas volume of 20, 100, 1000 and 5000 ml at a flow-rate of 20 mL/min.

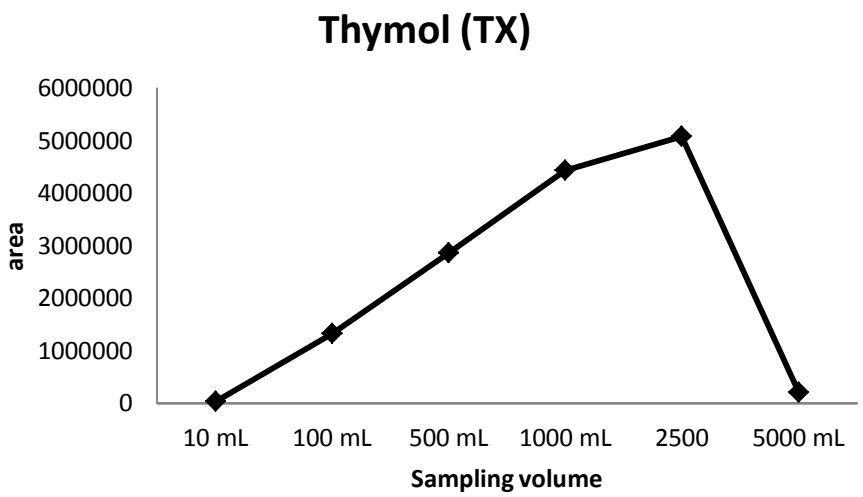
#	Compound	Tenax TA				CBS				PDMS			
		20 mL	100 mL	1000 mL	5000 mL	20 mL	100 mL	1000 mL	5000 mL	20 mL	100 mL	1000 mL	5000 mL
1	2,3-Pentandione	0.5	3.4	17.0	12.6	5.0	12.5	18.0	11.7	10.2	3.1	12.3	12.5
2	2-Methtylbutanal	10.2	16.1	2.3	15.4	9.1	10.8	8.3	9.6	7.5	11.4	10.9	12.3
3	Hexanal	13.1	12.8	7.5	13.6	8.9	13.5	9.1	12.8	6.1	11.3	10.7	11.1
4	2-Methylbutanol	5.5	14.9	14.0	10.6	13.9	14.0	16.4	18.1	5.5	12.4	9.0	15.1
5	2,5-Dimethylpyrazine	11.7	13.4	5.9	10.9	23.7	13.9	11.6	12.4	12.7	11.2	13.4	14.2
6	Thymol	7.6	8.3	17.2	11.9	11.9	12.7	15.6	11.2	9.2	3.9	13.1	16.1
7	α-Terpinene	10.5	9.8	9.1	12.7	6.1	13.6	11.1	13.6	10.5	9.8	14.2	15.0
8	Eucalyptol	14.0	15.2	3.5	11.4	26.8	12.4	3.5	12.6	14.0	9.1	10.8	11.9
9	Octanal	13.9	3.3	1.9	12.1	10.2	5.9	1.9	11.0	13.9	10.9	12.4	10.6
10	Limonene	3.2	9.1	3.6	15.0	9.1	9.7	10.5	15.4	3.2	10.5	10.3	11.2
11	2-Pentylfuran	14.0	10.7	14.2	11.6	18.4	13.6	14.2	12.8	14.0	11.0	9.9	15.2
12	E-2-Octenal	12.3	17.5	14.1	14.9	13.8	13.6	8.1	12.9	9.6	12.6	12.7	11.5
13	Linalyl acetate	12.4	15.4	18.0	13.6	7.4	12.9	17.0	17.9	12.4	12.8	16.2	14.8
14	Linalool	9.9	10.8	7.0	15.1	20.2	17.1	13.0	11.8	10.9	14.2	13.0	13.6
15	*E*-2-Decenal	12.1	8.7	15.6	12.5	19.9	15.3	17.2	13.9	9.2	6.2	11.9	17.2
16	t-β-Caryophyllene	14.8	12.4	8.1	16.4	9.2	12.5	14.1	13.8	10.6	14.1	14.6	12.3

Table 4. Concentration factors (CF) of 34 components sampled by fractionated D-HS calculated through their absolute areas *versus* the corresponding peak areas obtained with HS-SPME with CAR/PDMS/DVB fiber.

#	Compounds	VP (KPa)	CF TX 50°C	CF PDMS 75°C	Total CF
1	2-butanone + 2-methylfuran	13.065+21.464	3875		3875
2	1-methylpyrrole	3.693	4299		4299
3	pyridine	2.799	1403	1447	2850
4	2,3-butandione	2.750	1965		1965
5	acetic acid	1.870	1210	558	1768
6	pyrazine	1.710	2147		2147
7	hexanal	1.207	1696		1696
8	2,3-pentandione	1.034	2747		2747
9	furfural	0.710	221		221
10	2-methyl pyrazine	0.617	563	353	916
11	2-me-dihydro-3(2H) furanone	0.611	1641		1641
12	acetoxyacetone	0.605	229	117	346
13	1-hydroxy-2-propanone	0.393	3796	1104	4900
14	2,3-dimethyl pyrazine	0.365	668	864	1532
15	3-hydroxy-2-butanone	0.358	2472		2472
16	furfuryl formate	0.309	1494		1494
17	2,6-dimethyl pyrazine	0.199	565	877	1442
18	trimethylpyrazine	0.193	366	782	1148
19	limonene	0.193	948	583	1531
20	3-methylbutanoic acid	0.161	1481		1481
21	furfuryl acetate	0.134	298	672	970
22	2-ethyl-3,5-dimethyl pirazine	0.099	468	1255	1723
23	3-ethyl-2,5-dimethyl pyrazine	0.099		930	930
24	5-methyl furfural	0.091	260	854	1114
25	2-ethyl-6-methyl pyrazine	0.081	340	753	1093
26	furfuryl alcohol	0.057	554	1022	1576
27	butyrolactone	0.039	739	1742	2481
28	guaiacole	0.015		1291	1291
29	furfurylpyrrole	0.011		413	413
30	2-acetyl-3-methylpyrazine	0.007		6202	6202
31	2-acetyl pyrrole	0.003		1343	1343
32	ethylguaiacole	0.003		3186	3186
33	1-methylpyrrole carboxyaldehyde	0.001		2898	2898
34	vinylguaiacole	0.001		1543	1543

Figure 1


2,3-Pentandione (TX)
1000000
800000
600000
400000
200000
0
area
10 mL
100 mL
500 mL
1000 mL
2500 mL
5000 mL
Sampling volume


Thymol (TX)
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5000000
4000000
3000000
2000000
1000000
0
area
10 mL
100 mL
500 mL
1000 mL
2500
5000 mL
Sampling volume

Figure 2

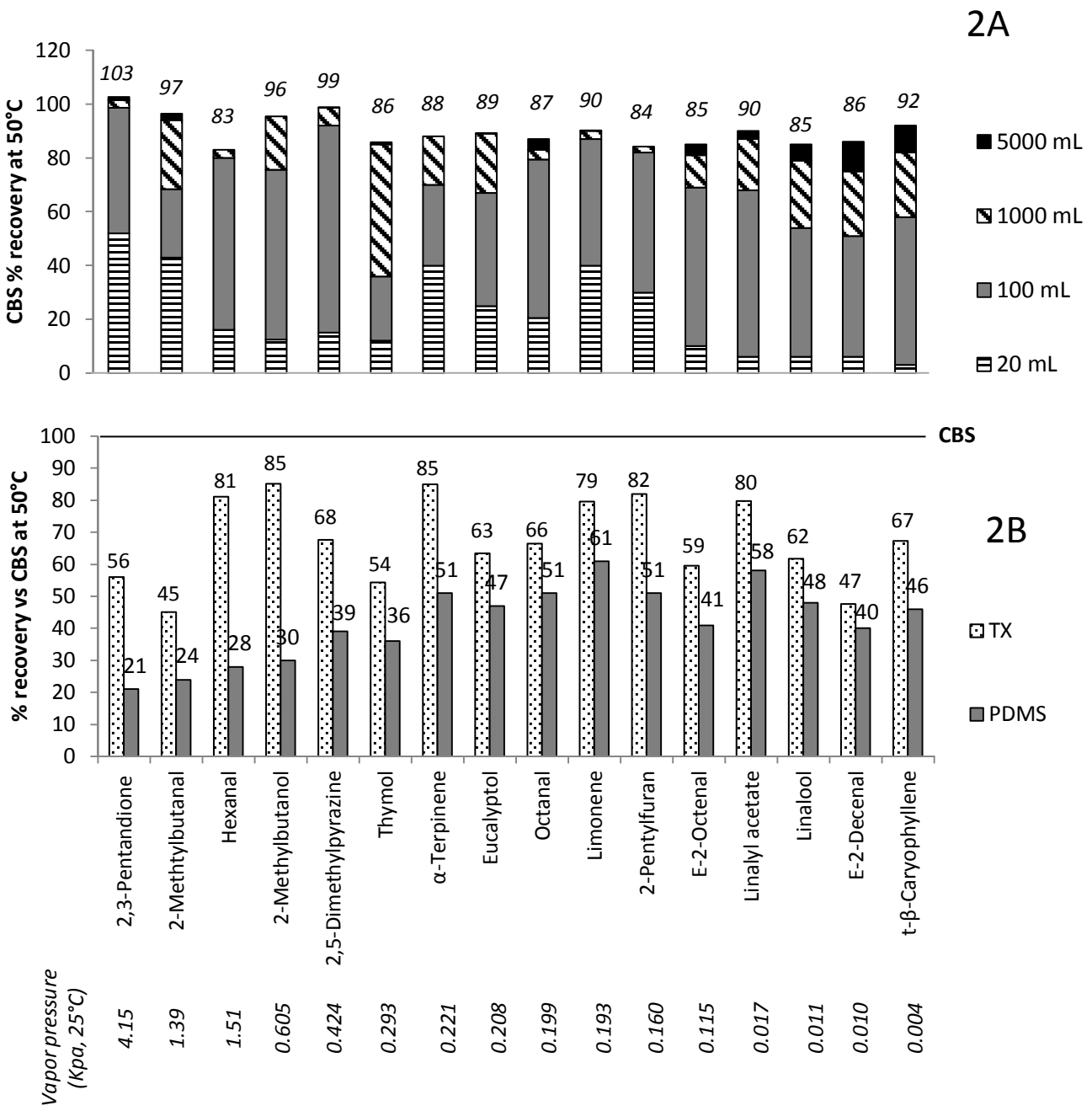

2A
CBS % recovery at 50°C
120
100
80
60
40
20
0
103
97
83
96
99
86
88
89
87
90
84
85
90
85
86
92
5000 mL
1000 mL
100 mL
20 mL
CBS
% recovery vs CBS at 50°C
100
90
80
70
60
50
40
30
20
10
0
2B
TX
PDMS
56
21
45
24
81
28
85
30
68
39
54
36
85
51
63
47
66
51
79
61
82
51
59
41
80
58
62
48
47
40
67
46
2,3-Pentandione
2-Methtylbutanal
Hexanal
2-Methylbutanol
2,5-Dimethylpyrazine
Thymol
α-Terpinene
Eucalyptol
Octanal
Limonene
2-Pentylfuran
E-2-Octenal
Linalyl acetate
Linalool
E-2-Decenal
t-β-Caryophyllene
Vapor pressure (Kpa, 25°C)
4.15
1.39
1.51
0.605
0.424
0.293
0.221
0.208
0.199
0.193
0.160
0.115
0.017
0.011
0.010
0.004

Figure 3

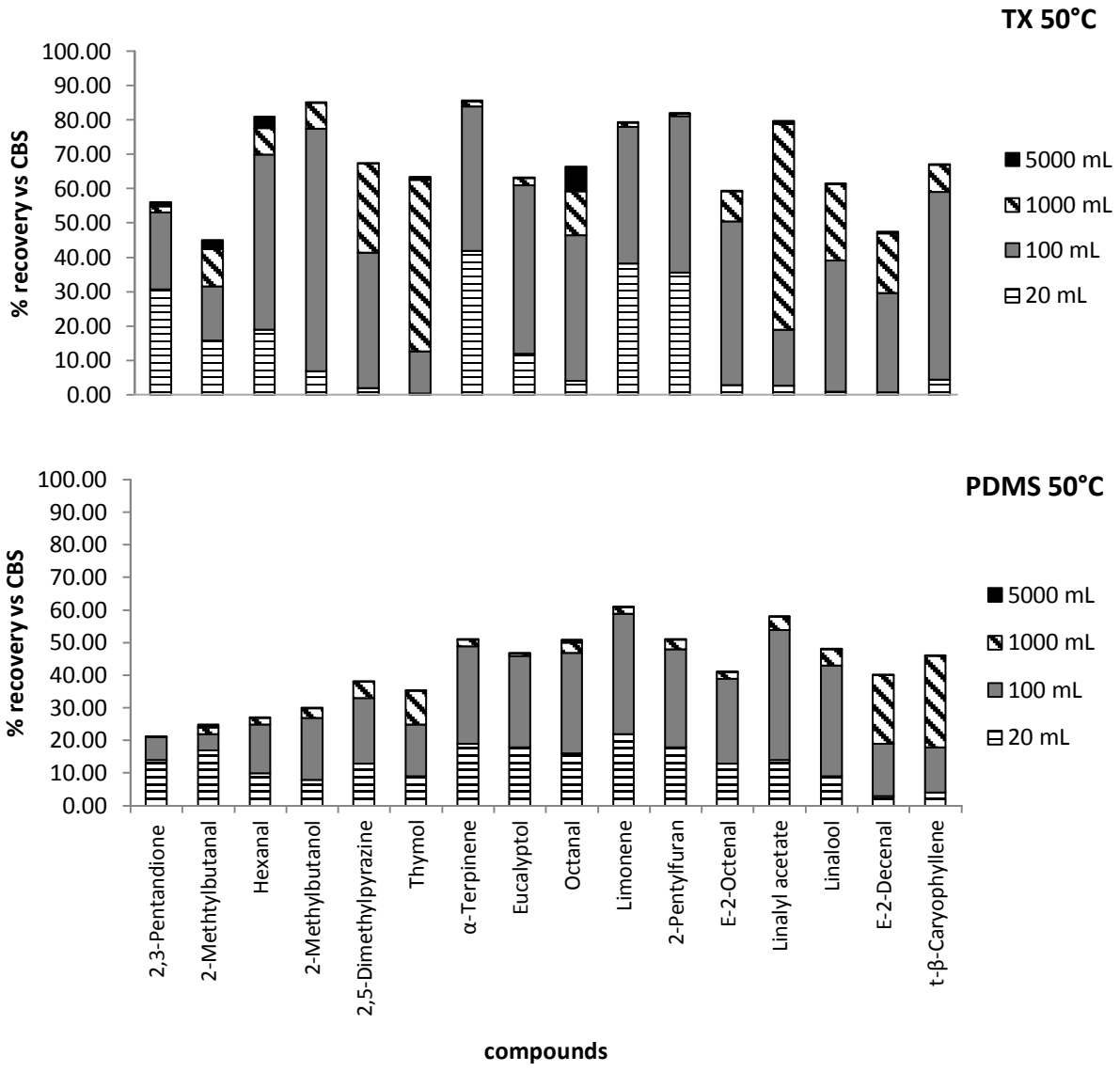

TX 50°C
% recovery vs CBS
100.00
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
5000 mL
1000 mL
100 mL
20 mL
PDMS 50°C
% recovery vs CBS
100.00
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
5000 mL
1000 mL
100 mL
20 mL
2,3-Pentandione
2-Methtylbutanal
Hexanal
2-Methylbutanol
2,5-Dimethylpyrazine
Thymol
α-Terpinene
Eucalyptol
Octanal
Limonene
2-Pentylfuran
E-2-Octenal
Linalyl acetate
Linalool
E-2-Decenal
t-β-Caryophyllene
compounds

Figure 4

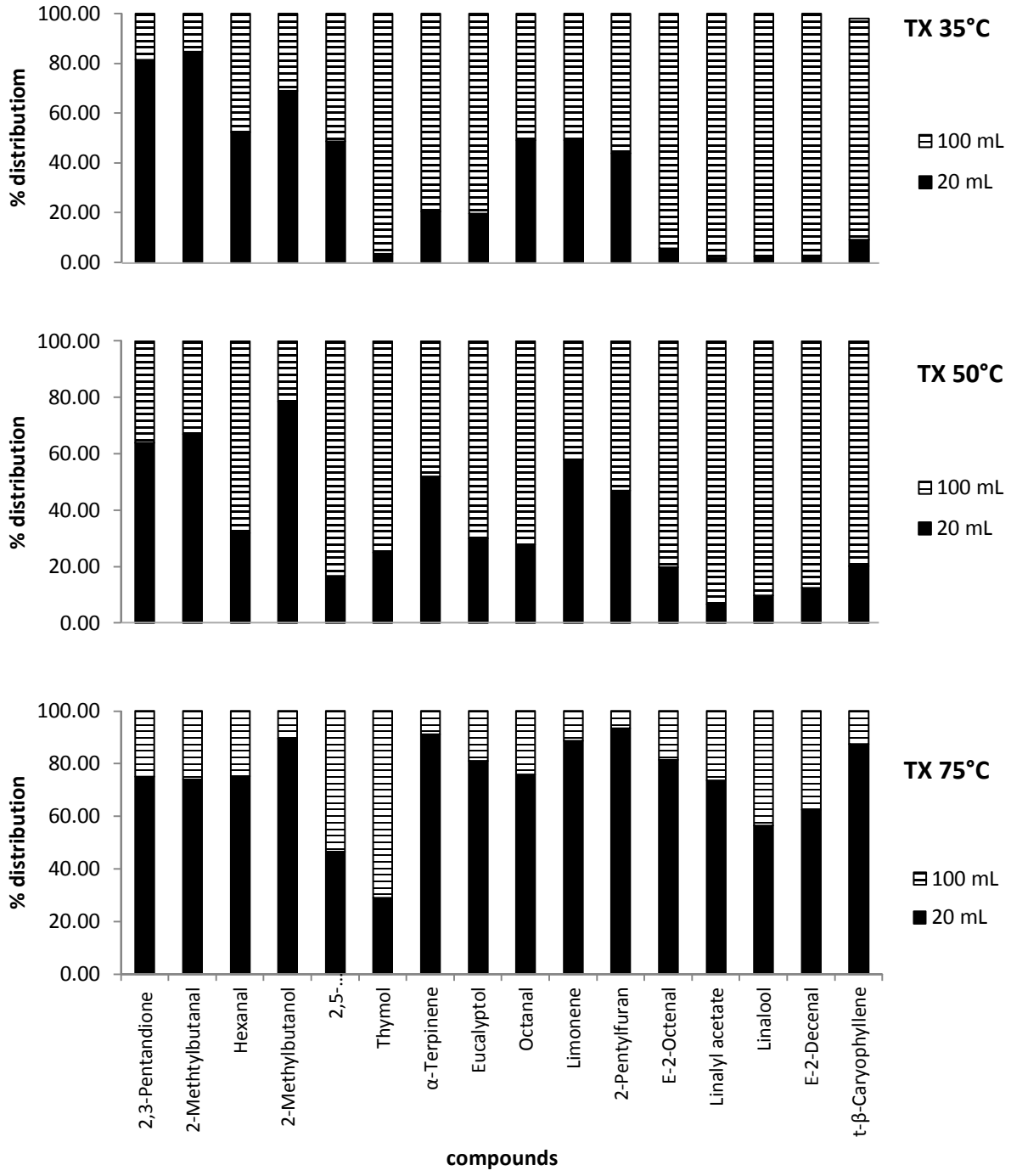
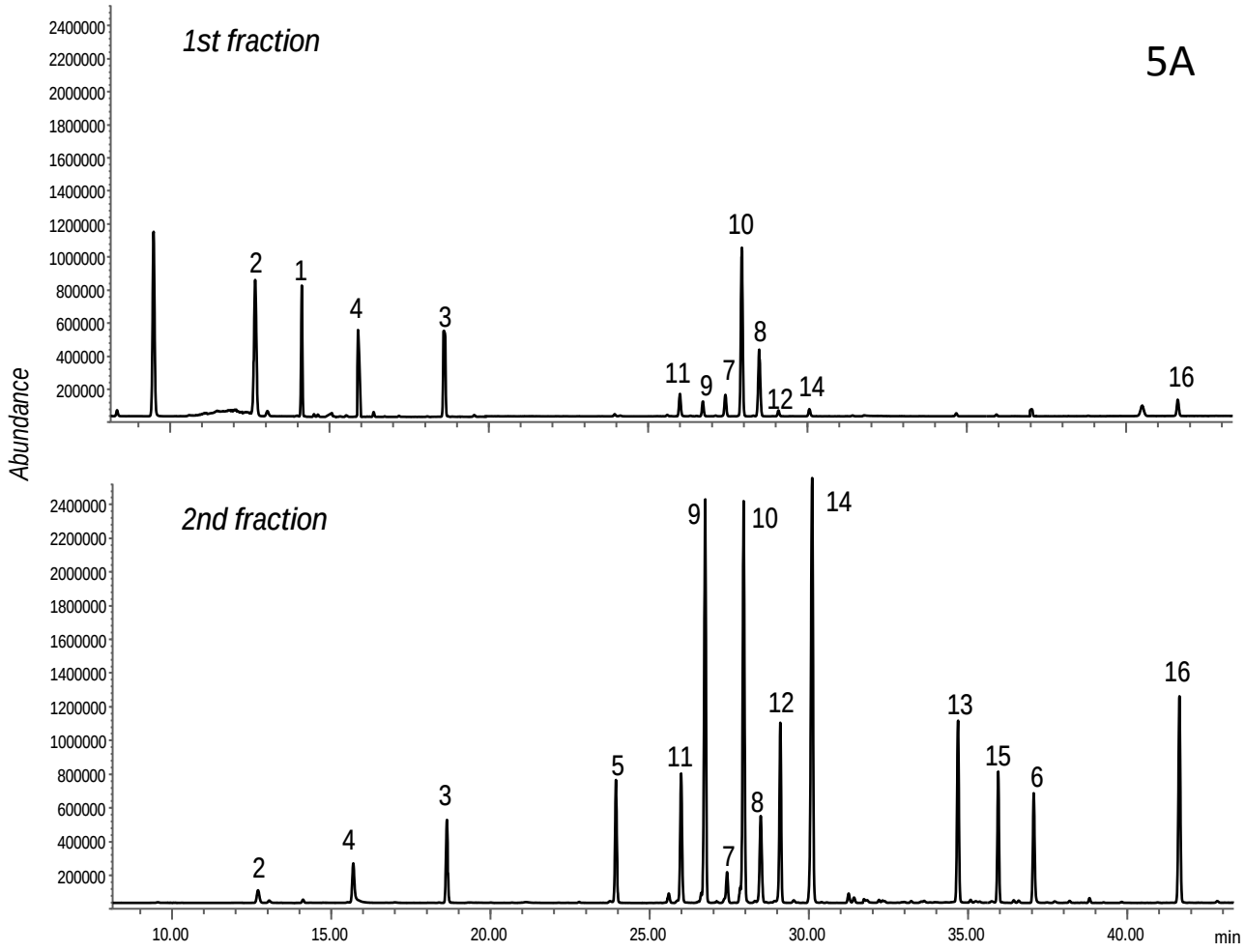

TX 35°C
TX 50°C
TX 75°C
% distribution
100.00
80.00
60.00
40.00
20.00
0.00
100 mL
20 mL
2,3-Pentandione
2-Methtylbutanal
Hexanal
2-Methylbutanol
2,5-...
Thymol
α-Terpinene
Eucalyptol
Octanal
Limonene
2-Pentylfuran
E-2-Octenal
Linalyl acetate
Linalool
E-2-Decenal
t-β-Caryophyllene
compounds

Figure 5


1st fraction
5A
Abundance
2400000
2200000
2000000
1800000
1600000
1400000
1200000
1000000
800000
600000
400000
200000
2
1
4
3
10
8
11
9
7
12
14
16
2nd fraction
2
4
3
5
11
9
10
14
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20.00
25.00
30.00
35.00
40.00
min

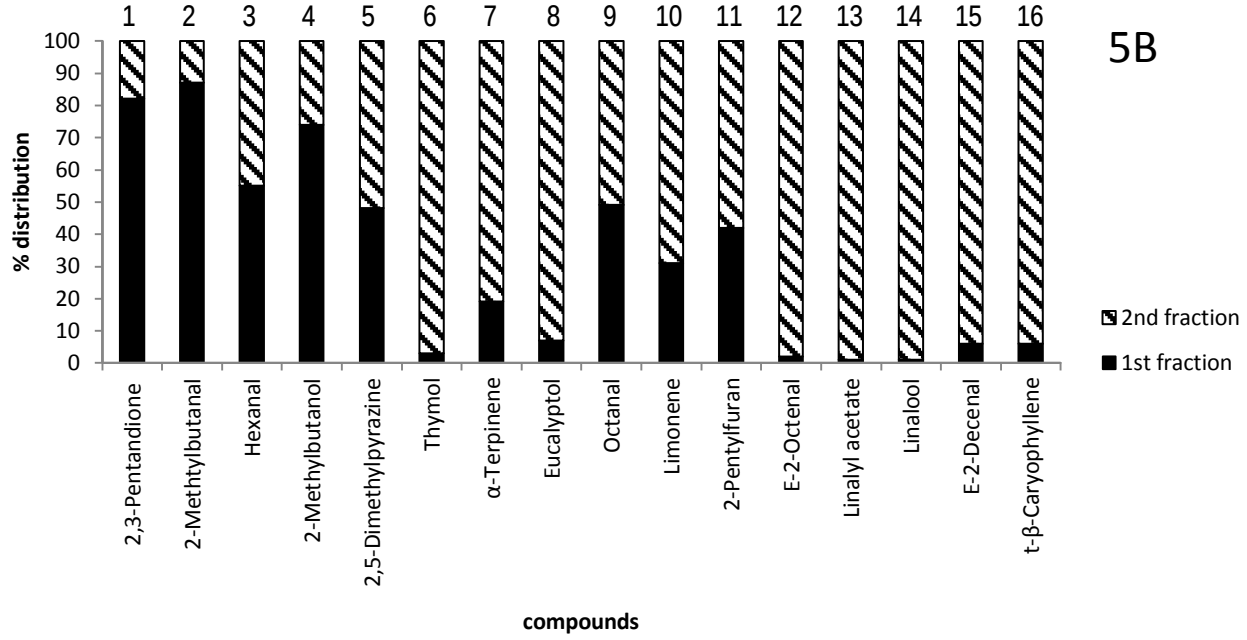
5B
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
% distribution
100
90
80
70
60
50
40
30
20
10
0
2nd fraction
1st fraction
2,3-Pentandione
2-Methtylbutanal
Hexanal
2-Methylbutanol
2,5-Dimethylpyrazine
Thymol
α-Terpinene
Eucalyptol
Octanal
Limonene
2-Pentylfuran
E-2-Octenal
Linalyl acetate
Linalool
E-2-Decenal
t-β-Caryophyllene
compounds

Figure 6