A Survey on High-Concentration-Capability Headspace Sampling Techniques in the Analysis of Flavors and Fragrances

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Abstract

This survey critically discusses high-concentration-capacity (HCC) headspace (HS) techniques applied to sample the volatile fraction of matrices of interest in the flavors and fragrance fields. In particular, the advantages, limits, and fields of application of HS solid-phase microextraction (SPME), high-capacity HS sorptive extraction (HSSE) and HS solid-phase dynamic extraction (SPDE) are evaluated. These techniques are discussed in view of the peculiar characteristic of HCC-HS techniques, from the standpoint that these techniques are a bridge between static (S-HS) and dynamic HS (D-HS) because they are as simple, fast, easy to automate, and reliable as S-HS, yet afford analyte concentration factors comparable to those of D-HS. Moreover, the different degree of their development is a consequence of the different times in which they were introduced into the market, because the potential of HS-SPME is now well known, having been introduced approximately 12 years ago, but that of HSSE has still to be fully explored, and HS-SPDE still has to be investigated because it is very recent.

Introduction

Headspace (HS) sampling is defined as a solvent-free sample preparation technique aiming at sampling the gaseous or vapor phase in equilibrium (or not) with a solid or liquid matrix in order to characterize its composition (1). Up to the end of the 1980s, most researchers thought that HS sampling could only be approached either statically (S-HS) or dynamically (D-HS); the early 1990s saw a remarkable revival of interest in HS sampling, partly because of the introduction of high-concentration-capacity HS approach (HCC-HS). HCC-HS techniques are really a bridge between S- and D-HS because they are as simple, fast, easy to automate, and reliable as S-HS, yet afford analyte concentration factors (CFs) that are comparable with those of D-HS. The first HCC-HS sampling technique to appear was HS solid-phase microextraction (SPME), introduced by Zhang and Pawliszyn in 1993 (2) as an extension of SPME, which had been developed by Arthur and Pawliszyn in 1990 (3) to sample organic pollutants from water. In 1999, Sandra introduced stir-bar sorptive extraction (SBSE) (4), again a technique to sample organic pollutants from water, which was applied to HS sampling by Tienpont et al. (5) and Bicchi et al. (6) almost simultaneously, under the name of high-capacity HS sorptive extraction (HSSE). Both HS-SPME and HSSE are based on the concentration of analytes onto a polymer via the S-HS approach; on the other hand, solid-phase dynamic extraction (SPDE), the most recently introduced technique, is based on the D-HS approach. SPDE, also known as "the magic needle", was introduced in 2001 by Lipinski (7) and is an inside-needle technique for vapor and liquid sampling. The success of these techniques was also made possible by developments in the knowledge of the basic phenomenon related to sorption (8–11) (in particular when polydimethylsiloxane is used), which, together with adsorption, are the main phenomena involved in the recovery of an analyte by a polymeric phase.

This survey offers a critical discussion of the authors' everyday experience with these three techniques, applied to HS sampling of the volatile fraction of matrices of interest in the flavors and fragrance fields. We would like to stress that this is not a review article, therefore the literature survey will not be exhaustive but only concerns the points under discussion.

Experimental

HS-SPME–gas chromatography–flame ionization detection analysis of Costa Rican roasted coffee sample

Sample preparation

The SPME device and a 75-µm fiber of carboxen (CAR)–polydimethylsiloxane (PDMS) were purchased from Supelco (Bellafonte, PA). Conditions for HS-SPME of the sampling of the Costa Rican roasted coffee were as follows: sample amount, 200 mg; vial volume, 12.5 mL; and equilibration and sampling time, 60 min at 50°C.

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Gas chromatographic analysis

A gas chromatographic (GC)–FID unit was used from Carlo Erba Mega 5360 GC (Milan, Italy); column, PEG 20M HTS-FSOT capillary column [25-m × 0.25-mm i.d., 0.25-µm film thickness, MEGA (Legnano Italy)]; injection temperature, 230°C; detector, FID at 250°C; carrier gas, hydrogen at a flow rate of 1.5 mL/min; and temperature program, from 20°C (2 min) to 40°C at 5°C/min, from 40°C to 180°C at 3°C/min, and from 180°C to 220°C at 5°C/min (5 min).

HSSE–GC–MS analysis of white pepper sample

Sample preparation

The PDMS stir bars with a volume of 55 µL were from Gerstel GmbH (Mülheim a/d Ruhr, Germany). Conditions for HSSE of sampling white pepper were as follows: sample amount, 1 mg; vial volume, 12.5 mL; and equilibration and sampling time, 30 min at 50°C.

Thermo desorption GC–MS and conditions

Analyte thermal desorption from the PDMS stir bar was achieved with a thermo desorption system unit [TDS-2, Gerstel GmbH Mülheim a/d Ruhr, Germany] installed on a GC–MS system (6890 GC-5973N MS system, Agilent Technologies, Little Falls, DE).

GC–MS analysis

The column used was a PEG 20M HTS-FSOT capillary column (25-m × 0.25-mm i.d., 0.5-µm film thickness, from MEGA). The chromatographic conditions were: temperature program, from 0°C (1 min) to 35°C at 10°C/min, from 35°C to 220°C at 3°C/min (10 min); carrier gas, helium; flow rate, 1 mL/min; injection temperature, 230°C; and MS, EI mode at 70 eV.

TDS conditions

The desorption program was from 0°C to 280°C (8 min) at 60°C/min; carrier gas, helium at a flow rate of 1.0 mL/min; flow mode, split at a split ratio of 1:10; transfer line, 280°C; injector, programmed temperature vaporizer (PTV) (CIS-4 PTV, Gerstel GmbH); PTV cryofocussing temperature, –10°C using liquid CO_2; injection, PTV in sample-remove mode; injection temperature, –10°C at 600°C/min to 280°C, (5 min); and inlet mode, splitless.

HS-SPDE–GC-MS analysis of a fresh banana sample

Sample preparation

A 2.5-mL gas-tight syringe (Chromtech, Idstein, Germany) provided with a stainless steel needle 5.5 cm long coated with a 50-µm film of PDMS and activated carbon (10%) was used. The conditions for HS-SPDE sampling of fresh, sliced banana were as follows: matrix volume, 2 mL; vial volume, 21.2 mL; equilibration time, 15 min; equilibration temperature, 35°C; agitator (sampling) temperature, 35°C; HS syringe temperature, 55°C; number of filling cycles, 50; plunger speed for extraction, 50 µL/s (each aspiration taking 40.5 s); helium volume for desorption, 1 mL; plunger speed for desorption, 15 µL/s; predesorption time, 30 s; and desorption temperature, 230°C.

HS-SPDE–GC–MS system

The SPDE equipment (syringes with attached SPDE needles and SPDE gas station) was from Chromtech (Idstein, Germany) and was installed in a CTC-Combi-PAL autosampler (Bender and Hobein, Zurich, Switzerland) that was assembled on a GC–MS system consisting of an Agilent model 6890 Series Plus/5973 N. The CTC-Combi-PAL autosampler included an incubator oven with one heated vial position and shaker (agitator) (Chromtech).

Analysis conditions: column, PEG 20M HTS-FSOT capillary column (25-m × 0.25-mm i.d., 0.25-µm film thickness, from MEGA). The temperature program was as follows: from 0°C (2 min) to 30°C at 30°C/min, then to 200°C (5 min) at 10°C/min. The injector temperature was 230°C; mode, splitless; transfer line, 250°C; carrier gas, helium at a flow rate of 1.0 mL/min; and MS, EI mode at 70 eV.

Results and Discussion

HS-SPME

SPME (2,3) is a solvent-free sampling technique in which the analytes from the liquid or gaseous sample are directly absorbed or sorbed (or both) onto a polymer-coated fused-silica fiber, which is part of the needle of a specially designed holder. The sampled analytes can be recovered either by thermal desorption directly into a GC injection port or by solvent elution into a modified high-performance liquid chromatographic (HPLC) injection valve (12). Figure 1 gives a diagram of the SPME holder and of a fused silica fiber. The state of the art concerning SPME theory, technology, evolution, applications, and specific topics have been reviewed by Pawliszyn et al. (13–21). They also investigated in depth the theory of SPME applied to HS sampling (2) and showed that the amount of analyte concentrated through HS-SPME in a fiber is the result of two closely related but distinct equilibria: the first is the matrix/HS equilibrium responsible for the HS composition, and the second is the HS/polymeric fiber coating equilibrium. The former equilibrium is conditioned by the volatility of each analyte and by the physical characteristics of the matrix, but the latter one concerns the diffusion of the analyte from the vapor phase to the fiber coating and is conditioned by the analyte interaction with the polymeric coating. The total HS-SPME recovery of an analyte from a solid or liquid matrix depends on the

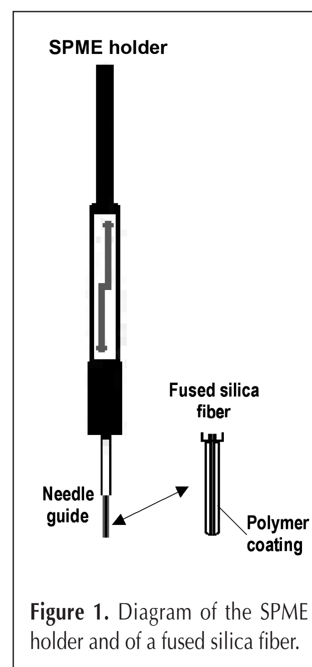

Figure 1. Diagram of the SPME holder and of a fused silica fiber.

overall partition coefficient (K) of the analyte between the SPME fiber coating and the matrix itself. K can be calculated from the equation: $K = K_1 K_2$, where K_1 is the analyte partition coefficient between SPME fiber coating and sample HS and K_2 is the partition coefficient between HS and sample matrix (2,22). Because K_2 is constant under standardized sampling conditions, K_1 can be assumed to be representative of the recovery of an analyte from the HS of a sample onto the polymeric coating of a fiber. K_1^i can be calculated from the following equation:

$$K_1^i = \frac{A_f V_g}{A_g V_f} \quad \text{Eq. 1}$$

where K_1^i is the partition coefficient for the analyte *I*, A_f is the area of analyte *i* after HS-SPME sampling with the fiber in question, V_g is the volume of the vapor phase, A_g is the area of analyte *i* after S-HS sampling, and V_f is the volume of the fiber polymeric coating.

The CF of an analyte *i* achieved by an SPME fiber versus the corresponding S-HS sampling is the ratio between the analyte areas obtained by HS-SPME (A_f) with that fiber and the corresponding area obtained by S-HS (A_g);

$$CF = \frac{A_f}{A_g} \quad \text{Eq. 2}$$

CF is not an absolute parameter because it depends on HS sampling conditions and the physical state of the matrix, but it may be used to evaluate the relative recovery efficiencies of different fibers for a given sample, provided that rigorous and reproducible standard conditions are applied.

One of the main problems in developing an SPME method is how to choose fiber and sampling conditions in order to maximize analyte recoveries. When a single analyte or a few analytes with similar physicochemical characteristics must be sampled, fiber polymeric coating and analysis conditions can be chosen in function (*i*) of the analyte structure and volatility, (*ii*) on the physicochemical characteristics of the polymeric coating and analyte/polymer affinity, and (*iii*) of K_1^i of the analytes investigated (2,23). On the other hand, when the HS of a complex composition is to be studied, as is often the case in the flavor and fragrance fields, the choice of fiber and sampling conditions becomes very difficult because recovery is conditioned by the polarity and volatility of the analytes investigated and, as a consequence, the composition and physical state of the matrix, HS equilibration temperature and time, and analyte diffusion from the vapor phase to the fiber surface (2,22,23). A different strategy is therefore necessary involving a nonequilibrium HS conditions in order to keep sampling time within reasonable limits. This requires rigorously standardized conditions. Moreover, it requires the use of fibers consisting of two or more components: a liquid (generally PDMS) for the less polar analytes mainly operating in sorption and a solid [divinylbenzene (DVB), CAR, or both] polymeric coating mainly operating in absorption, for the more polar analytes. Such fibers can sample analytes of a different nature simultaneously and effectively.

Bicchi et al. (23) investigated the difference in fiber performance for HS sampling in the aromatic and medicinal plant field. They determined the partition coefficient (K_1) and the relative CFs of eight commercially available SPME fibers (7 µm PDMS, 30 µm PDMS, 100 µm PDMS, 65 µm CAR–DVB, 75 µm CAR–PDMS, 85 µm polyacrylate, 65 µm PDMS–DVB, and 50/30 µm CAR–DVB–PDMS) using a standard solution of nine characteristic components of plant volatile fraction with different structures and volatilities dissolved in dibutyl phtalate. They also evaluated the abundance relative to S-HS of some components characteristic of the HS of four aromatic and medicinal plants: industrially dried rosemary (*Rosmarinus officinalis* L.), sage (*Salvia officinalis* L.), thyme *(Thymus vulgaris* L.), and naturally dried valerian (*Valeriana officinalis* L.). In general, the most effective fibers for HS-SPME are those consisting of two or more components (in particular, CAR–DVB–PDMS).

The mentioned study compared fibers on the basis of their performance on a single component. More recently, Zuba et al. (24) introduced a criterion function (F_{ij}) to evaluate the concentration capability of a given fiber in a set of fibers on the basis of a group of marker components characterizing the HS of the matrix investigated:

$$F_j = \frac{1}{n} \sum_{j=1}^{n} \frac{H_{ij}}{\frac{1}{k} \sum_{j=1}^{n} H_{ij}} \quad \text{Eq. 3}$$

where F_j is the concentration capability factor of the fiber *j, n* is the number of marker components characterizing the matrix under investigation, k is the number of fibers, and H_{ij} is the height of the peak of component *i* with the fiber *j*.

Tchapla et al. (25) recently simplified equation 3 as reported below:

$$F_{ij} = \frac{\sum_i H_{ij}}{\frac{1}{k} \sum_{i,j} H_{ij}} \quad \text{Eq. 4}$$

These equations are very useful, in particular, for routine analyses because they make it possible to choose the most effective polymeric coating for a given matrix or monitor the sampling capability of a fiber over time through a biased parameter (or both). Figure 2 reports the GC pattern of the HS of a Costa Rican roasted coffee sample after HS-SPME sampling with a 75-µm CAR–PDMS fiber. Table I lists F_{ij} values for five fibers calculated from equation 4 using the marker compounds reported in the caption of Figure 2.

HS-SPME is now a well-established technique and plays a fundamental role in the analysis of HS composition in flavors and fragrances. Many (possibly too many) applications have been described. Several factors have contributed to its success: (*a*) high extraction speed, stability, simplicity, flexibility, and ease of automation because analytes can reliably be concentrated onto the fiber in pseudo S-HS conditions; (*b*) parameters that must be tuned to maximize recovery are few

(mainly temperature, time, and phase ratio, β); (*c*) sampling and analysis can be carried out separately because storing the fiber in its holder keeps the sample safe over time, allowing field or process sampling; (*d*) sampled HS can be analyzed with conventional GC units; and (*e*) HS-SPME can successfully be used to monitor biological processes involving volatiles *in vitro*, keeping the system isolated from the surrounding atmosphere.

The main limits of SPME recognized after more than 10 years of everyday experience in the authors' laboratory are: (*a*) Limited concentration capability for trace analysis most probably attributable to the small volume of polymer coating the fiber, which ranges between 0.4 and 0.6 µL, and to the unfavorable phase ratio β (i.e., large HS volumes). The latter limit can be partially overcome through constant stirring (or vibration) of the sampling vial to improve the diffusion process and the HS/fiber analyte exchange (2); (*b*) quantitative analysis of HS components of a solid matrix is problematic because of the difficulty of building calibration curves; (*c*) fiber performance over time. Although HS is a "clean" sample and seldom influences fiber efficiency, in the authors' experience, the average lifetime of a fiber, in particular of the multicomponent ones, is approximately 50 sampling/reconditioning cycles. At this point, quantitative fiber performance with a relative standard deviation (RSD) below 10% is repeatable; this number includes the initial samplings in which the fiber performance is sometimes not constant. In any case, for reliable quantitative analysis, fiber efficiency must be checked against a reference sample at least every 10 samplings. Moreover, most fibers require longer conditioning time than that recommended by the manufacturer to obtain repeatable performance or to completely eliminate low volatility "ghost" peaks (or both) caused by the polymer coating, in particular when the special tool for fiber conditioning is not available (26); and (*d*) in some cases, fiber ingredients operating in absorption (in particular, charcoal) can produce artefacts (27). Some other minor disadvantages of SPME are fragility of the fused-silica, lack of protection of polymer coating, and limited flexibility of surface area.

Table I. Concentration Capability Indices of a Set of SPME Fibers for a Costa Rican Roasted Coffee Sample

Fibers	F_{ij}
100-µm PDMS	0.26
CW–DVB	1.01
PDMS–DVB	0.83
PDMS–CAR	1.80
PDMS–CAR–DVB	1.10

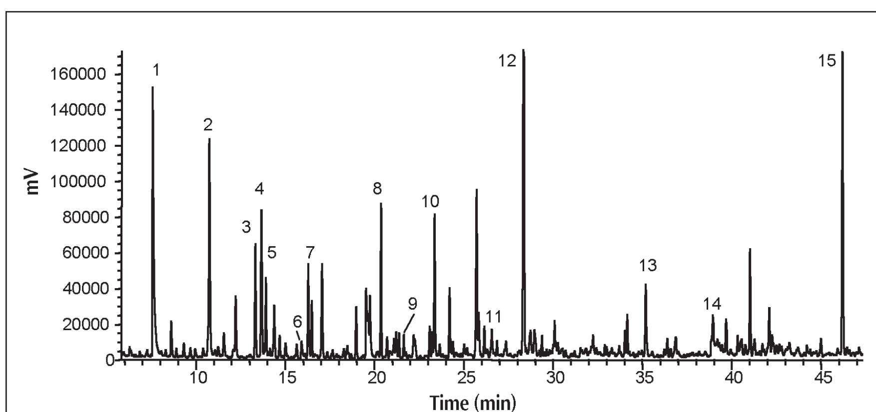

Figure 2. GC pattern of the HS of a Costa Rican roasted coffee sample after HS-SPME sampling with 75-µm CAR–PDMS fiber. See the text for analysis conditions. List of marker peaks and identification: (1) pyridine; (2) 2-methylpyrazine; (3) 2,5-dimethylpyrazine; (4) 2,6-dimethylpyrazine; (5) 2-ethylpyrazine; (6) 3-ethylpyridine; (7) 2-ethyl-5-methylpyrazine; (8) 1-acetyoxy-2-propanone; (9) furfuryl formate; (10) furfuryl acetate; (11) 6,7-dihhydro-^{5}H-cyclopentapyrazine; (12) furfuryl alcohol; (13) guaiacol; (14) 2-acethylpyrrole; and (15) *p*-ethylguaiacol.

HSSE

In the previous paragraph, it was said that one of the limits of HS-SPME was its reduced concentration capability, which is most probably caused by the small volume of polymer coating the fiber. In 1999, Sandra's group (4) introduced a technique enabling this limit to be overcome: SBSE. As for SPME, sorptive extraction was first developed for sampling liquids (SBSE), but its use was soon extended to HS sampling by Sandra's and Bicchi's groups (5,6) under the name HSSE. In sorptive extraction (SBSE and HSSE) an analyte (or analytes or a fraction) is sorbed onto a thick film of PDMS coating a glass-coated iron stir bar. The PDMS stir bar is introduced into the aqueous sample or suspended in the HS volume from where the analytes are recovered. After sampling the stir bar is placed in a glass tube and transferred to a thermodesorption system in which the analytes are thermally recovered and analyzed by GC or GC–MS. The volume of PDMS coated onto the stir bars ranges from 25 to 110 µL, depending on its size, which may vary from 1.0 to 2.0 cm in length and from 0.5 to 2.0 mm in thickness. PDMS volume is therefore between two and three orders of magnitude higher than that of the polymeric coating SPME fibers (0.4 to 0.6 µL). PDMS stir bars are marketed under the name "Twister" (Gerstel). Figure 3 shows HSSE sampling and a diagram of a PDMS stir bar.

HSSE is based on sorption, which is defined as the partition of an analyte between the sample and the bulk of a polymeric retaining phase, thus producing bulk retention instead of surface adsorption in the same way as for a partition chromatography process. The advantages of sorption over absorption include high inertness, absence of catalytic degradation reactions, better performance for polar or reactive compounds (or both), and linearity of sorption isotherms (11).

The theory advanced by Zhang and Pawliszyn for HS-SPME (2) has also been applied to HSSE, although HSSE mostly operates in the sorption mode, whereas HS-SPME operates in both sorption and adsorption modes. In this case, too, the

total HSSE recovery of an analyte from a solid or liquid matrix depends on the overall partition coefficient (K) of the analyte between the PDMS stir bar and the matrix itself. In its turn, K depends on K_1, the analyte partition coefficient between PDMS stir bar and sample HS, and on K_2, the partition coefficient between HS and sample matrix (2,6). In this case, too, K_1 can be assumed to be representative of the recovery process of an analyte from the HS of a sample onto the polymeric coating of the stir bar. K_1^i can be calculated from equation 1, replacing A_f with A_{SB} (area of analyte *i* obtained with PDMS stir bar) and V_f with V_{SB} (volume of the stir bar polymeric coating). Similarly, the CF can be calculated from equation 2.

The high volume of polymeric coating means that HSSE can achieve very high concentration capabilities, as shown by the minimum recoverable concentration of high volatility C_5 to C_7 components of a standard mixture in dibutyl phtalate (cyclohexane, propyl acetate, hexanal, 1-hexen-3-ol, isoamyl acetate and 2-heptanol), which ranged from 0.075µM for cyclohexane to 15µM for hexanal (6). The study also compared HSSE concentration capability with that of both S-HS and HS-SPME with different fibers, determining K_1 and CF values for the components of the mentioned C_5 to C_7 standard mixture and the relative abundances (RA) of the characterizing components of the volatile fractions of the same plants investigated for HS-SPME (i.e., rosemary, thyme, sage, and valerian). K_1^i values for the PDMS stir bar were in the 10^2–10^4 range, though CFs varied between 26.6 for cyclohexane and 828.0 for 1-hexen-3-ol. These results indicate that (also for HSSE) recovery is closely related to the volatility of the analyte investigated. Moreover, HSSE CFs were generally two orders of magnitude higher than those obtained by HS-SPME with a 100-µm PDMS fiber because they ranged from 0.6 for cyclohexane to 26.6 for 1-hexen-3-ol. This was expected because the volume of polymeric coating in HSSE with the adopted stir bars was approximately 100 times greater than that of the SPME fiber (55 vs. 0.6 µL). On the other hand, multicomponent fibers, in particular those containing CAR, showed recoveries comparable to and, in some cases, even higher than those of the PDMS stir bar with some of oxygenated analytes (propyl acetate and hexanal with 75-µm CAR–PDMS and hexanal and 2-heptanol with 50/30 µm CAR–DVB–PDMS). RA values of the characterizing HS components with different structures, volatilities, and polarities, from rosemary, thyme, sage and valerian, were determined using the areas obtained with the CAR–DVB–PDMS fiber as a reference. It was chosen as being the most effective fiber with the majority of the analytes investigated. The RAs for all analytes with PDMS stir bars were, in general, higher than those by HS-SPME with all sort of fibers. They were higher by a factor ranging from 5 to 20 for rosemary, thyme, and sage; valerian RAs varied from a factor of approximately 25 for α-terpinolene to approximately 180 with eudesma-2,6,8-triene, although 10 times less sample was analyzed (i.e., 60 mg instead of 600 mg). Similar results were obtained when HS-SPME and HSSE were applied to coffee analysis (28).

The concentration capability of HSSE is illustrated by the example in Figure 4, which shows the GC pattern of the HS obtained from 1 mg of a sample of commercial white pepper in a 12.5-mL vial after HSSE sampling with a PDMS stir bar.

Although the number of HSSE applications reported is not as high as those for HS-SPME, HSSE has been widely and successfully applied to the analysis of HS composition of flavors and fragrances (29). Several characteristics have contributed to its success:

(*a*) PDMS stir bars can successfully be applied to trace analysis and passive sampling because of their high concentration capability. (*b*) HSSE can successfully be applied to sample HSs with unfavorable β values or large HS volumes (or both). (*c*) HSSE concentration capability can be varied by changing the volume of PDMS coating the stir bar (stir bars of different lengths and thicknesses coated with PDMS volumes ranging from 25 to 110 µL are commercially available). (*d*) The absolute amounts of analytes sampled in the PDMS stir bar can easily be determined through analyte calibration curves constructed by direct injection of a standard solution of the analytes investigated through the TDS. Sorption is a partition phenomenon, therefore it is not (or only slightly) affected by the HS composition (8–11), but only by analyte solubility in

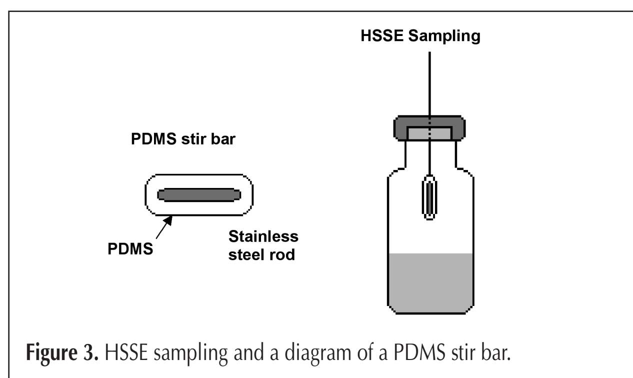

Figure 3. HSSE sampling and a diagram of a PDMS stir bar.

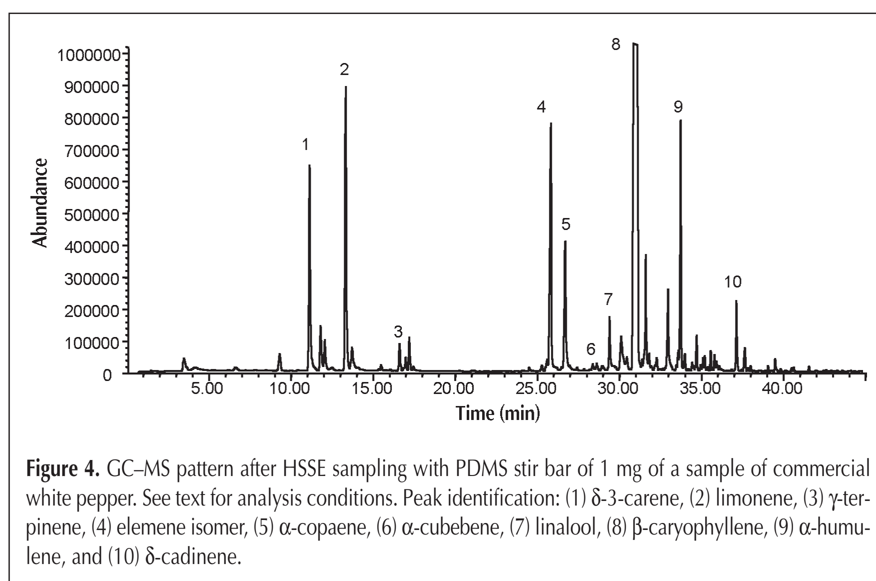

Figure 4. GC–MS pattern after HSSE sampling with PDMS stir bar of 1 mg of a sample of commercial white pepper. See text for analysis conditions. Peak identification: (1) δ-3-carene, (2) limonene, (3) γ-terpinene, (4) elemene isomer, (5) α-copaene, (6) α-cubebene, (7) linalool, (8) β-caryophyllene, (9) α-humulene, and (10) δ-cadinene.

PDMS provided that the analyte concentration in the HS does not saturate the PDMS coating. (*e*) PDMS is highly inert and does not induce catalytic degradation reactions. (*f*) Sampling and analysis can be carried out separately thus allowing field or process sampling, as for HS-SPME. (*g*) Parameters that must be tuned to maximize recovery are few (mainly temperature, time, and phase ratio, β) as for SPME.

The main limits of HSSE as found in the everyday experience in the authors' laboratory are:

(*a*) HSSE requires dedicated instrumentation for reliable quantitative analysis (i.e. a thermodesorber in combination with a cryoconcentration system). Cryoconcentration is indispensable for high volatility compounds as is very often the case in the flavour and fragrance field. (*b*) Only PDMS is at present available as polymeric coating for stir bars, which limits the effectiveness of HSSE concentration capability when medium-to-high polarity compounds must be sampled. Although highly polar constituents are rare in HSs in the flavor and fragrance field, stir bars coated with material with a better affinity to polar compounds would increase the method's flexibility and selectivity while keeping, or even increasing, its concentration capability. "Polar" stir bars would extend the use of HSSE to ultratrace analysis or selective sampling (or both) of specific analytes in complex or multi-ingredients matrices (i.e., acrylamide residues in cooked foods) or to passive sampling in very large volumes (i.e., pollutants in indoor pollution analysis or studies on modelling diffusion of odorants in large volumes).

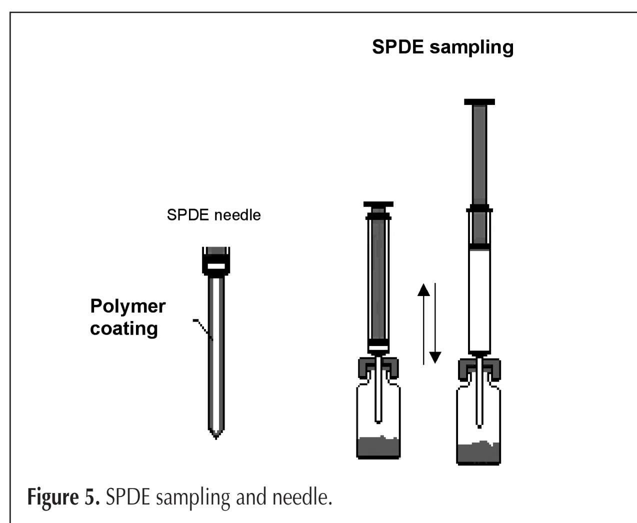

Figure 5. SPDE sampling and needle.

SPDE

HS-SPME and HSSE are based on the S-HS approach. Analyte recovery is conditioned by the HS/fiber or stir bar coating partition coefficient (K_1^i), which depends on several factors, including analyte diffusion through the matrix and polymer coating boundary layers, analyte convection through the vapor phase (which can be made consistent by stirring), and the nature of the polymer coating (solid or liquid) that characterizes the recovery mode (sorption or adsorption) (2,14,16). A new technique, SPDE (also known as "the magic needle"), was recently introduced by Lipinsky (7). SPDE is an inside-needle technique that can be used indifferently for sampling in liquid (IS-SPDE) or vapor phases (HS-SPDE); the analytes are concentrated on a thick film (50 µm) of a polymer coated onto the inside wall of the stainless steel needle (5.5 or 7.5 cm long) of a gas-tight syringe (2.5 mL). In HS-SPDE, analytes are accumulated in the polymer coating the inside needle wall of the gas-tight syringe; a fixed volume of the sample HS is pulled in and pushed out of the syringe, at a specific rate, a suitable number of times. The trapped analytes are then thermally desorbed and online transferred by a fixed volume of carrier gas into the GC injector body where they are analyzed by GC or GC–MS. The diagram in Figure 5 shows the SPDE sampling and needle. The volume of polymer coated on the 5.5-cm SPDE needle wall is approximately 4.5 µL, versus approximately 0.4–0.6 µL coating an SPME fiber. Several polymeric coatings are available: PDMS, PDMS–activated charcoal, PDMS–OV 225, PDMS–phenyl-methyl polysiloxane, polyethylenglycole (PEG), PDMS, 7% phenyl, and 7% cyanopropyl. (OV 1701). HS-SPDE is closer to the D-HS approach because the vapor phase flowing over the polymeric layer is continuously renewed. The number of reported HS-SPDE applications is still small. It has been successfully applied to analysis of cannabinoids and amphetamines in hair samples of drug abusers by Musshoff et al. (30,31) and to food matrices and aromatic plants—in particular rosemary, banana, green and roasted coffee, and red and white wine—by Bicchi et al. (32). Figure 6 shows the GC–MS pattern of the HS obtained from a fresh banana sample after HS-SPDE sampling.

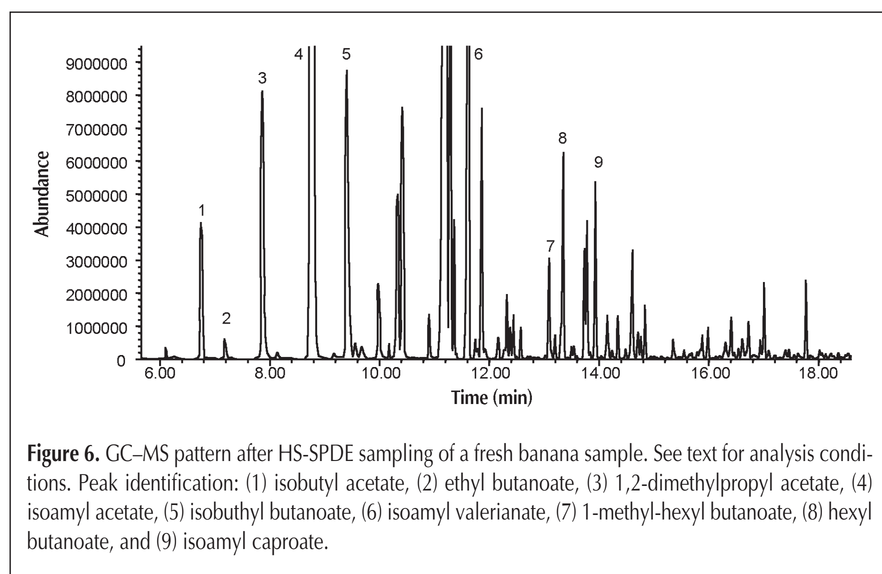

Figure 6. GC–MS pattern after HS-SPDE sampling of a fresh banana sample. See text for analysis conditions. Peak identification: (1) isobutyl acetate, (2) ethyl butanoate, (3) 1,2-dimethylpropyl acetate, (4) isoamyl acetate, (5) isobuthyl butanoate, (6) isoamyl valerianate, (7) 1-methyl-hexyl butanoate, (8) hexyl butanoate, and (9) isoamyl caproate.

A series of experiments is still in progress to evaluate HS-SPDE performance and limits (32). The influence of sampling parameters on recovery was investigated by analyzing a standard mixture consisting of β-pinene (900 ng/mL), isoamyl acetate (250 ng/mL), and linalool (750 ng/mL) in HPLC-grade water; in particular, sampling temperature, number of aspiration cycles, plunger speed and

volume aspired for each cycle, total volume of sampled HS, helium desorption volume, and plunger speed for desorption. All experiments were carried out with a stainless steel needle coated with a 50 µm film of PDMS and activated carbon (10%). Preliminary experiments showed that for these analytes good recoveries can be obtained (*a*) at a moderately low temperature (50°C) to displace the analyte partition equilibrium towards the polymeric coating, (*b*) with a moderate number of aspiration cycles (50) to avoid unwanted loss of the most volatile sampled components during the pushing out step, (*c*) with a relatively small aspiration volume (0.5–1 mL), (*d*) with a medium-to-low plunger speed (50 µL/s), (*e*) with a helium desorption volume of 1 mL, and (*f*) with a desorption plunger speed of 15 µL/s. Several points remain to be studied in depth, in particular the influence of temperature, number, and volume of aspiration cycles on the recovery of analytes with different polarities and volatilities; and the effect of the polymeric coating composition on recovery. In the same study, HS-SPDE–GC–MS repeatability and intermediate precision and concentration capability were also evaluated on 13 characteristic components of the HS of a dried rosemary sample. Repeatability was good because the RSDs percent ranged from 4.1 for α-pinene to 9.6 for β-ionone. The same was true for intermediate precision in which the RSDs percent values were just slightly higher than repeatability for all analytes investigated ranging from 6.7 for α-pinene to 9.7 for β-ionone. These values are fully comparable with those obtained by HS-SPME–GC–MS. The concentration capability of HS-SPDE was also determined and compared with that of HS-SPME with a 100-µm PDMS fiber by determining the CFs (equation 2) calculated versus S-HS of a group of analytes characteristic of Costa Rican roasted coffee. As expected, because the volume of PDMS coating the needle wall is approximately 8 times higher than the coating of the SPME fused silica fiber (V_{SPDE} of 4.5 µL vs. V_{SPME} of 0.6 µL), CFs by HS-SPDE were all higher than those obtained by HS-SPME (three- to five-fold) and ranged from approximately 10 for furfuryl formate to 30 for 3-ethylpyridine (32).

HS-SPDE is a very new technique and, consequently, a lot of work still has to be done to understand its advantages, limits, and fields of application. At present, some general points can be emphasised: (*a*) unlike HS-SPME and HSSE, HS-SPDE concentration capability seems to be higher with highly volatile compounds; (*b*) the role played by sampling parameters and inside needle polymeric coating composition on recovery has yet to be evaluated in depth; and (*c*) when a specific analyte (or analytes) must be sampled, HS-SPDE concentration capability can be varied in function of its (their) concentration in the vapor phase by applying a suitable number of pulling in/pushing out cycles.

The main limits of HS-SPDE noted to date are: (*a*) HS-SPDE requires dedicated instrumentation (i.e., a CTC-Combi-PAL autosampler including an incubator oven with one heated vial position and shaker); (*b*) cryoconcentration is indispensable because of the low transfer speed of the thermally desorbed analyte to the GC system, in particular with high volatility compounds commonly found in the flavor and fragrance field; (*c*) several parameters have to be tuned to maximize analyte recovery, unlike HS-SPME and HSSE; and (*d*) to the best of the authors' knowledge, time required for the sampling is fixed by the number of cycles required; a 1-mL cycle take between from 30 s and 1 min. Time for pulling/pushing cycles is only partially compensated by the fact that HS-SPDE is a technique close to D-HS and thus requires only short equilibration times.

Conclusion

HS-SPME, HSSE, and HS-SPDE have strongly contributed to the success of HCC-HS sampling techniques, although, of course, to different extents. The main difference between them is that theory and practice, as well as advantages and limitations of HS-SPME, are now well known, but the potential of HSSE has not yet been fully explored, particularly for non-conventional applications (e.g., passive sampling, air, or indoor pollution studies). The potential of HS-SPDE still has to be investigated in depth because it is still in its infancy. The three techniques have several characteristics in common, although they are based on different approaches (HS-SPME and HSSE are close to S-HS, and HS-SPDE is close to D-HS) including versatility, possibility of automation, repeatability, and reproducibility. One of the most important characteristics is their flexibility, because they can all be used indifferently for liquid or vapor-phase samplings. This property is particularly important in the flavor and fragrance field because it allows direct correlations of (*i*) the composition of the HSs of a matrix to that of the corresponding water extract (*ii*) the composition of these HSs with that of the extract itself, and (*iii*) it allows sensory analysts to correlate reliably HS and matrix chemical compositions to taste or odors (or both) detected via both the ortho- and the retronasal pathway.

At the present state of our knowledge, these three techniques are complementary and very often interchangeable. The main advantage of HS-SPME is probably that it can be used with any type of instrumentation without modification and in any conditions. HSSE is very effective for trace analysis, and HS-SPDE is useful when a set of samples with different analyte concentration (i.e., requiring a technique with a variable concentration capability) must be analyzed.

One of the points that still requires in-depth investigation is how to apply these techniques to fast sampling, because sampling time is still too long. This makes it irrational to combine them with high-speed GC reliably. In-depth investigation on nonequilibrium HS sampling (in particular, for HS-SPME and HSSE) is necessary to make HCC-HS techniques suitable as fast sample preparation techniques for fast analysis of volatile fractions.

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