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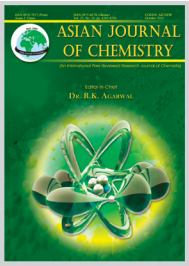

REVIEW

Analysis of Floral Volatiles by Using Headspace-Solid Phase Microextraction: A Review

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Headspace solid phase microextraction (HS-SPME) is a relatively new technique for analysis of floral volatiles. Volatile components emitted from the flowers are pre-concentrated on solid phase microextraction fiber by using field sampling manual holder. The volatiles are identified by GC-MS and percentage composition determined by GC-FID. Solid phase microextraction is a technique that can be used to analyze the fragrance of flowers in different time intervals without solvent treatment. The technique is simple and pre-concentration is easily achieved in the field without sophisticated experimental arrangement. The basic concepts of solid phase microextraction along with the existing methods of floral extraction are discussed. Headspace solid phase microextraction analysis of the volatile compounds emitted by plants and plant products in general and by flowers in particular has been reviewed. The volatile composition of live and picked flowers of *Jasminum sambac, Michelia champaca, Murraya paniculata* and *Quisqualis indica* are discussed.

Key Words: HS-SPME, PDMS fiber, GC-MS, *Jasminum sambac*, *Michelia champaca*, *Murraya paniculata*, *Quisqualis indica*.

INTRODUCTION

Background

Despite tremendous advances and sophistication in development, synthesis and manufacturing of aroma chemicals, essential oils still remain absolutely necessary for the fragrances from the most sophisticated to cosmetic and even to household products making them materials of economic importance for the flavour and fragrance industry. With application in the production of specialized fragrances and in aromatherapy, essential oils from floral materials have attained considerable importance. The so called absolutes from *Jasminum grandiflorum*, *Rosa damascena*, *Polyanthus tuberosa* and several other flowers produced through solvent extraction are traded in large quantities. Production of essential oils from flowers using the traditional equipment is largely discontinued or has undergone significant modifications elsewhere. However, they are still produced in India by distillation with water from flowers of *Rosa damescena*, *Pandanus fascicularis*, *Jasminum sambac*, *Michelia champaca* and other flowers.

The pioneering work of Mookherjee *et al.*[1] has focused a new dimension in fragrance research. Following dynamic head space technique of purge and trap, these authors enclosed the plant part flowers, fruits or spice in a container and removed the emitted volatiles by purging with purified air over 6-12 h and collected the vapours by adsorbing on a Tenax trap. The volatiles were then desorbed from Tenax with an organic solvent for analysis by GC/MS. Mookherjee *et al.*[1] have analyzed the head space volatiles from several flowers, fruits and spices and have demonstrated that the composition of the volatiles emitted by them when live and attached to the mother plant differ considerably in at least some of the major components from the volatiles emitted by the same material when they are picked and detached from the plant. The advantage of solid phase micro extraction (SPME) analysis over the headspace trapping with solid adsorbents is the speed of analysis and simplicity.

Different methods are used for extraction and isolation of essential oils/volatiles from plant materials which may include distillation with water or steam[2], solvent extraction[3], supercritical fluid extraction (SFE)[4-7] and microwave assisted process (MAP) extraction[8]. However all these processes always lead to loss of some components, incomplete extraction, solvent residues and are time consuming.

Alternative techniques for sampling and analysis of the volatiles are static headspace gas chromatography (HS-GC), dynamic purge and trap GC and solid phase trapping solvent extraction (SPTE-GC)[8]. But each technique gives a different picture of fragrance profile[9-12].

Solid phase micro extraction is a novel approach in sorbent extraction. It totally excludes use of organic solvents and is very simple to operate. The technique integrates extraction, concentration and sampling into a single step. In SPME, a fused silica fiber is coated with a stationery phase and the fiber is exposed to an aqueous or gaseous sample till equilibrium is achieved between the analyte in the sample and on the fiber. The analyte is then thermally desorbed from the fiber in the injection chamber of a gas chromatograph and analyzed.

The method was first reported by Arthur and Pawliszyn[13] using optical fibers of fused silica and coated with polyimide stationery phase. Originally it has developed for pre-concentration and analysis of environmental samples. It is now widely accepted and employed in a wide range of areas. Review articles published using SPME on different topics including environmental analysis[14-18], pesticide analysis[19], determination of organic vapours[20], indoor air analysis[21], vapours from cooking[22] and analysis of live biological samples[23]. Reviews have also appeared on technology evolution[24-27] and derivatization[28]. A comprehensive review by Ouyang and Pawliszyn[29] and review on calibration methods for SPME of same authors[30] have recently appeared. Solid phase micro extraction is now firmly established technique that has benefited from many recent developments. The development of needle trap devise for monitoring air samples in both free and particle bound concentration. The development of cold fiber headspace SPME has addressed the issues of analyte desorption from interfering particles present in complex solid matrices[31].

The concept of SPME may have been derived from the idea of an immersed GC capillary column[27]. The SPME apparatus is a very simple device. It looks like modified syringe (Fig. 1) consisting of a fiber holder and a fiber assembly, the latter containing 1-2 cm long extractable SPME fiber. The SPME fiber itself is a thin fused silica optical fiber. Solid phase micro extraction (SPME) was originally named after the first experiment with a device that used solid fused silica fibers as the extracting phase, though it is well known that the extraction phase is not always technically a solid. Fibers coated with poly dimethyl siloxane (PDMS) or polyimide stationery phases are mainly used. However, other phases such as carbowax-divinyl benzene (CW/DVB), PDMS/DVB are also used in various applications. The fibers are mounted on a holder and are usable up to 100 extractions or more depending upon the extraction conditions.

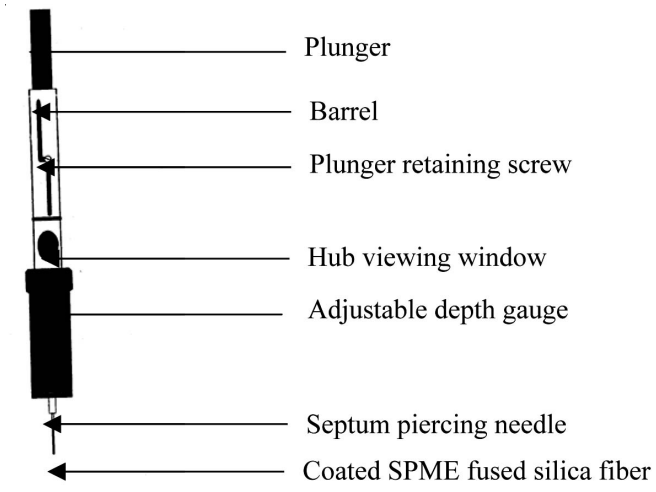

Fig. 1. SPME fiber with manual holder

Basic principle

The basic principles behind SPME are adequately presented by Pawliszyn and coworkers[32-35] and have been summarized by Prosen and Zupancic-Kralj[25]. In a SPME procedure, the fiber is exposed to the sample matrix or its headspace until equilibrium is reached between the concentration of the analyte in the adsorbing phase with its concentration in the sample matrix. The amount of the analyte extracted on to the fiber is linearly proportional to its initial concentration in the sample matrix[35]. In SPME plant volatiles are concentrated onto a coated fiber and the quantity of the given compound adsorb onto the fiber depends on both the partitioning of the compound into the headspace from the plant matrix and the partitioning of the compound into the fiber coating from the headspace. Ficks first law of diffusion can be used for the calibration of the fiber-retracted SPME device when the inner diameter of the diffusion path is very small and the coating is "Zero sink" for target analytes[36].

The objective of the present review is to study the applicability of SPME to the determination of volatile components emitted by plants in general and flowers in particular. It is also our objective to present the results of our work on analysis of extractives using SPME of a few common Indian flowers while still attached to the branch and after picking.

Before the actual results are presented, it will be relevant to briefly describe theoretical aspects of SPME applicable to extraction of headspace and gaseous samples.

Solid phase micro extraction is an equilibrium process, whether the sample is aqueous or gaseous. Partition of the analytes between the sample matrix and fiber coating takes place. In such a case, the partition coefficient is expressed as

$$K_{fs} = C_f/C_s$$

where C_f and C_s are concentrations of the analyte in the fiber stationery phase and sample, respectively. The amount of the analyte on the fiber coating is given by

$$n_f = V_f \times C_f = V_f \times K_{fs} \times C_s$$

where V_f is the volume of the stationery phase or coating. This equation is valid if the volume of the sample is practically infinity compared to V_f. Otherwise a more general equation applies:

$$n_f = K_{fs} \times V_f \times V_s \times C_s/(K_{fs} \times V_f + V_s)$$

where, V_s is the volume of the sample.

According to the above model, in a uniformly mixed sample, there is diffusion in the fiber coating, but there is no diffusion in the sample. The analyte concentration in the fiber increases rapidly at first, then more slowly till equilibrium is reached. The equilibrium time is inversely proportional to the square of thickness of the fiber coating *i.e.* stationery phase. On the other hand, the amount of adsorbed analyte is directly proportional to coating thickness.

For analytes of lower K_{fs} values, the equilibrium time can be very long even in homogeneous or mixed samples. There is a linear relationship between the amount of analyte absorbed and its original concentration in the sample even before the

equilibrium time is reached. If the required analyte concentration is lower than detection limits, sampling under the same conditions of temperature and stirring and same time that is shorter than equilibrium time can be used.

Solid phase micro extraction from the gaseous phase means extraction from gaseous samples or from headspace. Equilibrium between the analyte in the stationery phase and the gaseous sample is reached in much shorter time because of the diffusion coefficient for the transport of the analyte in the gaseous phase is much greater. The humidity of the air has some impact on the extraction from headspace. When the relative humidity is above 90 % adsorption may be reduced by as much as 10 %[37].

Quantitative determination of analytes in the gas phase using SPME is possible using external calibration, standard addition or the partition coefficient K_{fg} of the analyte between the fiber coating and gas phase[35].

It has been shown that there is linear relationship between log K_{fg} and linear temperature programmed retention index (LTPRI) at least for alkanes and aromatic hydrocarbons[37,38].

$$\log K_{fg} = \alpha\,(\text{LTPRI}) + \beta$$

where α is the slope and β is the intercept. This relationship is valid for all compounds in the gas phase when the fiber coating is the same as the stationery phase of the column used to obtain the LTPRI values[35,39]. Once the K_{fg} value for an analyte is known, its concentration in the headspace can be determined by the equation.

$$C_{headspace} = C_f/K_{fg}$$

where, $C_{headspace}$ and C_f are the analyte concentration in the headspace and fiber coating respectively. C_f can be determined easily by measuring the analyte quantity extracted onto the fiber coating and knowing the coating volume.

To determine the α and β values in the above relationship for a category of analytes, K_{fg} must be experimentally measured for a few selected model compounds. By performing HS-SPME and static headspace analysis under equilibrium and on identical samples under similar conditions, K_{fg} is measured according to the following equation[39,40].

$$K_{fg} = (A_f V_g)/(A_g V_f)$$

where A_f and A_g are peak areas obtained by HS-SPME and static head-space, respectively. Similarly V_f and V_g are the volumes of fiber coating and static head-space injected into the GC, respectively. The K_{fg} values thus obtained are reliable when the volume of vial used for head-space is relatively large. By plotting the K_{fg} values of the selected compounds from the same group with their LPTRI values, it is possible to measure α and β values. From them, the K_{fg} values of other compounds belonging to the same group can be calculated for use in quantitative determination.

Desorption of analytes from the SPME fiber: Most injection ports in modern chromatographs are suitable for direct introduction of the fiber for affecting thermal desorption. The capillary GC should not contain any packing and split less mode is used. Linear volume should be small to avoid peak tailing.

Desorption of the analytes from the fiber is achieved thermally in the GC injector at higher temperature, nearly 250 ºC. The partition coefficient for stationary phase/gas decreases rapidly at higher temperature, resulting in a diminished capacity of the fiber to retain the adsorbed analytes. These diffuse from the stationary phase to the GC carrier gas. The process is fast, because the diffusion coefficients are higher at elevated temperature[25].

Generally the optional desorption temperature is approximately equal to the boiling point of the least volatile analyte[32]. To prevent broadening of chromatographic peaks, the initial temperature of the GC column should be kept low or even cooled (cryofocussing). An initial temperature of 80-100 ºC below the boiling point of the most volatile analyte is recommended[32]. Thus concentration of analytes at the head of the column is achieved.

The desorption time depends on desorption temperature, but in general it is less than 1second and so a 1 min desorption in the injector should be more than adequate[32]. However, it is better to leave the fiber in the GC injector for 5-10 min in order to eliminate the memory effects.

Effect of different fiber coatings: The choice of the fiber coating is an important parameter since partition of the analyte from the sample depends on physico-chemical characteristics of the stationery phase and film thickness. The affinity of the fiber for an analyte depends on the principle 'like dissolves like' and appropriate fiber or thickness should be selected in accordance with target compounds[41-43]. Non-polar polydimethyl siloxane (PDMS) fiber is preferred for extraction of non-polar analytes, whereas more polar polyacrylate (PA) fiber is more appropriate for the extraction of polar analytes. Mixed coating fibers containing divinyl benzene (DVB) copolymers or carboxene (CAR) increase retention capacity. PDMS/DVB and CAR/DVB can be used for extraction of low molecular weight volatiles and polar analytes respectively. The properties of the fibers are given in Table-1.

Some variations in use of SPME technique: Matisova *et al.*[44] have studied on the precision and repeatability of

TABLE-1
COMPOSITION AND PROPERTIES OF THE STANDARD SPME FIBERS

SPME fiber coating	Thickness (μm)	Polarity	Length of the fiber (cm)	Volume of the coating (μL)	Maximum operating temperature (ºC)	Compounds to be analyzed
PDMS	7	Low	1	0.026	340	Medium to non-polar semivolatiles
PDMS	30	Low	1	0.132	280	Non-polar semivolatiles
PDMS	100	Low	1	0.612	280	Volatiles
PA	85	High	1	0.521	320	Polar semivolatiles (Phenols)
CW/DVB	65	High	1	0.357	265	Polar analytes (alcohols)
CAR/PDMS	75	High	1	0.436	320	Gases and volatiles
PDMS/DVB	65	Medium	1	0.357	270	Polar volatiles
CAR/DVB/PDMS	50/30	Medium	2	1.0	270	Odours

PDMS: Polydimethylsiloxane; PA: Polyacrylate; CW: Carbowax; DVB: Divinylbenzene; CAR: Carboxen

measurements of the HS-SPME with open-cap vials in comparison with septum-seal vials using PDMS fiber. Precision of SPME with open cap vials is reported very high, but is dependent on sample volume. Ishikawa *et al.*[45] have reported the modified SPME known as solid phase aroma concentrate extraction (SPACE), with increased area of the adsorbent to enable more sensitive analysis of volatiles. The SPACE rod used in the technique is fabricated from stainless steel coated with a graphite carbon adsorbent mixture, which is fixed in the headspace of a closed flask to adsorb the volatiles. Desorption is achieved in a TDS-2 (thermo desorption system) mounted on Agilent GC and sample transfer to column of a GC/MS *via* cryofocussing tool.

Watkins and Wijesundera[46] studied on sample pre-concentration of volatiles of grape aroma compounds by purge and trap using Tenax-TA trapping and SPME. The sample concentration in P & T is dependent on the sample purge time, where as SPME is dependent on the fiber exposure time.

Giardina and Olesik[42] have used low temperature glassy carbon (LTGC) films as a sorbent coating for SPME. The selectivity of these coatings is primarily controlled by shape characteristics of the solute molecule and the final processing temperature used to form the LTGC. The LTGC films are prepared by first coating porous silica particles with a diethynyl oligomer precursor and then by heat curing at temperatures between 300 and 1000 ºC to form LTGC. Then, using sol-gel process, the LTGC coated silica particles are immobilized onto stainless steel fibers and subsequently used for headspace and liquid extractions followed by GC-FID analysis. The selectivity of LTGC over a variety of aromatic hydrocarbons as well as taste and odour contaminants geosmin, 2-methyl isoborneol and 2,4,6-trichloroanisole commonly found in water samples. For comparison 100 µm PDMS and 65 µm PDMS/DVB SPME fibers are used. Interestingly, the PDMS fibers have selectivity similar to LTGC processed at low temperature (< 400 ºC).

Fiber coated with PDMS for extraction: In the foregoing discussion we have briefly described development of SPME as a solvent less extraction technique and the various aspects involved in it as an analytical tool. As has been mentioned earlier SPME is widely used in various applications and some reviews have appeared. To our best of knowledge, no review has appeared on the application of HS-SPME on determination of organic volatiles from plant sources including flowers. The present work is an attempt to address this issue.

Analytes extracted by SPME from the volatiles of ginger (*Zingiber officinale*) were analyzed by Shao *et al.*[12] using 2D-GC using different fibers for SPME. Among four fibers were investigated for the study are 30 µm PDMS, 100 µm PDMS, 75 µm CAR/PDMS and 65 µm PDMS/DVB, the 100 µm PDMS fiber gave better results. These studies come to the conclusion that the adsorption of volatiles is dependent on porosity and chemical nature of the coating. The fibers investigated were porous polar fibers: 75 µm CAR/PDMS and 65 µm PDMS/DVB and non-polar fibers 30 µm PDMS and 100 µm PDMS. It was found that 75 µm CAR/PDMS coating has higher signal response for more volatile components and very poor recovery for larger molecular compounds. The carboxene phase has a total pore volume 0.78 mL/g and mean micropore diameter is 10 Å, is ideal for extracting small molecules in the C2-C12 range. Molecules larger than C12 are strongly retained on the surface of the carboxene particle and are imbedded in the fiber coating and difficult to desorb[47]. In 65 µm PDMS/DVB has larger mean micropore diameter of 17 Å. It produced sufficient extraction yields for all volatiles, which increases the total capacity of the fiber. But high carryover from the previous extraction was observed when using the standard GC injection conditions. Increasing desorption time and temperature were unable to totally eliminate the carryover for analytes which were retained more tightly. In such cases, an extra through cleaning step before each analysis was required. It is observed that PDMS fibers generally show similar GC results in compared to 65 µm PDMS/DVB fibers. 100 µm PDMS having higher extraction efficiency in compared to 30 µm PDMS fiber and additionally no carry over effect was observed with 1 min desorption.

Di *et al.*[48] have used HS-SPME and 2D GC for the chemical profiling of essential oils in ginseng and three other herbs. Within the two contour plots more than 20 marker compounds in American ginseng were identified which helped in authentication and quality control. These authors have used 100 µm PDMS fiber for SPME of headspace volatiles. They used the following conditions for extraction: extraction temperature 70 ºC, extraction time 0.5 h, desorption temperature 250 ºC and desorption time 1 min. The detection sensitivity of less volatile compounds was significantly improved at elevated temperature because of more chemical substances from the herbs partitioned into the headspace of the sample. However, a decrease of peak areas were observed for more volatile compounds at elevated temperature, most likely as a result of reduced fiber-headspace distribution coefficient.

Zabaras and Willie[40] studied the relation between the fiber coating/gas phase partition coefficient of terpenoids and their linear temperature programmed retention index is derived using a group of selected monoterpenoids. The relationship established the simple and accurate determination of terpenoids in the gas phase using SPME with 100 µm PDMS fiber. Goncalves *et al.*[49] have analyzed the *in vitro* shoot culture, micropropagated plants and field grown mother plant of *Lavandula viridis* by using 100 µm PDMS fiber. The major compound were 1,8-cineole (52-74 %) and camphor (2.9-15.3 %) in the headspace of these samples. These oxygenated monoterpenes have better selectivity over PDMS fiber.

Then several authors have studied the adsorption characteristics of the volatiles of different plant species[50-55]. Kovacevic and Kac[50] have evaluated different varieties of hop cones for their aroma components by means of SPME analysis using 100 µm PDMS fibers. Solid phase micro extraction parameters like extraction temperature, extraction time and sample mass were studied. RSD for 11 components used for finger printing was ± 5 %.

Tammela *et al.*[50a] studied on the volatile compound profiles of aging *Pinus sylvestris* L. seeds using 100 µm PDMS fiber. Similarly Santos *et al.*[51] have evaluated the volatile compounds emitted by phloems of four *Pinus* species: *P. halepensis, P. sylvestris, P. pinaster* and *P. pinea* over 100 µm PDMS fiber and discriminated the pine species based on monoterpene emissions. Hern and Dorn *et al.*[52] have monitored seasonal variation in apple fruit volatile emissions *in situ* using SPME on 100 µm PDMS fiber.

Flamini *et al.*[53] have analyzed the essential oil from the aerial parts of *Viola etrusca* growing wild in Monte Labbro (South Tuscany, Italy). Flower volatiles were analyzed using SPME on 100 μm PDMS fiber. The floral volatiles by SPME analysis of yellow flower contained mainly 1,8-cineole (38.9 %), sabinene (16.7 %), (E,E)-α-farnesene (15.2 %), benzyl acetate (3.5 %), α-pinene (3.5 %) and myrcene (3.4 %), while violet flowers contained mainly E-ocimene (43.4 %), limonene (20.6 %), β-sesquiphellandrene (8.0 %), (E,E)-α-farnesene (6.9 %). There were significant composition variation occurred in yellow and violet flowers. The essential oil contained methyl salicylate (96 %). In another work they were sampled the volatiles emitted by fresh whole flowers as well as isolated flower plants of mandarin, *Citrus deliciosa* using SPME with 100 μm PDMS fiber[54]. They have demonstrated emission of distinctive volatile compounds from different parts of the flower. The advantage of SPME over dynamic headspace technique has also been discussed. They have concluded that differences in strength or quality of emitted volatiles between the flower organs may assist the insects in finding food and position themselves suitably for effective pollination.

Pizzolatti *et al.*[55] have analyzed the flowers and roots of the two *Polygala* species by headspace/SPME using 100 μm PDMS fiber. Methyl salicylate (89.1 %, 97.8 %) was found as the main volatile constituent in both the roots of the *P. paniculata* and *P. cyparissias*, respectively. However in the flowers, bornyl acetate (26.0 %, 19.2 %) and 1,8-cineol (13.0 %, 7.2 %) were found as the main compound in *P. paniculata* and *P. cyparissias*, respectively.

Li *et al.*[56] have carried out microwave-distillation HS-SPME (MD-HS-SPME) for determination of volatile organic compounds in traditional Chinese medicines (TCMs). Iron carbonyl powder was used as medium for absorption of microwaves as traditional Chinese medicines contained too little water. Using pulverized rhizomes of *Atradylodes lancea* DC and compared the results with conventional HS-SPME showed that more compounds were isolated and identified by MD-HS-SPME than those by HS-SPME. They have selected 100 μm PDMS, 65 μm PDMS/DVB, 65 μm CW/DVB and 75 μm CAR/PDMS fibers. Headspace extraction was performed at 80 ºC for 0.5 h. The five active compounds α-pinene, E-caryophyllene, γ-elemene, atractylon and β-elemol were present in the *Atradylodes lancea* sample determined in optimal conditions. PDMS fiber has more extraction efficiencies to α-pinene, E-caryophyllene, atractylon and β-elemol, where as only CAR/PDMS has better extraction efficiency to γ-elemene. So authors were concluded the results that PDMS fiber was superior in comparison to other fibers.

An *et al.*[57] have used 100 μm PDMS fiber for SPME and collected the volatile from flowering tops of living lavender (*Lavendula aryastufolia* L.) and also headspace volatiles of a commercial sample of the essential oil. The peak area was increasing steadily upto 0.5 h, so indicating that the equilibrium was not reached. Equilibrium period of 1 h and desorption time of 10 min was found to be adequate. The headspace composition of the living flowers obtained by SPME compared very well with most of the major components in the hexane extract of the flowers, but differs distinctively with headspace of the essential oil with respect to 3-octanone, terpinen-4-ol and E-caryophyllene.

Nakamura *et al.*[58] have attempted to identify bedding plant *Petunia hybrida* cultivars using 100 μm PDMS fiber in SPME. The floral volatiles emitted by several cultivars were detected. The quantitative analysis was carried out for seven benzenoid compounds *i.e.*, isoeugenol, methyl benzoate, benzyl benzoate are found to be major compounds and benzaldehyde, benzyl alcohol, β-phenyl acetaldehyde, phenyl ethyl alcohol are minor volatile compounds.

Hoit[59] studied the equilibrium constants over 100 μm PDMS fibers that describe the distribution of various volatile components of strawberries between the three phases: sample, headspace and fiber coating. For qualitative analysis of strawberry volatile flavours, SPME has proven to be a valuable sample preparation technique. Solid phase micro extraction reveals that all the key flavour components in the berries and no artifacts due to sample preparation are formed, as seen for the purge and trap method. For quantitative analysis, standard solution containing ethyl butanoate (48.4 mg mL^{-1}), E-2-hexen-1-ol (12.2 mg mL^{-1}), ethyl pentanoate (15.7 mg mL^{-1}), methyl hexanoate (13.5 mg mL^{-1}), 3-methylbutyl propanoate (13.8 mg mL^{-1}), ethyl hexanoate (12 mg mL^{-1}), linalool (45 mg mL^{-1}), E-2-hexenyl butanoate (11.6 mg mL^{-1}) and geraniol (48.2 mg mL^{-1}) was taken in a vial for SPME and direct gas phase sampling. The volumes of the headspace and liquid sample were 12 and 5 mL, respectively. The mass transfer of each component from the sample to the fiber is fitted to a transport model, taking assumption that the rate-controlling step is the diffusion of the components within the fiber. The experimental results agreed well with the model for most of the components but very few components do not agree with the model. So mass adsorbed in the fiber coating is proportional to the amount of the sample. In compared to headspace analysis, the components *i.e.*, linalool, E-2-hexenyl benzoate and geraniol are hardly or not at all, detectable by injection of the headspace gas but all three components are present in SPME analysis in easily detectable amounts.

Deng *et al.*[60] have developed the pressurized hot water extraction (PHWE) with HS-SPME for determination of essential oil in *Fructus amomi*. Parameters of PHWE and SPME and method validation were studied. 0.05 g of *Fructus amomi* sample was extracted at three different temperatures 125, 150 and 175 ºC with pressure 20, 50 and 80 bar. The aqueous extract after PHWE was cooled in refrigerator at 25 ºC and collected in 10 mL vial. The analytes in the aqueous extract (2 mL) were headspace extracted by 100 μm PDMS, 65 μm PDMS/DVB, 65 μm CW/DVB and 75 μm CAR/PDMS fibers at 80 ºC for 15 min. In comparing the extraction efficiencies of eight compounds, the 100 μm PDMS fiber was found suitable. The analytes adsorbed on the fiber were desorbed at 270 ºC for 2 min. There are 35 compounds were separated and identified. The volatiles constituents present as camphor, camphene, limonene, borneol, bornyl acetate and caryophyllene, the active compounds camphor, borneol and bornyl acetate were found to be present in the essential oil. PHWE-HS-SPME was applied to quantitative analysis of active compound *i.e.*, camphor. In same experimental set up and conditions, Dong

et al.[61] have quantitatively estimated the three active compounds of eucalyptol, camphor and borneol in the chrysanthemum flower collected from four geographical locations: Huangshan, Bozhou, Chuzhou and Hangzhou. The experimental result was agreed with Dong *et al.*[61] that, the PDMS fiber was found suitable at 80 ºC for 15 min. Diaz *et al.*[62] study the optimization of truffle aroma analysis by SPME and compared the extraction with different polarity fibers. A response surface experimental design was applied considering three factors such as extraction temperature, equilibrium time and extraction time. Results presented in this study showed that truffle volatiles compounds analysis by HS-SPME is mainly influenced by extraction temperature, independently of the polarity and nature of fiber used. A non-polar fiber, a quadratic effect of temperature was observed, being the optimum response found at medium temperature (~50 ºC). On the other hand polar fiber has moderate quadratic effect temperature, the response found at high temperature (~60 ºC). To characterize the *T. aestivum* aroma, it seemed more appropriate to use PDMS fiber, since although the sum of areas was smaller than with the CW/DVB fiber.

Kurkcuoglu and Baser[63] studied the HS-SPME analysis of Turkish rose concrete, absolute and hydrosol by using PDMS fiber. Phenyl ethyl alcohol and citronellol were characterized as main constituents. However HS-SPME of rose concrete showed highly comparable results with absolute.

The floral volatiles mainly contain monoterpenes (C10) and sesquiterpenes (C15) and their derivates. So PDMS is the suitable liquid phase for extraction of floral fragrance and easy to analysis without carryover of residue.

Fiber coated with liquid phase (PDMS) and porous solid (CAR or DVB or both): Zhao *et al.*[64] have analyzed the volatile components during potherb mustard (*Brassica juncea* Coss) pickle fermentation using SPME-GC-MS. The 100 µm PDMS coating as chosen because of the high reproducibility presented and lower coefficients of variance obtained as compared to CAR/PDMS fiber. Solid phase micro extraction sampling parameters *i.e.*, 1 h equilibrium between sample and headspace was sufficient to reach partition equilibrium and 5 min sufficient for fiber adsorption. The reproducibility of the method was tested by the peak area abundance of internal standard in six replicate analysis of the same sample. It indicated that the proposed method was satisfied for analysis of volatile components in the fresh vegetable and their pickles. The analysis of fresh potherb mustard and their pickles, total 87 components were identified. In these conditions, the extraction method was reproducible and almost identical chromatograms were observed with very small variability in relative peak area composition. HS-SPME-GC-MS can provide an excellent methodology for qualitative analysis of flavour components in fresh potherb mustard and pickles in compared to Tenax-GC-MS (purge & Trap) method[47]. HS-SPME with PDMS fiber was suitable for determination of volatiles in potherb mustard and their pickles, on the other hand only few components were identified by Tenax-GC-MS method with extensive sampling.

Yu *et al.*[65] have analyzed and compared the volatiles compounds obtained by simultaneous-distillation extraction (SDE) and SPME of *Pinus densiflora* (red wine) needles. They have used 100 µm PDMS and 75 µm CAR/PDMS fibers for SPME. The adsorption of monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes are comparable over both the fibers. The CAR/PDMS fiber showed higher flavour dilution factor, which had higher adsorption efficiency for aliphatic aldehyde *i.e.*, Z-3-hexenal in comparison to PDMS fiber.

Zini *et al.*[66] have monitored biogenic volatile compounds (BVOC) emitted by *Eucalyptus citriodora* leaves from living plants using SPME. They have investigated 65 µm PDMS/DVB, 75 µm CAR/PDMS and 100 µm PDMS and selected porous polymer coated fibers PDMS/DVB and CAR/PDMS for detail studies. Diffusion based quantification model was used for determination of isoprene from healthy and damaged leaves of *E. citriodora*. Isoperene, citronellol, citronellal and β-caryophyllene were detected in more than 10 % relative abundance and Z- and E- rose oxides along with other monoterpenes were detected in more than 1 % RA.

Bicchi *et al.*[67] have studied influence of fiber coating in the analysis of aromatic and medicinal plants by using HS-SPME. The performance of eight commercially available SPME fibers of different polarities and structures was compared in the recovery of some characteristic components of the plants: rosemary, sage, thyme and valerian. The relative capacity of each fiber on the same analyte of a plant was determined by comparing their abundance with that obtained by classical static headspace analysis. They have selected different fibers for study were 7 µm PDMS, 30 µm PDMS, 100 µm PDMS, 65 µm CAR/DVB, 75 µm CAR/PDMS, 85 µm PA, 65 µm PDMS/DVB, 50/30 µm CAR/DVB/PDMS fibers. The profiles of three PDMS fibers (7 µm PDMS, 30 µm PDMS, 100 µm PDMS) were qualitatively similar for all the components, but variation of relative abundance was not directly related to the volume of the polymeric coating. As a general consideration, all the most effective fibers contained PDMS, a liquid phase favouring adsorption of non-polar analytes as well as a porous solid (CAR or DVB or both) that favours the adsorption of polar analytes. In a study of microwave distillation of *Acorus tatarinowii* for extraction of essential oil, Ye *et al.*[68] were analyzed the volatiles by SPME-GC/MS using different fibers *viz.*, 100 µm PDMS, 65 µm PDMS-DVB, 65 µm CW-DVB, 75 µm CAR-PDMS. Among these fibers 65 µm PDMS-DVB gave better extraction efficiency.

Esteban *et al.*[69] have studied the combination of different fiber coatings and extraction conditions on dry cured ham volatiles. The fiber coatings tested were 85 µm CAR/PDMS, 50/30 µm DVB/CAR/PDMS, 100 µm PDMS and 65 µm PDMS/DVB using different extraction times: 15, 30 and 60 min. The fiber coatings with CAR/PDMS and DVB/CAR/PDMS extracted more than 100 volatile components and have shown the highest area counts for most of the volatile components of dry cured ham. Similarly the fibers coated with CAR/PDMS extracted preferably low molecular weight compounds, while DVB/CAR/PDMS extracted preferably high molecular weight compounds.

Porto *et al.*[70] have analyzed the orange spirit distillate by direct injection GC-MS and headspace SPME coupled to GC-MS70. Among 100 µm PDMS, 65 µm PDMS/DVB, 75 µm CAR/PDMS and 50/30 µm DVB/CAR/PDMS, the 50/30 µm DVB/CAR/PDMS showed the lowest amount of variation

among replicates for volatile compounds considered. It has also exhibited high affinity for non-polar and semi-polar volatiles and poor sensitivity to polar compounds. Headspace SPME GC/MS selectivity was found to be comparable to GC direct injection for most orange spirit volatile components.

Kim and Lee[71] studied the volatiles from the dried lemon verbena (*Aloysia triphylla*) using six different commercially available SPME fibers *viz.*, 100 μm PDMS, 30 μm PDMS, 7 μm PDMS, 85 μm PA, 65 μm CW/DVB and 50/30 μm DVB/CAR/PDMS fibers using 1 h equilibrium time. To optimize the experimental conditions, the isolation of volatile compounds from *Lemon verbena* was performed using a mixture of standards containing limonene, cineole, geranial, neral, α-terpineol and β-caryophyllene. The 50/30 μm DVB/CAR/PDMS fiber afforded better HS-SPME recoveries of the fragrance compounds and closely comparable with 100 μm PDMS fiber. The mixture of limonene, geranial, neral and β-caryophyllene was attained equilibrium on 100 μm PDMS fiber in 0.5 h, whereas, 1,8-cineole and α-terpineol were attained equilibrium in 40 min. The more polar coating of CAR/PDMS and CAR/DVB/PDMS produced better efficiencies for geranial, neral and geranyl acetate.

Perestrelo *et al.*[72] have studied the volatile flavour constituent patterns of *Terras madeireneses* red wines by HS-SPME. They tested three types of fibers 100 μm PDMS, 85 μm PA and 50/30 μm DVB/CAR/PDMS at 30 ºC and 1 h extraction time. Under these conditions DVB/CAR/PDMS fiber had a low sorption capacity. The more polar fiber (PA), showed effective extraction for polar compounds such as higher alcohols and fatty acids, whereas PDMS fiber favours the extraction of less polar compounds like ethyl esters, monoterpenoids, sesquiterpenoids, acetates and isoamyl esters.

Deng *et al.*[73] studied the on-fiber derivatization involved headspace extraction and determination of plant signaling compounds: C6 aldehydes hexanal, Z-3-hexenal and E-2-hexenal in tomato plant emission by SPME-GC/MS with on fiber derivatization. They first adsorbed 2,3,4,5,6-pentaflurobenzyl hydroxylamine (PFBHA) over PDMS/DVB fiber and then used it for headspace extraction of tomato plant emission. PDMS/DVB fiber was selected because it adsorbed PFBHA with greater reproducibility in comparison to 100 μm PDMS fiber. The method had low detection limit values for the three aldehydes from 0.1 to 0.5 ng/L and good precision (RSD < 10 %). Similarly Mu *et al.*[74] studied the microwave extraction (MAE) and HS-SPME of *Toona sinensis* over PDMS/DVB fiber. Total 45 compounds were identified with major compound E-caryophyllne (21.4 %) and good precision (RSD < 9 %).

Li *et al.*[75] have studied the volatile composition of *Syringa oblata* flowers by using HS-SPME-GC/MS. The optimum condition of a 65 μm PDMS/DVB, extraction temperature 25 ºC and extraction time 0.5 h were obtained and applied to extraction of volatile compounds emitted by fresh flowers of *S. oblate*. The 65 μm CW/DVB fiber coating is least preferable in comparison to 30 μm PDMS and 65 μm PDMS/DVB fibers. The 65 μm PDMS/DVB fibers adsorbed some compounds preferably over the other compounds and total absorption capacity is highest in comparison to 30 μm PDMS fibers. It may be happened due to less surface area of the 30 μm PDMS fiber.

Musteata and Pawliszyn[76,77] have summarized their work in application of SPME on bioanalysis and divided in eight main groups according to the type of analytes: toxicological and forensic analysis, drugs of abuse, clinical chemistry, analysis of pharmaceuticals in biological samples, biochemical analysis, semiochemical analysis and analysis of natural products. The fiber coated with PDMS or PDMS/DVB was most suitable for extraction of analytes, in the chemical groups discussed above. The nature of target analytes and complexity of sample matrix determined the level of difficulties in performing successful extraction. The following steps were usually required when a new SPME method developed are: selecting the fiber coating, selection of extraction mode, selection of agitation method, selection of separation/detection technique, optimization of desorption conditions, optimization of sample volume, determination of extraction time, optimization of extraction conditions (pH, salt, temperature), determination of linear range for pure matrix at optimum extraction conditions, optimization of extraction conditions for heterogeneous samples, determination of method detection limit[77].

Zhang *et al.*[43] have analyzed the volatile compounds in the dry rhizome of *Ligusticum chuanxiong* Hort by using 100 μm PDMS, 65 μm PDMS/DVB and 50/30 μm DVB/CAR/PDMS fibers. They were separated and identified 73 components in a polar capillary column by HS-SPME-GC-MS. The qualitative analyses of above three fibers were comparable. Their observation was the extraction capacity of DVB/CAR/PDMS fiber was more due to the length of DVB/CAR/PDMS was twice in comparison to the other two fibers. There was hardly any difference between the extraction capacities of 100 μm PDMS and 65 μm PDMS/DVB fibers. Similarly Bothe *et al.*[78] determine the perfume oil in household products by HS-SPME by using 100 μm PDMS, 70 μm CAR/DVB, 85 μm PA and 50/30 μm DVB/CAR/PDMS fibers. Among these fibers, 100 μm PDMS and 50/30 μm DVB/CAR/PDMS were most suitable fiber coatings for headspace extraction of fragrances from the household product analyzed. The DVB/CAR/PDMS fiber, however proved unstable, leaving PDMS as the coating of choice.

Practical application: Finally taking in depth consideration of all reported work on essential oil, it was concluded that 100 μm PDMS or 65 μm PDMS/DVB fibers were suitable for extraction of floral volatiles emitted by flowers. The head space analysis of some flowers using HS-SPME has been carried out by us using 100 μm PDMS fiber[79-87]. We present here the live and picked flower composition of *Jasminum sambac*, *Michelia champaca*, *Murraya paniculata*, *Quisqualis indica* and *Gardenia jasminoides*.

Experimental set up: Head-space solid phase micro extraction was carried out as follows: A 100 mL conical flask provided with a B40 and a B14 joint was used for equilibrating the fragrance emitted by the flowers in the headspace with a PDMS fiber. The B14 joint was sealed with a screw cap provided with a silicone rubber septum for introducing the SPME manual holder (Fig. 2). The thickness of stationary phase (PDMS) coating was 100 μm and the length is 10.6 mm.

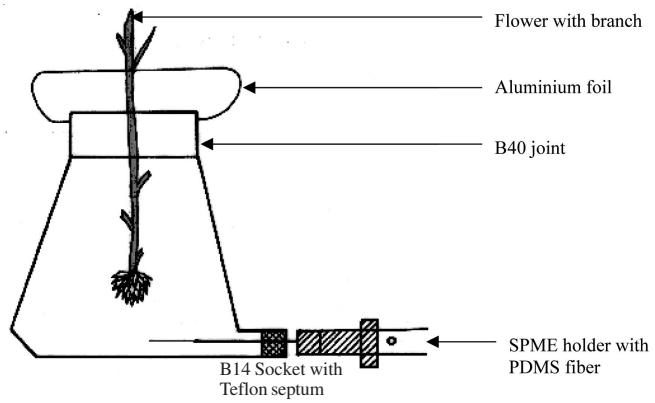

Fig. 2. SPME set up for floral volatiles pre-concentration over PDMS fiber

Before use, the fibers were conditioned at 250 ºC for 2 h. A branch of the tree carrying two flowers (living flowers) was carefully introduced into the flask through the wider mouth and the mouth was closed with aluminum foil and then parafilm. The headspace was allowed to attain equilibrium with the volatile components emitted by the flowers during the next 0.5 h. Then SPME manual sample holder used for outdoor sampling was introduced through the septum within 2 cm distance from the flowers and the PDMS fiber exposed for 1-2 h for achieving equilibrium. The fiber was withdrawn into the needle and then the needle was removed from the septum and inserted directly into the injection port of the GC. The analytes from the fiber coating were desorbed by heating the fiber in the injection port at 250 ºC for 4-5 min. The analytes were transferred directly into the chromatographic column for analysis. Between extractions, the fiber was conditioned for 10 min at 250 ºC to prevent carryover of residues. The fragrance emitted by freshly picked flowers (without branch) was analogously carried out. A second equilibration with PDMS fiber of the same live and picked flowers was carried out after carefully transferring the flowers to a fresh setup by means of a forceps. After withdrawing the fiber into the holder, it was brought to the laboratory for analysis. The percentage composition was determined by GC-FID and compounds were identified by GC/MS. The compound identification was finally confirmed by comparison of their relative retention indices with literature values[7,88].

Analysis results: The chemical compositions of selected flowers are given in Tables 2-6. A comparison of the different classes of compounds identified in present studies clearly indicated that higher amounts of sesquiterpenes were present in the floral headspace (Table-1). The live and picked flowers contained 62-87 and 43-64 % of sesquitepene hydrocarbons respectively. The 100 µm PDMS fiber was extracted effectively the sesquiterpene hydrocarbons and oxygenated sesquiterpenoids[65]. The sesquiterpene hydrocarbons like (E,E)-α-farnesene, germacrene-D, δ-cadinene and E-caryophyllene were some of the major components in these analyzed flowers. The picked flowers contained higher percentage of monoterpenoids, whereas the live flowers contained higher amounts of sesquiterpenoids. While the headspace of the living flowers contained higher amounts of methyl benzoate, hexyl tiglate, benzyl tiglate, hexenyl phenyl acetate, benzyl salicylate along with germacrene-D, (E,E)-α-farnesene and E-caryophyllene, the picked flowers were found to contained more amounts of E-β-ocimene and linalool. Linalool was released significantly in picked flowers in comparison to live flowers. Mookherjee *et al.*[1] have observed the increase of indole, Z-jasmone, decrease of benzyl alcohol, E-β-ocimene, linalool in the living flowers of *Jasminum grandiflorum* in comparison to the picked flowers. Indole and methyl anthranilate present in higher amounts in the flower headspace contributes to the sweet fragrance of the flower. Z and E-linalool oxides, benzyl acetate, ethyl benzoate, benzyl benzoate, myrcene and linalool were released more in the picked flowers[1]. In Table-1, the percentage of benzenoids in live and picked flowers is closed except Jasmine. In a similar HS-SPME study Edris *et al.*[89] have analyzed the volatile aroma compounds from the concrete headspace and absolute of *Jasminum sambac* (L.) flowers grown in Egypt by using 100 µm PDMS fiber. The volatile profile of July flowers were characterized by the presence of seven major components in both the concrete HS and absolute respectively, including benzyl acetate, indole, (E,E)-α-farnesene, Z-3-hexenyl benzoate, benzyl alcohol, linalool and methyl anthranilate. There is no significant difference between the major components *viz.*, indole, methyl anthranilate and (E,E)-α-farnesene in concrete HS and absolute. The same finding was previously reported for Chinese *J. sambac* in which indole and methyl anthranilate were also found in comparable proportions in concrete and in the absolute[90]. The benzyl acetate present in significant amount in picked flowers of jasmine in compared to live flowers. HS-SPME analyses of these live and picked flowers[78-87] were reported first time.

Conclusion

HS-SPME is a very simple, solvent less extraction technique and the extraction is readily performed in the field without requirement of electricity and special arrangement. It is a new method of sample preparation, which combines extraction and concentration in a single step and in one device. Poly dimethyl siloxane is a non-polar liquid phase having more affinity for

TABLE-2
HS-SPME PROXIMATE COMPOSITIONS OF LIVE AND PICKED FLORAL FRAGRANCE

Flowers	Proximate compositions									
	Monoterpene hydrocarbons		Oxygenated monoterpenes		Sesquiterpene hydrocarbons		Oxygenated sesquiterpenes		Benzenoids	
	Live	Picked	Live	Picked	Live	Picked	Live	Picked	Live	Picked
Jasminum sambac	0.4±0.1	6.3±0.1	2.4±0.2	13.6±0.1	87.0±1.7	47.7±1.3	0.9±0.1	0.3±0.1	6.2±0.4	14.2±0.3
Michelia champaca	1.0	0.5	-	-	66.9	64.8	0.6	0.8	31.9	28.2
Murraya paniculata	1.8±0.2	2.2±0.2	3.1±0.1	9.4±0.2	72.4±2.7	61.2±1.8	t	0.1	5.8±0.5	6.3±0.5
Quisqualis indica	-	-	13.7±0.5	28.3±0.2	62.6±0.4*	51.2±2.3*	1.3±0.2	0.7±0.1	14.2±1.7	12.2±1.7

*Sesquiterpene hydrocarbons and unidentified hydrocarbons

TABLE-3
COMPARATIVE PERCENTAGE OF LIVE AND PICKED FLOWERS OF *Jasminum sambac* Linn

Compound	A	B	C	D	E	RRI lit
Ethyl acetate	-	-	0.8	3.5	2.0	807
3-Hexen-1-ol	-	-	< 0.1	< 0.1	0.2	857
Benzaldehyde	-	-	-	-	0.1	961
6-Methyl-5-hepten-2-one	t	-	-	0.1	<0.1	991
Z-3-Hexenyl acetate	0.9	0.9	1.0	12.0	11.9	1007
Benzyl alcohol	0.1	-	-	0.1	0.1	1032
E-β-ocimene	0.5	0.5	< 0.1	6.5	6.0	1050
Linalool	0.9	0.8	< 0.1	13.7	12.8	1098
Benzyl acetate	2.4	1.8	0.1	9.0	7.4	1163
α-Terpineol	0.1	0.1	< 0.1	0.2	0.1	1189
Methyl salicylate	0.4	0.4	< 0.1	2.0	2.0	1190
Z-3-hexenyl-3-methyl butyrate*	0.2	0.2	0.1	0.9	0.8	-
Geraniol	0.1	< 0.1	< 0.1	< 0.1	0.1	1255
Ethyl salicylate	-	-	-	0.1	<0.1	1267
Indole	0.4	0.3	< 0.1	0.7	0.7	1288
Methyl anthranilate	1.9	1.9	0.6	2.0	2.4	1337
δ-Elemene	0.3	0.2	0.2	0.4	0.4	1339
Butyl benzoate*	0.2	0.2	< 0.1	0.2	0.1	-
β-Elemene	0.4	0.5	0.4	0.5	0.7	1391
Z-Jasmone	0.1	0.1	< 0.1	0.4	0.4	1393
Z-Caryophyllene	0.6	0.6	< 0.1	1.0	1.0	1404
β-Gurjunene	< 0.1	0.2	0.1	0.2	0.2	1432
α-Humulene	0.1	0.2	0.1	0.2	0.1	1454
E-β-farnesene	0.3	0.4	0.4	0.3	0.2	1458
Germacrene-D	1.4	1.4	1.1	2.4	2.4	1480
(Z,Z)-α-farnesene	2.0	1.4	1.7	1.0	1.0	-
(E,E)-α-farnesene	77.7	78.1	80.5	37.6	42.2	1508
δ-Cadinene	2.0	1.5	1.7	0.4	0.7	1524
Cadina-1,4-diene	0.2	< 0.1	0.1	< 0.1	< 0.1	1532
Z-Nerolidol	0.1	< 0.1	0.1	< 0.1	< 0.1	1534
E-Nerolidol	0.2	0.1	0.2	< 0.1	< 0.1	1564
Z-3-Hexenyl benzoate	2.2	2.4	3.0	0.8	1.4	1570
Hexyl benzoate	0.1	0.1	0.2	0.1	0.1	1576
Cedrol	0.4	0.6	0.6	0.2	0.2	1596

*Tentative identification based on mass fragmentation pattern; A: Headspace analysis of live flowers with branch and equilibrium over the PDMS fiber for 2 h; B: Transferring the flowers of "A" to a new set up and equilibrium over the PDMS fiber for 2 h; C: Transferring the flowers of "B" to a new set up and equilibrium over the PDMS fiber for 16 h; D: Headspace analysis of picked flowers equilibrated over the PDMS fiber for 1.5 h; E: Transferring the flowers of "D" to a new set up and equilibrium over the PDMS fiber for 1.5 h; RRI lit: Relative retention indices literature reported

TABLE-4
COMPARATIVE PERCENTAGE OF LIVE AND PICKED FLOWERS OF *Michelia champaca* Linn

Compound	A	B	RRI lit
E-β-Ocimene	1.0±0.3	0.5±0.2	1050
Methyl benzoate	23.4±1.4	21.2±1.2	1091
Phenyl ethyl alcohol	1.3±0.4	0.8±0.2	1110
Phenyl acetonitrile	4.6±0.6	4.0±0.5	-
Indole	3.5±0.3	3.3±0.4	1288
Methyl anthranilate	1.0±0.2	0.7±0.2	1337
δ-Elemene	1.0±0.2	0.7±0.2	1339
α-Copaene	1.5±0.4	0.9±0.3	1376
β-Copaene	0.8±0.3	0.6±0.2	1390
β-Elemene	5.7±0.7	3.3±0.5	1391
E-Caryophyllene	4.2±0.6	2.8±0.5	1418
γ-Elemene	0.6±0.2	0.7±0.3	1433
α-E-Bergamotene	1.3±0.4	1.1±0.3	1436
epi-α-Muurolene	1.9±0.4	1.7±0.3	1441
E-β-Farnesene	1.2±0.3	0.9±0.2	1458
9-epi-E-Caryophyllene	0.8±0.2	0.6±0.2	1467
Germacrene-D	7.3±0.7	4.8±0.6	1480
E-β-Ionone	0.6±0.2	0.8±0.2	1485
Zingiberene	2.1±0.6	1.6±0.3	1495
(E,E)-α-Farnesene	32.7±1.8	44.6±2.0	1508
δ-Cadinene	0.7±0.2	0.7±0.2	1524
Methyl palmitate	-	0.2±0.1	1927

A: Headspace analysis of live flowers with branch and equilibrium over the PDMS fiber for 1 h; B Headspace analysis of picked flowers equilibrated over the PDMS fiber for 1 h.

non-polar compounds. The equilibrium is attained quickly between the volatile compounds and liquid phase of the fiber due to faster diffusion of floral components in the vapour phase. The amount of analyte extracted into the fiber is linearly proportional to its initial concentration in the sample matrix[43]. The polymeric film on the fiber concentrates the organic analytes on its surface either through adsorption or absorption or both. However, when dealing with a multi-component system such as floral volatile compounds, as the components collect on the PDMS fiber, its partition behaviour can no more be the same as the original coating. When the headspace is in equilibrium with the floral compounds given by the blooming flower, the composition of the volatiles collected and concentrated on the PDMS fiber may be considered close to the headspace composition. The suitability of PDMS for such studies has been documented by previous workers[72,76]. The extraction condition was optimized by several workers; the extraction was carried at nearly room temperature (25-35 ºC) and equilibrium was achieved within a short period of time *i.e.*, 10-60 min[12,71,75].

Head-space solid phase micro extraction is a simple and convenient way to analyze the fragrance composition emitted by flowers at different stages of their development. The composition of headspace of live and picked flowers vary

TABLE-5
COMPARATIVE PERCENTAGE OF LIVE AND PICKED FLOWERS OF *Murraya paniculata* Linn

Compound	A	B	C	D	RRI lit
Benzaldehyde	-	-	0.10	0.10	961
Myrcene	0.7±0.14	0.3±0.15	1.1±0.30	1.3±0.25	991
E-β-ocimene	0.6±0.10	1.7±0.20	0.5±0.05	1.1±0.10	1050
Linalool	2.0±0.20	4.0±0.30	7.3±0.45	11.2±0.20	1098
Phenyl ethyl alcohol	0.7±0.10	0.9±0.10	1.2±0.10	3.0±0.35	1110
Phenyl acetonitrile	0.4±0.05	0.10	0.3±0.10	0.6±0.05	-
Ethyl benzoate	0.6±0.10	1.6±0.15	3.9±0.20	1.1±0.15	1170
Methyl salicylate	0.9±0.10	0.9±0.05	0.5±0.10	0.8±0.10	1190
Ethyl salicylate	0.4±0.10	0.10	<0.10	0.2±0.10	1267
Indole	6.6±0.70	6.0±0.90	4.3±0.40	5.2±0.80	1288
α-Copaene	0.3±0.10	0.4±0.10	0.5±0.10	1.8±0.20	1376
β-Elemene	2.2±0.14	1.8±0.10	2.2±0.10	2.8±0.20	1391
Z-Jasmone	0.3±0.10	0.2±0.04	0.3±0.03	0.4±0.06	1394
E-Caryophyllene	7.4±0.60	7.3±0.50	7.0±0.30	6.9±0.20	1418
Z-β-Farnesene	3.9±0.20	2.5±0.10	2.5±0.24	3.1±0.20	1443
E-β-Farnesene	2.0±0.10	1.5±0.10	2.0±0.20	3.3±0.35	1458
Germacrene-D	39.6±2.50	43.4±3.90	37.5±1.70	27.5±1.90	1480
Bicyclogermacrene	6.9±0.80	6.7±0.70	6.3±0.20	5.4±0.30	1494
(E,E)-α-Farnesene	5.8±0.15	3.8±0.20	3.5±0.40	3.0±0.30	1508
δ-Cadinene	2.1±0.15	2.1±0.20	2.2±0.20	1.4±0.24	1524
E-Nerolidol	-	< 0.10	0.10	< 0.10	1564
Benzyl benzoate	1.1±0.20	1.2±0.40	2.5±0.30	0.6±0.15	1762
Phenethyl benzoate	0.4±0.10	0.3±0.05	0.3±0.10	0.2±0.04	-
Benzyl salicylate	0.8±0.20	0.3±0.20	0.4±0.30	< 0.10	1863
Methyl palmitate	-	0.6±0.24	0.4±0.20	0.6±0.20	1927

A: Headspace analysis of live flowers with branch and equilibrium over the PDMS fiber for 1.5 h; B: Transferring the flowers of "A" to a new set up and equilibrium over the PDMS fiber for 1.5 h; C: Headspace analysis of picked flower equilibrated over the PDMS fiber for 1.5 h; D: Transferring the flowers of "C" to a new set up and equilibrium over the PDMS fiber for 1.5 h.

TABLE-6
COMPARATIVE PERCENTAGE OF LIVE AND PICKED FLOWERS OF *Quisqualis indica* Dent

Compound	A	B	C	D	E	RRI lit
E-Hexenyl acetate	<0.1	0.3±0.1	<0.1	-	-	1007
E-Linalool oxide	0.6±0.1	1.4±0.2	5.8±0.4	4.2±0.4	4.6±0.5	1088
Methyl benzoate	0.6±0.1	1.5±02	3.7±0.4	4.4±0.4	3.0±0.2	1091
2,2,6-Trimethyl-6-vinyl-tetrahydro pyran-3-one	7.8±0.4	5.6±0.4	14.7±1.2	19.5±1.1	17.7±1.4	-
Hexenyl isobutyrate	-	-	-	0.4±0.1	-	1145
2,2,6-Trimethyl-6-vinyl-tetrahydro pyran-3-ol	0.8±0.1	1.2±0.1	1.3±0.2	1.2±0.2	1.2±0.2	-
Z-3-Hexenyl butyrate	-	-	-	-	0.3±0.1	1186
Z-3-Hexenyl-2-methyl butyrate	1.3±0.25	0.5±0.1	-	-	0.3±0.1	1134
Hexyl-3-methyl butyrate	-	-	2.9±0.3	3.3±0.4	1.4±0.2	1143
Unidentified hydrocarbon	35.1±0.5	30.5±0.4	35.8±0.6	31.0±0.5	33.3±1.0	-
Hexyl tiglate	4.5±0.5	3.0±0.3	3.4±0.2	4.2±0.5	3.6±0.4	1331
Quinoline-4-carbonitrile	5.8±0.4	5.6±0.5	1.7±0.2	1.0±0.2	1.3±0.1	-
Germacrene-D	0.3	0.3	0.2±0.1	-	0.1	1480
δ-Decalactone	1.2±0.4	1.0±0.2	0.7±0.1	-	0.7±0.2	1493
Benzyl tiglate	1.0±0.3	1.3±0.3	0.8±0.2	0.6±0.1	0.8±0.2	1496
(E,E)-α-Farnesene	28.0±0.3	30.3±0.5	16.8±0.5	16.5±0.5	13.1±0.4	1508
Z-3-Hexenyl benzoate	2.0±0.2	2.2±0.2	5.1±0.5	2.6±0.3	3.1±0.5	1570
Hexyl benzoate	1.6±0.3	2.5±0.3	1.2±0.2	2.4±0.3	1.4±0.2	1576
E-2-Phenyl ethyl tiglate	0.8±0.2	1.3±0.3	2.0±0.2	<0.1	2.4±0.3	1584
Hexenyl phenyl acetate	0.2±0.05	0.3±0.05	0.2±0.05	-	0.1±0.05	1631
Benzyl benzoate	0.5±0.3	1.2±0.4	-	0.2±0.1	-	1762
Methyl palmitate	0.3±0.1	0.8±0.3	0.4±0.15	0.5±0.15	0.3±0.1	1927
Octadecadienol	1.3±0.3	1.6±0.4	-	0.8±0.2	0.4±0.1	2092

*Tentative identification based on mass fragmentation pattern; A: Headspace analysis of live flowers with branch and equilibrium over the PDMS fiber for 1.5 h; B: Transferring the flowers of "A" to a new set up and equilibrium over the PDMS fiber for 1.5 h; C: Headspace analysis of picked flowers equilibrated over the PDMS fiber for 1.5 h; D: Transferring the flowers of "C" to a new set up and equilibrium over the PDMS fiber for 1.5 h; E: Transferring the flowers of "D" to a new set up and equilibrium over the PDMS fiber for 2 h.

significantly and it appears that a major biological change occurs at the time of picking[1].

Solid phase micro extraction is an alternative quick method for extraction and concentration of floral volatiles present in the headspace. It needs fewer samples, shorter extraction time, simple procedure and no solvents. Solid phase micro extraction combined with GC/MS has been developed as a powerful method to analyze the volatile constituent from flower. Application of SPME technique permitted us to collect and analyze the volatiles emitted from live and picked flowers and avoiding the formation of artifacts due to damage of the plant. Though PDMS fiber is a non-polar liquid phase and has higher affinity for non-polar components, due to fast equilibrium in the vapour phase and presence of several components, at equilibrium the analytes composition collected and concentrated on the PDMS fiber may be considered comparable to the headspace composition of flowers and the technique is suitable for analysis of floral volatiles. Finally SPME integrates sampling, extraction, concentration and sample introduction into a single solvent free step for analysis of floral volatiles.

REFERENCES

1. B.D. Mookherjee, R.W. Trenkle and B.A. Wilson, *Pure Appl. Chem.*, **62**, 1357 (1990).
2. E. Geunther, The Essential Oils, D. Van Nostrand Co. Inc., New York (1952).
3. J.T. Knudsen, L. Tousten and G. Bergstoim, *Phytochemistry*, **33**, 253 (1993).
4. K.C. Zancan, M.O.M. Marques, A.J. Petenate and M.A.M. Meirles, *J. Supercrit. Fluids*, **24**, 57 (2002).
5. J.P. Bartley, *J. Sci. Food Agric.*, **68**, 215 (1995).
6. J.P. Bartley and P. Foley, *J. Sci. Food Agric.*, **22**, 365 (1994).
7. P.K. Rout, S.N. Naik, Y.R. Rao, G. Jadeja and R.C. Maheshwari, *J. Supercrit. Fluids*, **42**, 334 (2007).
8. M.J. Alfaro, J.M.R. Belanger, F.C. Padilla and J.R.J. Pare, *Food Res. Int.*, **36**, 499 (2003).
9. F. Augusto, A.L. Lopes and C.A. Zini, *Trends Anal. Chem.*, **22**, 160 (2003).
10. N.-S. Kim and D.-S. Lee, *J. Sep. Sci.*, **27**, 96 (2004).
11. H.-J. Kim, K. Kim, N.S. Kim and D.S. Lee, *J. Chromatogr. A*, **902**, 389 (2000).
12. Y. Shao, P. Marriott, R. Shellie and H. Hugel, *Flav. Fragr. J.*, **18**, 5 (2003).
13. C.L. Arthur and J. Pawliszyn, *Anal. Chem.*, **62**, 2145 (1990).
14. A. Penalver, E. Pocurull, F. Borrull and R.M. Marce, *Trends Anal. Chem.*, **18**, 557 (1999).
15. T. Gorecki and J. Nameisnik, *Trends Anal. Chem.*, **21**, 276 (2002).
16. J. Nameisnik, B. Zabeigala, A. Kot-wasik, M. Partyka and A. Wasik, *Anal. Bioanal. Chem.*, **381**, 279 (2005).
17. B. Verna, G.A. Mills, I.J. Allan, E. Dominiak, K. Swensson, J. Knutsson, G. Morrison and R. Greenwood, *Trends Anal. Chem.*, **24**, 845 (2005).
18. C.J. Koesten and A. Moulik, *Anal. Chem.*, **77**, 3737 (2005).
19. J.S. Aulakh, A. Malik, V. Kaur and P. Schmitt-Kopplin, *Crit. Rev. Anal. Chem.*, **35**, 71 (2005).
20. J. Namcisnik, B. Zygmunt and A. Jastrzebska, *J. Chromatogr. A*, **885**, 405 (2000).
21. J.A. Koziel and I. Novak, *Trends Anal. Chem.*, **21**, 840 (2002).
22. C. Dietz, J. Sanz and C. Camara, *J. Chromatogr. A*, **1103**, 183 (2006).
23. F. Augusto and A.L.P. Valente, *Trends Anal. Chem.*, **21**, 428 (2002).
24. H. Lord and J. Pawliszyn, *J. Chromatogr. A*, **885**, 153 (2000).
25. H. Prosen and L. Zupancic-Kralj, *Trends Anal. Chem.*, **18**, 272 (1999).
26. M.F. Plpendurada, *J. Chromatogr. A*, **889**, 3 (2000).
27. G. Vas and K. Vekey, *J. Mass Spectrom.*, **39**, 233 (2004).
28. E.E. Stashenko and J.R. Mantinez, *Trends Anal. Chem.*, **23**, 553 (2004).
29. G. Ouyang and J. Pawliszyn, *Trends Anal. Chem.*, **25**, 692 (2006).
30. G. Ouyang and J. Pawliszyn, *Anal. Chem. Acta*, **627**, 184 (2008).
31. S. Risiceric, V.H. Niri, D. Vuckovic and J. Pawliszyn, *Anal. Bioanal. Chem.*, **393**, 781 (2009).
32. C.L. Arthur, L.S. Killiam, K.D. Buchholz, J. Pawliszyn and R.B. John, *Anal. Chem.*, **64**, 1960 (1992).
33. D. Louch, S. Motlagh and J. Pawliszyn, *Anal. Chem.*, **64**, 1187 (1992).
34. Z. Zhang and J. Pawliszyn, *Anal. Chem.*, **65**, 1843 (1993).
35. J. Pawliszyn, Solid Phase Microextraction-Theory and Practice, Wiley-VCH, New York, USA (1997).
36. G. Ouyang, Y. Chen and J. Pawliszyn, *Anal. Chem.*, **75**, 2004 (2003).
37. P.A. Mantos and J. Pawliszyn, *Anal. Chem.*, **69**, 206 (1997).
38. P.A. Mantos, A. Saraullo and J. Pawliszyn, *Anal. Chem.*, **69**, 402 (1997).
39. A.J. Matich, in ed.: J. Pawliszyn, Applications of Soild Phase Microextraction, Royal Society of Chemical, Cambridge, pp. 349-357 (1999).
40. D. Zabaras and S.G. Wyllie, *Flav. Fragr. J.*, **16**, 411 (2001).
41. H. Kataoka, H. Lord and J. Pawliszyn, *J. Chromatogr. A*, **880**, 35 (2000).
42. M. Giardina and S.V. Olesik, *Anal. Chem.*, **73**, 5841 (2001).
43. C. Zhang, M. Qi, Q. Shao, S. Zhou and R. Fu, *J. Pharm. Biomed. Anal.*, **28**, 464 (2007).
44. E. Matisova, M. Medvedova, J. Vraniakova and P. Simon, *J. Chromatogr. A*, **960**, 159 (2002).
45. M. Ishikawa, O. Ito, S. Ishizaki, Y. Kurobayashi and A. Fujita, *Flav. Fragr. J.*, **19**, 183 (2004).
46. P. Watkins and C. Wijesundera, *Talanta*, **70**, 595 (2006).
47. S. Li and G.W. Stephen, in ed.: J. Pawliszyn, Application of Solid Phase Microextraction, Royal Society of Chemistry, Cambridge (1999).
48. X. Di, A. Shellie, J. Marriott and C.W. Huie, *J. Sep. Sci.*, **27**, 451 (2004).
49. S. Goncalves, H. Serra, J.M.F. Nogueira, R. Almeida, L. Custodio and A. Romano, *Biol. Plant.*, **52**, 1331 (2008).
50. M. Kovacevic and M. Kac, *J. Chromatogr. A*, **918**, 159 (2001).

50a. P. Tammela, M. Nygren, I. Laakso, A. Hopia, H. Vuorela and R. Hiltunen, *Flav. Fragr. J.*, **18**, 290 (2003).

51. A.M. Santos, T. Vasconcelos, E. Mateus, M.H. Farrall, M.D.R.G. Silva, M.R. Paiva and M. Branco, *J. Chromatogr. A*, **1105**, 191 (2006).
52. A. Hern and S. Dorn, *Phytochem. Anal.*, **14**, 232 (2001).
53. G. Flamini, P.L. Cioni and I. Morelli, *Flav. Fragr. J.*, **17**, 147 (2002).
54. G. Flamini, P.L. Cioni and I. Morelli, *J. Chromatogr. A*, **998**, 229 (2003).
55. M.G. Pizzolatti, B.G. Mendes, C. Soldi, F.C. Missau, J.H. Bortoluzzi and E. Carasek, *J. Essent. Oil Res.*, **21**, 255 (2009).
56. N. Li, C. Deng, Y. Li and X. Zhang, *J. Chrmmatogra. A*, **1133**, 29 (2006).
57. M. An, T. Haig and P. Hatfield, *J. Chromatogr. A*, **917**, 245 (2001).
58. K. Nakamura, K. Matsubara, H. Watanabe, H. Kokubun, Y. Ueda, N. Oyama-Okubo, M. Nakayama and T. Ando, *Sci. Horticul.*, **108**, 61 (2006).
59. R.U. Hoit, *J. Chromatogr. A*, **937**, 107 (2001).
60. C. Deng, A. Wang, S. Shen, D. Fu, J. Chen and X. Zhang, *J. Pharm. Biomed. Anal.*, **38**, 326 (2005).
61. L. Dong, J. Wang, C. Deng and X. Shen, *J. Sep. Sci.*, **30**, 86 (2007).
62. P. Diaz, E. Ibanez, G. Reglero and F.J. Senorans, *LWT-Food Sci. Technol.*, **42**, 1253 (2009).
63. M. Kurkcuoglu and K.H.C. Baser, *Chem. Nat. Comp.*, **39**, 457 (2003).
64. D. Zhao, J. Tang and X. Ding, *LWT-Food Sci. Tech.*, **40**, 439 (2007).
65. E.J. Yu, T.H. Kim, K.H. Kim and H.J. Lee, *Flav. Fragr. J.*, **19**, 532 (2004).
66. C.A. Zini, F. Augusto, E. Christensen, B.P. Smith, E.B. Caramao and J. Pawliszyn, *Anal. Chem.*, **73**, 4729 (2001).
67. C. Bicchi, S. Drigo and P. Rubiolo, *J. Chromatogr. A*, **892**, 469 (2000).
68. H. Ye, J. Ji, C. Deng, N. Yao, N. Li and X. Zhang, *Chromatographia*, **63**, 591 (2006).
69. M.G. Esteban, D. Ansorena, I. Astiasaran and J. Ruiz, *Talanta*, **64**, 458 (2004).
70. C.D. Porto, L. Pizzale, M. Bravin and L.S. Conte, *Flav. Fragr. J.*, **18**, 66 (2003).
71. N.-S. Kim and D.-S. Lee, *J. Sep. Sci.*, **27**, 96 (2004).
72. R. Perestrelo, M. Caldeira, F. Rodrigues and J.S. Camara, *J. Sep. Sci.*, **31**, 1841 (2008).
73. C. Deng, N. Li, W. Zhu, J. Qian, X. Yang and X. Zhang, *J. Sep. Sci.*, **28**, 172 (2005).
74. R. Mu, X. Wang, S. Liu, X. Yuan, S. Wang and Z. Fan, *Chromatographia*, **65**, 463 (2007).
75. Z.-G. Li, M.-R. Lee and D.-L. Shen, *Anal. Chim. Acta*, **576**, 43 (2006).

76. F.M. Musteata and J. Pawliszyn, *J. Biochem. Biophys. Methods*, **70**, 181 (2007).
77. J. Pawliszyn, Sampling and Sample Preparation for Filed and Laboratory: Fundamentals and New Directions in Sample Preparation, Analytical Comprehensive Chemistry, Elsevier, pp. 1131 (2002).
78. F. Bothe, K. Dettmer and W. Engwald, *Chromatographia*, **57**, 199 (2003).
79. Y.R. Rao and P.K. Rout, *Indian Perfum.*, **46**, 49 (2002).
80. P.K. Rout, S.N. Naik and Y.R. Rao, *J. Essent. Oil Res.*, **22**, 398 (2010).
81. P.K. Rout, S.N. Naik and Y.R. Rao, *Flav. Fragr. J.*, **21**, 906 (2006).
82. P.K. Rout, S.N. Naik and Y.R. Rao, *J. Supercrit. Fluids*, **56**, 249 (2011).
83. P.K. Rout, Y.R. Rao, A. Sree and S.N. Naik, *Flav. Fragr. J.*, **22**, 352 (2007).
84. P.K. Rout, Y.R. Rao and S.N. Naik, *Ind. Crops Prod.*, **32**, 338 (2010).
85. Y.R. Rao and P.K. Rout, The Fragrance Components of Flowers of *Quisqualis indica* and the Composition of Essential Oils, 32nd International Symposium of Essential Oils, Wroclaw, Poland, p. 42 (2001).
86. P.K. Rout, S.N. Naik and Y.R. Rao, *J. Supercrit. Fluids*, **45**, 200 (2008).
87. P.K. Rout, Ph.D. Thesis, Solid Phase Microextraction, In: Development of Process for Extraction of Floral Fragrance by Subcritical Carbondioxide, Indian Institute of Technology, Delhi, India, pp. 166-199 (2008).
88. R.P. Adams, Identification of Essential Oils by Ion Trap Mass Spectroscopy, Academic Press, San Diego, pp. 17-28 (1989).
89. A.E. Edris, R. Chizzola and C. Franz, *Eur. Food Res. Technol.*, **226**, 621 (2008).
90. C. Wu, D. Zhao, W. Sun, P. Ma, Q. Wang and C. Lu, *Zhiwu Xuebao*, **29**, 636 (1987); B. Lawrence, *Perfum Flav.*, **19**, 64 (1994).