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Determination of floral fragrances of *Rosa hybrida* using solid-phase trapping-solvent extraction and gas chromatography–mass spectrometry

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Abstract

Floral fragrances emanated from *Rosa hybrida* were determined by solid-phase trapping extraction and GC–MS. A novel protocol of sampling technique was established. There is a variation in the recoveries depending on the adsorbent and components. A total of 41 compounds were identified in the floral fragrances of *Rosa hybrida*. These include alcohols, aldehydes, alkanes, monoterpenes, sesquiterpene, esters, ether and ketones. Citral, *n*-nonane, *n*-butyl acetate, *n*-decane, β-phenylethyl acetate and hexadecanol were major components. Floral fragrances differ between rose species and sample to sample within a single species. Interestingly, endocrine disruptors such as bis(2-ethylhexyl) phthalate were detected simultaneously.

Keywords: *Rosa hybrida*; Fragrance components; Gas chromatography–mass spectrometry; Solid-phase trapping extraction; Endocrine disruptors

1. Introduction

Several thousand compounds have been identified from various floral fragrances. Most of these compounds are terpenes, esters, alcohols, aldehydes, ketones or alkanes. An excellent review of the useful literature on floral scents was given by Knudsen et al. [1], and there have been some reports on the fragrance compositions of rose flowers. Dobson et al. [2] found a total of 31 fragrance compounds including 2-phenylethanol, citronellol, benzyl alcohol, methyleugenol and geraniol from *Rosa rugosa*. Some other workers [3–11] also reported fragrance components of Rosaceae species including *Rosa chinensis* and *Rosa damascena*.

Recently, floral fragrances have become pervasive in modern life, including cosmetics, foods, aromatherapy, household products, and many other consumer goods [12]. Annual consumption of flavours and fragrances is estimated at US$9687 million worldwide [13]. The rapid expansion of the fragrance industry worldwide has been driven by the many demands for all natural fragrances. Many perfumers still survey natural sources for novel

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fragrance compounds, this information is most often used in directing organic syntheses to imitate natural fragrances or create new combinations [14,15]. Also, the biological natures and functions of floral fragrances have been interesting from the botanical and entomological points of view [16,17]. Therefore, separations and analyses of floral fragrances are very important and useful in many fields.

Traditional methods to obtain odorous components from natural sources were enfleurage (pommade method), expression (cold pressing), extraction (maceration or percolation) and distillation with steam [18]. Separations of these kinds are necessary in not only manufacturing operations but also analytical procedures. In the analytical scale combined with gas chromatography–mass spectrometry (GC–MS), fragrance compounds are generally obtained from flowers by solvent extraction, steam distillation or headspace trapping. The major limitation of solvent extraction is that it is useful only on samples that do not contain any lipids. Both solvent extraction and steam distillation are liable to produce artifacts by isolating non-volatile materials from tissues or by partial decompositions [1]. Therefore, the sampling technique used in a majority of the studies was headspace adsorption method, and adsorbed compounds were either thermally desorbed or eluted with organic solvents prior to GC–MS [19–27]. Tenax TA, Porapak Q and charcoal are commonly used as adsorbent to trap fragrance compounds. Charcoal is an extremely powerful and non-selective adsorbent that traps a wide range of organic compounds very efficiently. However, because its adsorption is so strong, desorption can become something of problem. An alternate technique for enriching headspace volatiles that has grown in popularity is the use of milder porous polymer adsorbents as trapping media. Tenax (poly[2,6-diphenyl-*p*-phenylene oxide]) was first introduced for use as a GC stationary phase by Van Wijik [28]. This material exhibited a high adsorptive capacity towards volatile and semi-volatile organic compounds and low affinity for water vapor. Since these porous polymers have large surface areas (Tenax TA, 35 m^2/g; Porapak Q, 582 m^2/g) [19] and the thermal stability, they are usually used in preference to adsorbent for solid-phase extraction. The use of other adsorbents soon followed. On the other hand headspace methods produce an aroma isolate that is very biased towards some aroma constituents, and analytical data one receives is on the amount of an aroma constituent only in the headspace. It thus appears that quantitative analysis is difficult by the headspace method. Nevertheless, compared with conventional liquid–liquid extraction, solid-phase extraction using porous polymers is convenient, easy to use and less time consuming, requires much smaller amounts of solvents and is capable of producing cleaner extracts [29,30]. There are extensive reports upon the application of Tenax and Porapak for the collection of volatile fragrances of flowers [1–11,19–27]. The choice of adsorbent and trapping method is important parameter, which governs the range of fragrance compounds that can be effectively trapped. Numerous investigations have compared adsorbents with regard to trapping efficiencies and breakthrough volumes for various classes of organic compounds [31–36]. However, practically it is difficult to choose a perfect method for isolating and concentrating the volatile compounds.

In this study, floral fragrances emanated from Rosaceae were analyzed by solid-phase trapping extraction (SPTE) and GC–MS. A novel protocol of SPTE by a modification of earlier techniques [37–39] was described. Collection efficiencies of the various adsorbents such as Tenax TA, Porapak Q, Chromosorb P, and W, Sep-Pak plus C_{18}, CN and NH_2 cartridges were compared. Our final objective is to characterize the rosy floral fragrances in the *Rosa hybrida* species, which are the most popular domestic cultivars. Variation in floral fragrances among the three closely related species of *Rosa hybrida* was also investigated.

2. Experimental

2.1. Materials

The freshly picked flower samples of *Rosa hybrida* (‘‘Sandra’’, ‘‘Cardinal’’ and ‘‘Silva’’) were gathered during from June to October and watered when necessary. Tenax TA (2,6-diphenyl-*p*-phenylene oxide polymer, 250–177 μm), Porapak Q (ethylvinylbezene divinyl benzene copolymer, 149–125 μm), and Sep-Pak plus C_{18} (octadecyl silane)

cartridge were purchased from Waters (Milford, MA, USA). Chromosorb P (diatomite firebrick), and W (diatomite, 177–149 μm) were obtained from Supelco (Bellefonte, PA, USA). CN cartridge and NH_2 cartridge (100 mg/1 ml) were from Alltech (Deerfield, IL, USA). Both farnesene and farnesol purchased from Tokyo Kasei (Japan) were mixtures of isomers, all other fragrance standards were of analytical grade (purity, 99.9%) were purchased from Sigma (St. Louis, MO, USA) or Tokyo Kasei. All organic solvents were of analytical grade were purchased from Sigma. Water used in the experiments was distilled-deionized and then purified using an E-pure water purification system (Barnstead/Thermolyne, Dubuque, IA, USA). The specific conductivity of this water was 1.8×10^{-7} Ω^{-1} cm^{-1}.

2.2. Collection techniques of fragrance compounds from flowers

Fragrance compounds were collected from the rose flowers by the following SPTE methods. Freshly cut rose flower samples (ca. 50 g) just after anthesis were enclosed in a clean, dry barrel of the intravenous glass syringe (50 ml, 3 cm I.D.×14 cm long) which removed its plunger and needle. And then a couple of barrels were fitted together with a polytetrafluoroethylene (PTFE, Teflon) spacer gasket and held by a joint clip, as illustrated in Fig. 1. An available cartridge or the Pasteur pipet (0.565 cm I.D.×15 cm long) was used as a trap-housing in which packed with adsorbent (500 mg) and glass wool plugs. Seven kinds of adsorbents such as Tenax TA, Porapak Q, Chromosorb P, and W, Sep-Pak plus C_{18}, CN and NH_2 cartridges were used for the comparison. This adsorbent trap was activated prior to use by pre-rinsing with 2 ml of diethyl ether. The Luer lock inlet at the end of cartridge housing or the inlet of the Pasteur pipet was attached to the Luer taper tip of the barrel containing the flower cut. An oil-free electric vacuum pump (Vacuubrand, Wertheim, Germany, diaphragm ME2 model, 2.4 m^3/h) and a PTFE valve restrictor were connected with Tygon tubing to the outlet end of the trap. A purified nitrogen gas (purity, 99.99%) flow at ca. 400 ml/min was passed into a couple of barrels and out through the adsorbent trap under reduced pressure. The collection was continued for 3 h at ambient temperature. After a run, the trap was then removed and the trapped fragrance compounds were eluted by two extractions with 2 ml of diethyl ether in portions to the new syringe to which the trap was attached and forcing the solvent through with the syringe plunger. The extract obtained in a small vial (2 ml) was further concentrated to final volume of approximately 200 μl on a water bath at 80°C by using a Kuderna-Danish concentrator jointed with a Snyder column. Aliquots were analyzed by GC.

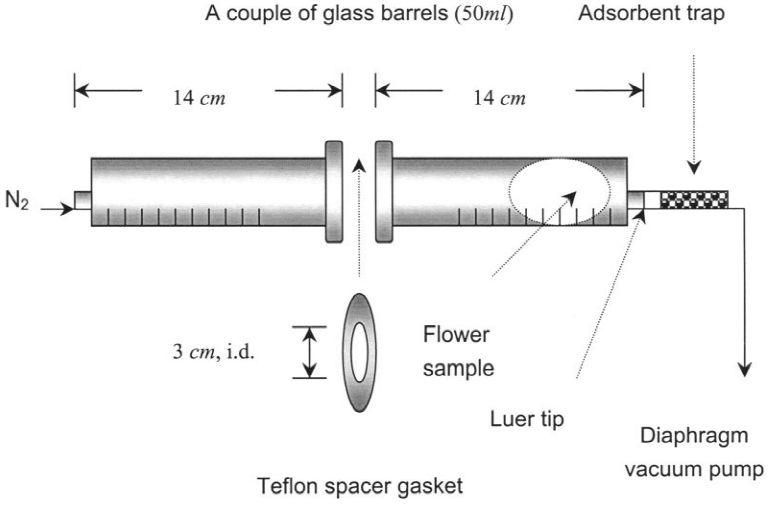

Fig. 1. A trapping apparatus to collect floral fragrances.

2.3. Gas chromatography

All samples were analyzed by a HP 5890 series II gas chromatograph (Hewlett-Packard, Avondale, PA, USA) using a 2-μl sample, 250°C split injection (split ratio 1:30), a flame ionization detector at 250°C, crosslinked 5% phenyl poly(dimethylsiloxane) wall-coated open tubular (WCOT) column (Ultra 2, Hewlett-Packard, 25 m×0.2 mm I.D., 0.33 μm film thickness). Gas flow-rates were kept as follows: nitrogen carrier gas, 2 ml/min; hydrogen, 30 ml/min; air, 300 ml/min. The column oven temperature was held 70°C for 3 min and then programmed to 240°C at a rate 5°C/min, and held at final temperature for 20 min. GC peak areas were integrated with a HP 3396A integrator (Hewlett-Packard).

2.4. Gas chromatography–mass spectrometry

Trace GC with GC-Q Plus ion trap MS^n (Thermoquest-Finnigan, Austin, TX, USA) gas chromatograph–mass spectrometer with Xcalibur software system (Thermoquest-Finnigan) was used for separation and identification. Identification was based on comparison of mass spectral information and retention indices with 46 authentic standards. Peak identification was confirmed by the use of Kovats retention indices (I) on a polar liquid phase and on a nonpolar phase. Structural assignments were based on searching against the NIST and Wiley library data. A crosslinked 5% phenyl poly(dimethylsiloxane) WCOT (SPB-5, Supelco, 60 m×0.25 mm I.D., 0.25 μm film thickness) column was used as a non-polar phase and a poly(ethylene glycol) WCOT (Supelcowax-10, Supelco, 30 m×0.32 mm I.D., 0.25 μm film thickness) column was used as a polar phase. In the case of a non-polar column, injector temperature was 240°C, oven temperature was held 70°C for 8 min and then programmed to 240°C at a rate of 5°C/min, and held at final temperature for 20 min. The carrier gas was He at 1.0 ml/min flow-rate. The sample volume injected was 1 or 2 μl, and the split ratio was 1:30. The electron impact (EI) ionization mass spectrometer was operated as follows: ionization voltage, 70 eV; ion source temperature, 200°C; transfer line temperature, 275°C. The oven temperature program of a polar column was 40°C (5 min)−4°C/min−150°C−8°C/min−240°C; injector, 230°C; transfer line, 230°C; all other conditions were the same as those of a non-polar column.

3. Results and discussion

3.1. Gas chromatography–mass spectrometry

Fig. 2 shows a total ion chromatograms (TIC) of the 46 authentic standards of the identified constituents of *Rosa hybrida*. A standard mixture was prepared containing an accurately known amount of about 0.1 g of each standard in 20 ml of diethyl ether. This mixture was analyzed by GC or GC–MS. Fig. 2A is a TIC separated by using a crosslinked 5% phenyl poly(dimethyl siloxane) column, B is a TIC by a poly(ethylene glycol) column. The peak numbers in Fig. 2 correspond to the numbers indicated in the first column of Table 1.

The retention factor (k) and retention indices (I) on non-polar and polar columns for standard mixture are summarized in Table 1, in order of increasing t_R on a non-polar phase. The characteristic mass spectral ions (m/z) of each peak are summarized in Table 2. Among the 46 constituents, farnesol, farnesene, and 2,6-dimethoxy toluene were identified when steam distillation technique with reduced pressure instead of SPTE was used to collect fragrances from *Rosa hybrida*. Six peaks of farnesene and four peaks of farnesol were observed, respectively, because a mixture of isomers for these compounds was used in this work. However, their geometric isomerisms are uncertain.

It should be noted that butylated hydroxy toluene, 2,6-dimethoxy toluene and bis(2-ethylhexyl) phthalate were thought to be a contaminants or pollutants. Butylated hydroxy toluene is used as an antioxidant for synthetic rubbers and plastics. Bis(2-ethylhexyl) phthalate has been widely used as a plasticizer. Recently these compounds have been known as the endocrine disruptor [40–45]. It is unknown whether these compounds were polluted through a certain insect from air, polyvinylchloride sheets and polystyrene containers used in the farmland or by any other reasons. Our results evidenced an example of

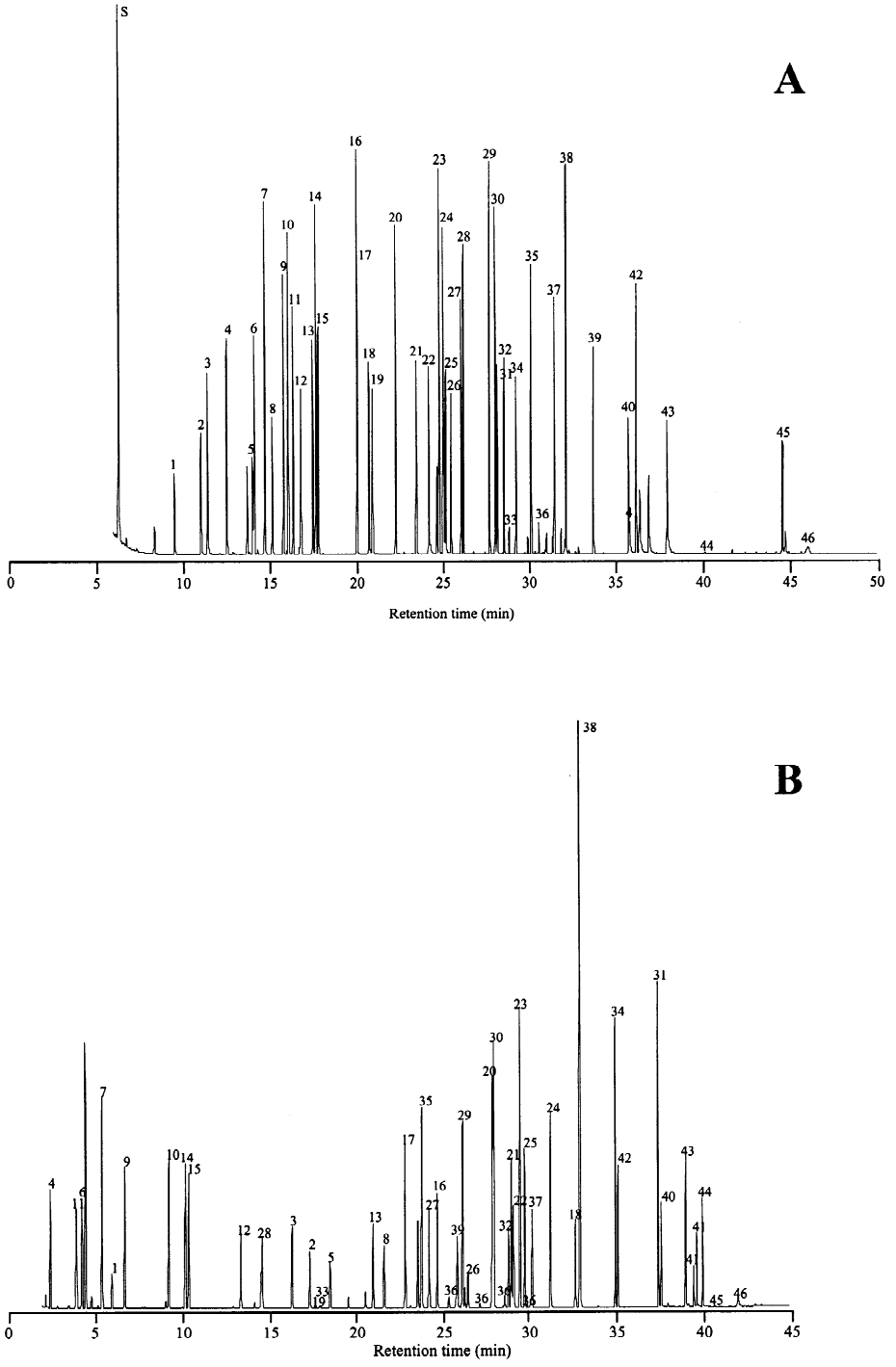

Fig. 2. Total ion chromatograms of authentic standards of the identified constituents of *Rosa hybrida*: obtained by using (A) 5% phenyl poly(dimethylsiloxane) (Supelco SPB-5, 60 m×0.25 mm×0.25 μm) column and (B) polyethylene glycol (Supelcowax-10, 30 m×0.32 mm×0.25 μm) column. Peak numbers correspond to the numbers indicated in Table 1. For analytical conditions, see Section 2.

Table 1
The retention factors (k) and retention indices (I) on non-polar and polar columns for standards mixture identified from floral fragrances of *Rosa hybrida*

Peak no.	Compound	Non-polar column: 5% phenylpoly(dimethylsiloxane)[d] (Supelco SPB-5) 60 m×0.25 mm×0.25 μm			Polar column: polyethylene glycol[e] (Supelcowax-10) 30 m×0.32 mm×0.25 μm			ΔI[f]
		t_R	k	I	t_R	k	I	
1	Butyl acetate	9.35	0.99	808	6.07	3.30	1047	239
2	*cis*-3-Hexen-1-ol	11.05	1.36	857	17.43	11.36	1376	519
3	Hexanol	11.44	1.44	868	16.41	10.64	1348	480
4	Nonane	12.55	1.68	900	2.55	0.81	900	0
5	2-Cyclohexen-1-one	14.04	1.99	939	18.60	12.19	1410	471
6	α-Pinene	14.14	2.01	942	4.35	2.09	1005	63
7	Camphene	14.75	2.14	958	5.51	2.91	1033	75
8	Benzaldehyde	15.17	2.23	969	21.73	14.41	1507	538
9	β-Pinene	15.82	2.37	986	6.83	3.84	1065	79
10	β-Myrcene	16.12	2.44	993	9.33	5.62	1139	146
11	Decane	16.37	2.49	1000	4.12	1.92	1000	0
12	Hexyl acetate	16.84	2.59	1017	13.47	8.55	1268	251
13	2-Ethyl hexanol	17.51	2.73	1042	21.09	13.96	1490	448
14	Limonene	17.68	2.77	1049	10.29	6.30	1176	127
15	Cineole	17.83	2.80	1054	10.49	6.44	1183	129
16	Methyl benzoate	20.09	3.28	1124	24.80	16.59	1575	451
17	Linalool	20.13	3.29	1125	22.95	15.28	1534	409
18	2-Phenylethanol	20.76	3.43	1140	32.76	22.23	1859	719
19	Isophorone	20.98	3.47	1146	17.87	11.67	1388	242
20	Benzylacetate	22.31	3.76	1177	28.00	18.86	1703	526
21	Methyl salicylate	23.51	4.01	1208	29.08	19.62	1749	541
22	β-Citronellol	24.24	4.17	1232	29.20	19.71	1755	523
23	β-Phenylethyl acetate	24.83	4.34	1252	29.59	19.99	1771	519
24	Geraniol	25.06	4.29	1260	31.34	21.23	1826	566
25	2,6-Dimethoxy toluene[a,b]	25.22	4.38	1266	29.86	20.18	1783	517
26	Citral	25.54	4.45	1276	26.59	17.86	1632	356
27	2-Undecanone	26.10	4.57	1295	24.33	16.26	1564	269
28	Tridecane	26.24	4.59	1300	14.67	9.40	1300	0
29	Citronellyl acetate	27.76	4.92	1353	26.28	17.64	1616	263
30	Neryl acetate	28.09	4.99	1365	28.07	18.91	1706	341
31	Eugenol	28.20	5.01	1369	37.53	25.62	1994	625
32	Geranyl acetate	28.59	5.10	1382	28.91	19.50	1742	360
33	Tetradecane	29.10	5.20	1400	18.29	11.97	1400	0
34	Methyl eugenol	29.28	5.24	1410	35.07	23.87	1918	508
35	Caryophyllene	30.17	5.43	1460	23.92	15.96	1555	95
36	Farnesene	31.16	5.64	1510	25.44	17.04	1589	79
	(mixture of isomer)[b,c]	31.81	5.78	1532	26.36	17.70	1620	88
		32.60	5.95	1560	27.23	18.31	1664	104
		33.10	6.06	1577	27.88	18.77	1698	121
		33.31	6.10	1584	28.66	19.33	1731	147
		34.10	6.27	1611	29.19	19.70	1754	143
37	2-Tridecanone	31.50	5.72	1521	30.29	20.48	1801	280
38	Butylated hydroxy toluene[a]	32.19	5.86	1545	33.06	22.45	1867	322
39	Hexadecane	33.77	6.20	1600	25.96	17.41	1600	0
40	Tetradecanol	35.77	6.63	1668	37.70	25.74	1999	331

Table 1. Continued

Peak no.	Compound	Non-polar column: 5% phenylpoly(dimethylsiloxane)[d] (Supelco SPB-5) 60 m×0.25 mm×0.25 μm			Polar column: polyethylene glycol[e] (Supelcowax-10) 30 m×0.32 mm×0.25 μm			ΔI^f
		t_R	k	I	t_R	k	I	
41	Farnesol (mixture of isomer)[b,c]	36.11	6.70	1680	39.10	26.73	2042	362
		37.73	7.04	1762	39.61	27.09	2057	295
		37.77	7.05	1764	39.81	27.23	2063	299
		38.27	7.16	1795	40.16	27.48	2074	279
42	2-Pentadecanone	36.23	6.72	1684	35.22	23.98	1923	239
43	Pentadecanol	37.98	7.10	1778	39.11	26.74	2042	264
44	Hexadecanol	40.11	7.55	1839	40.06	27.41	2071	232
45	2-Dodecen-1-yl-succinic anhydride	44.61	8.51	1966	40.30	27.58	2078	112
46	Bis(2-ethylhexyl)phthalate[a]	46.08	8.83	2019	42.40	29.07	2143	124

[a] Contaminants.

[b] Identified from a sample collected by the steam distillation under reduced pressure.

[c] Their geometric isomerisms are uncertain.

[d] Operating conditions: column oven, 70°C (8 min)−5°C/min−240°C (20 min); injector, 240°C; transfer line, 275°C; ion source, 200°C; EI, 70 eV; carrier (He) flow, 1 ml/min; split ratio, 30:1; injection volume, 1 μl instrument, Thermoquest-Finnigan Trace GC with GC-Q plus ion trap MS^n.

[e] Operating conditions: column oven, 40°C (5 min)−4°C/min−150°C−8°C/min−240°C/min (5 min); injector, 230°C; transfer line, 230°C; all other conditions are the same as a 5% phenyl poly(dimethylsiloxane) column.

[f] $\Delta I = I_{(polar)} - I_{(non\text{-}polar)}$.

the serious problems of the current environmental pollution.

3.2. Comparison of relative trapping efficiency by different adsorbent traps

A series of trapping experiments were carried out to assess the relative trapping performances of the various adsorbents. One μl of the standards mixture (0.1 g of each standard in 20 ml) was added to 50 mg of pure cotton enclosed in a couple of syringe barrels, and then SPTE using the chosen adsorbent trap was implemented according to the experimental procedure. After SPTE implements, aliquots were analyzed by GC. Separately, a standards mixture was analyzed with GC by the direct injection without SPTE procedures. The relative trapping efficiency percent based on the relative GC peak area ratio was calculated as follows:

Relative trapping efficiency (%) = 100

× (Peak area of compound by SPTE)/

(Peak area of compound without SPTE)

Trapping efficiencies of the various adsorbents were compared. Table 3 lists the relative trapping efficiency percent of the authentic standards of the identified constituents of *Rosa hybrida* ''Sandra'' fragrance by using SPTE with different adsorbents. It can be seen that SPTE adsorbents used in this study gave the low efficiencies within 13%. And there is a considerable variation in the efficiencies observed. Tenax TA and Porapak Q were the better efficient adsorbents while Chromosorb P, Chromosorb W were the least effective. CN and NH_2 cartridges showed the selectivities to farnesol, 2-ethyl hexanol, and linalool but efficiencies of many other compounds were poor. When Tenax TA was used as the adsorbent 45 compounds were trapped except hexadecanol was hardly detected. The relative efficiencies of α- or β-pinenes, β-myrcene, decane, 2-ethyl hexanol, limonene, cineol, and linalool on Porapak Q were higher than on Tenax TA. Neither Tenax TA nor Porapak Q alone effectively trapped the full range of floral fragrance compounds. The relative efficiencies of the present study were lower than previous report by Patt et al. [20].

The relative trapping efficiencies of the selected standards were repeated for different trapping times using Porapak Q and Tenax TA as the adsorbent

Table 2
Characteristic mass spectral ions of volatile compounds identified from floral fragrances of *Rosa hybrida* using a 5% phenyl poly(dimethylsiloxane) column (Supelco SPB-5, 60 m×0.25 mm×0.25 μm)[a]

Peak no.	Compound	M_r	Base peak m/z (100%, species)	Characteristic mass spectral ions (EI) m/z (relative abundance %, species)
1	Butyl acetate	116	43(CH_3CO)	41(32, C_3H_5), 56(9, C_4H_8), 73(0.1, C_4H_9O), 116(0.07, M^+)
2	*cis*-3-Hexen-1-ol	100	67($M-H_2O$ & CH_3)	41(69,C_3H_5) 31(20, CH_2OH), 82(3.5,$M-H_2O$), 69(1, $M-CH_2OH$), 100(0.07, M^+)
3	Hexanol	102	41(C_3H_5)	56(24, $M-H_2O$ & C_2H_4), 69(9.9, $M-H_2O$ & CH_3), 84(0.1, $M-H_2O$), 102(0.1, M^+)
4	Nonane	128	41(C_3H_5)	57(34, C_4H_9), 43(32, C_3H_7), 85(1.6, C_6H_{13}), 71(1.3, C_5H_{11}), 128(0.04, M^+)
5	2−Cyclohexen-1-one	96	68($M-CO$)	39(91, C_3H_3), 42(35, C_3H_6), 28(5, CO), 96(3, M^+)
6	α-Pinene	136	91(C_7H_7)	77(37, C_6H_5), 93(25, C_7H_9), 65(7, C_5H_5), 41(5, C_3H_5), 136(0.67, M^+), 137(0.27, M+1)
7	Camphene	136	91(C_7H_7)	93(66, C_7H_9), 77(52, C_6H_5), 39(29, C_3H_3), 65(19, C_5H_5), 41(14, C_3H_5), 136(0.81, M^+), 137(1, M+1)
8	Benzaldehyde	106	77(C_6H_5)	105(99, M−1), 51(95, C_4H_3), 106(10, M^+)
9	β-Pinene	136	91(C_7H_7)	77(50, C_6H_5), 93(31,C_7H_9), 41(24, C_3H_5), 65(9, C_5H_5), 136(1.1, M^+), 137(0.48, M+1)
10	β-Myrcene	136	91(C_7H_7)	41(53, C_3H_5), 77(42, C_6H_5), 93(30,C_7H_9), 65(9, C_5H_5), 136(0.58, M^+), 137(0.47, M+1)
11	Decane	142	41(C_3H_5)	43(35, C_3H_7), 57(35, C_4H_9), 71(6, C_5H_{11}), 85(1.38, C_6H_{13}), 142(0.03, M^+)
12	Hexyl acetate	144	43(CH_3CO)	41(70, C_3H_5), 39(50, C_3H_3), 56(28, C_4H_8), 145(2.25, M+1), 101(0.65, M−43) 144(0.12, M^+)
13	2-Ethyl hexanol	130	41(C_3H_5),	55(56, C_4H_7), 57(30, C_4H_9), 29(27, C_2H_5), 84(1.3, $M-C_2H_4$ & H_2O), 112(0.18, $M-H_2O$)
14	Limonene	136	67(C_5H_7)	91(64, C_7H_7), 93(29, C_7H_9), 41(18, C_3H_5), 136(1.03, M^+), 137(0.78, M+1)
15	Cineole	154	43(C_3H_7)	81(57, $M-CH_3CH_2OCH_2CH_2$), 154(1.47, M^+), 155(4.19, M+1)
16	Methyl benzoate	136	77(C_6H_5)	105(86, C_6H_5CO), 136(20, M^+), 137(81, M+1)
17	Linalool	154	43(C_3H_7)	91(71, C_7H_7), 81(58, $M-CH_3CH_2OCH_2CH_2$), 93(44, C_7H_9), 55(37, C_4H_7), 80(31, C_6H_8), 136(7, $M-H_2O$)
18	2-Phenylethanol	122	91($M-CH_2OH$)	65(22, C_5H_5), 77(4, C_6H_5), 31(4, CH_2OH), 104(0.89, $M-H_2O$), 122(0.59, M^+)
19	Isophorone	138	39(C_3H_3)	82(58, C_6H_{10}), 95(8, $M-C_3H_7$), 138(1.62, M^+), 139(1.39, M+1)
20	Benzylacetate	150	79(C_6H_7)	91(98,C_7H_7), 108(71,$M-CH_2CO$), 43(42, CH_3CO), 150(2.42, M^+)
21	Methyl salicylate	152	92(C_6H_4O)	63(55, C_5H_3), 120(43, $M-CH_3OH$), 152(12, M^+), 153(1.3, M+1)
22	β-Citronellol	156	67(C_5H_7)	81(34, C_6H_9), 79(33, C_6H_7), 69(7, C_5H_9), 138(0.5, $M-H_2O$), 156(0.06, M^+)
23	β-Phenylethyl acetate	164	91(C_7H_7)	65(20, C_5H_5), 105(3, $C_6H_5CH_2CH_2$), 43(0.2, CH_3CO), 104(0.03,$C_6H_5CH_2CH$), 164(0.63, M^+)
24	Geraniol	154	41(C_3H_5)	67(47, C_5H_7), 91(22, C_7H_7), 69(12, C_5H_9), 154(2, M^+)
25	2,6-Dimethoxy toluene[b,c]	152	77(C_6H_5)	91(88, $C_6H_5CH_2$), 152(45, M^+), 121(32, $M-CH_2O$), 137(13, $M-CH_3$), 153(6, M+1), 151(4, M−1)
26	Citral	152	41(C_3H_5)	39(97, C_3H_3), 69(11, C_5H_9), 109(10, $M-CH_2CHO$), 123(3, M−CHO), 43(3, CH_2CHO), 152(0.35, M^+)

Table 2. Continued

Peak no.	Compound	M_r	Base peak m/z (100%, species)	Characteristic mass spectral ions (EI) m/z (relative abundance %, species)
27	2-Undecanone	170	43(C_3H_7)	58(24, CH_3COCH_3), 171(2.94, M+1), 170(0.27, M^+), 155(0.21, M−CH_3)
28	Tridecane	184	41(C_3H_5)	57(37, C_4H_9), 71(12, C_5H_{11}), 184(0.05, M^+)
29	Citronellyl acetate	198	67(C_5H_7)	81(47, C_6H_9), 41(47, C_3H_5), 43(26, CH_3CO), 95(21, C_7H_{11})
30	Neryl acetate	196	41(C_3H_5)	91(70, C_7H_7), 43(39, CH_3CO), 93(37, C_7H_9) 69(12, C_5H_9)
31	Eugenol	164	77(C_6H_5)	91(90, $C_6H_5CH_2$), 164(37, M^+), 149(16, M−CH_3), 94(15, C_6H_5OH), 165(5, M+1), 163(2, M−1)
32	Geranyl acetate	196	39(C_3H_3)	41(90, C_3H_5), 43(82, CH_3CO), 67(94, C_5H_7), 196(0.01, M^+)
33	Tetradecane	198	41(C_3H_5)	57(67, C_4H_9), 71(35, C_5H_{11}), 198(1.12, M^+), 199(0.07, M+1)
34	Methyl eugenol	178	91($C_6H_5CH_2$)	77(71, C_6H_5), 178(32, M^+),147(24, M−OCH_3), 163(11, M−CH_3), 179(5, M+1), 177(1, M−1)
35	Caryophyllene	204	91(C_7H_7)	77(52, C_6H_5), 79(49,C_6H_7), 41(37, C_3H_5),105(30,C_8H_9), 204(0.63, M^+), 205(0.18, M+1)
36	Farnesene[c,d]	204	91(C_7H_7)	41(84, C_3H_5), 39(61, C_3H_3), 77(47, C_6H_5), 93(44, C_7H_9), 204(0.22, M^+)
37	2-Tridecanone	198	43(CH_3CO)	58(26, CH_3COCH_3), 71(8, C_5H_{11}), 198(0.2, M^+)
38	Butylated hydroxy toluene[b]	220	57(C_4H_9)	205(95, M−CH_3), 220(56, M^+), 221(11, M+1)
39	Hexadecane	226	41(C_3H_5)	55(91, C_4H_7), 39(75, C_3H_3), 67(47, C_5H_7), 226(0.04, M^+)
40	Tetradecanol	214	43(C_3H_7)	41(29, C_3H_5), 39(25, C_3H_3), 31(20, CH_2OH)
41	Farnesol[c,d]	222	41(C_3H_5)	39(89, C_3H_3), 67(69, C_5H_7), 79(53, C_6H_7), 91(46, C_7H_7), 69(20, C_5H_9), 222(0.03, M^+)
42	2-Pentadecanone	226	41(C_3H_5)	55(91, C_4H_7), 39(75, C_3H_3), 67(47, C_5H_7), 226(0.04, M^+), 227(0.09, M+1)
43	Pentadecanol	228	41(C_3H_5)	55(93, C_4H_7), 67(77, C_5H_7), 31(20, CH_2OH), 182(0.1, M−H_2O & C_2H_4), 210(0.03, M−H_2O)
44	Hexadecanol	242	41(C_3H_5)	55(91, C_4H_7), 67(50, C_5H_7), 31(22, CH_2OH)
45	2-Dodecen-1-yl-succinic anhydride	266	67(C_5H_7)	39(75, C_3H_3), 41(77, C_3H_5), 79(68, C_6H_7), 55(50, CH_2CHCO)
46	Bis(2-ethylhexyl)phthalate[b]	390	149($C_6H_5(CO)_2OH$)	41(94, C_3H_5), 55(52, C_4H_7), 77(24, C_6H_5), 57(22, CH_3CH_2O), 390(0.01, M^+)

[a] Operating conditions: column oven, 70°C (8 min)−5°C/min−240°C (20 min); injector, 240°C; transfer line, 275°C; ion source, 200°C; EI, 70 eV; carrier (He) flow, 1 ml/min; split ratio, 30:1; injection volume, 1 μl instrument, Thermoquest-Finnigan Trace GC with GC-Q plus ion trap MS^n.

[b] Contaminants.

[c] Identified from a sample collected by the steam distillation under reduced pressure.

[d] Their geometric isomerisms are uncertain.

with the results shown in Figs. 3 and 4. The trappings of α-pinene, butyl acetate, nonane are complete after 1 h, benzaldehyde and tridecane after 2 h, β-phenylethyl acetate, hexadecane, tetradecanol, 2-phenylethanol, citral, citronellol, and caryophyllene after 3 h. It was impossible to achieve quantitative trapping for all standards in a chosen trapping time.

3.3. Floral fragrance composition of Rosa hybrida

TIC of the floral fragrances of *Rosa hybrida*

Table 3
Relative trapping efficiencies of standard compounds by different adsorbent traps (mean efficiency %)

Peak no.	Compound	Adsorbent[a]						
		A-1	A-2	A-3	A-4	A-5	A-6	A-7
1	Butyl acetate	0.06	0.16	0.01	0.01	0.01	0.14	0.01
2	*cis*-3-Hexen-1-ol	0.30	1.57	0.02	0.01	0.02	0.58	0.44
3	Hexanol	0.41	2.25	0.07	0.04	0.09	0.79	0.69
4	Nonane	0.17	1.29	0.03	0.03	0.04	0.23	0.07
5	2-Cyclohexan-1-one	1.20	2.01	0.01	0.01	0.01	0.59	0.28
6	α-Pinene	0.21	1.28	0.01	0.01	0.01	0.14	0.04
7	Camphene	1.20	1.42	0.01	0.01	0.01	0.55	0.04
8	Benzaldehyde	1.89	3.77	0.01	0.02	0.03	0.26	0.56
9	β-Pinene	0.28	3.19	0.01	0.01	0.01	0.17	0.08
10	β-Myrcene	0.81	10.09	0.01	0.02	0.36	0.25	0.21
11	Decane	0.47	12.67	0.01	0.02	0.04	0.31	0.25
12	Hexyl acetate	1.71	6.08	0.02	0.02	0.11	0.65	0.46
13	2-Ethyl hexanol	0.95	10.61	0.08	0.03	0.43	1.10	2.18
14	Limonene	0.96	12.53	0.04	0.02	0.05	0.22	0.21
15	Cineole	1.48	8.92	0.01	0.03	0.12	0.39	0.35
16	Methyl benzoate	4.84	10.73	0.02	0.03	0.28	0.38	0.52
17	Linalool	1.44	4.88	0.04	0.03	0.66	0.88	1.91
18	2-Phenylethanol	2.78	2.18	0.05	0.04	0.40	0.18	0.36
19	Isophorone	2.22	4.16	0.05	0.03	0.64	1.26	1.21
20	Benzylacetate	7.87	6.05	0.03	0.04	0.65	0.65	0.84
21	Methyl salicylate	6.75	6.19	0.02	0.05	0.66	0.37	0.76
22	β-Citronellol	1.64	1.38	0.13	0.03	1.46	0.14	0.39
23	β-Phenylethyl acetate	4.55	4.46	0.03	0.04	2.15	0.50	0.92
24	Geraniol	4.77	1.66	0.12	0.03	1.51	0.12	1.08
25	2,6-Dimethoxy toluene	4.55	1.96	0.04	0.05	2.15	0.50	0.92
26	Citral	4.63	1.97	0.08	0.04	1.55	0.48	0.05
27	2-Undecanone	3.97	2.29	0.08	0.04	2.63	0.40	1.28
28	Tridecane	2.84	4.08	0.01	0.07	4.04	0.49	1.76
29	Citronellyl acetate	3.15	1.86	0.05	0.04	3.01	0.29	1.02
30	Neryl acetate	3.01	1.58	0.04	0.04	2.72	0.25	0.90
31	Eugenol	1.59	0.84	0.10	0.04	1.24	0.14	0.37
32	Geranyl acetate	2.39	1.53	0.04	0.04	2.88	0.23	0.81
33	Tetradecane	2.91	1.92	0.02	0.05	4.81	0.22	1.08
34	Methyl eugenol	1.23	0.76	0.11	0.03	1.70	0.12	0.41
35	Caryophyllene	1.47	2.33	0.01	0.05	3.40	0.28	1.22
36	Farnesene	1.80	0.66	0.01	0.01	2.47	0.20	0.42
37	2-Tridecanone	0.87	0.63	0.11	0.03	1.10	0.07	0.22
38	Butylated hydroxy toluene	0.99	0.63	0.01	0.02	1.45	0.08	0.28
39	Hexadecane	0.75	0.47	0.02	0.02	0.89	0.05	0.17
40	Tetradecanol	0.06	1.06	0.03	0.01	0.04	0.01	0.01
41	Farnesol	0.84	0.01	0.01	0.01	0.01	2.40	0.01
42	2-Pentadecanone	0.07	0.08	0.08	0.02	0.16	0.02	0.02
43	Pentadecanol	0.15	0.01	0.01	0.01	0.01	0.10	0.01
44	Hexadecanol	0.01	0.01	0.01	0.01	0.01	0.02	0.02
45	2-Dodecen-1-yl-succinic anhydride	0.16	0.10	0.01	0.01	0.07	0.07	0.03
46	Bis(2-ethylhexyl)phthalate	0.17	0.13	0.02	0.08	0.33	3.85	0.13

[a] Adsorbent symbols: A-1, Tenax TA; A-2, Porapak Q; A-3, Chromosorb P; A-4, Chromosorb W; A-5, C_{18} cartridge; A-6, CN cartridge; A-7, NH_2 cartridge. $n=3$.

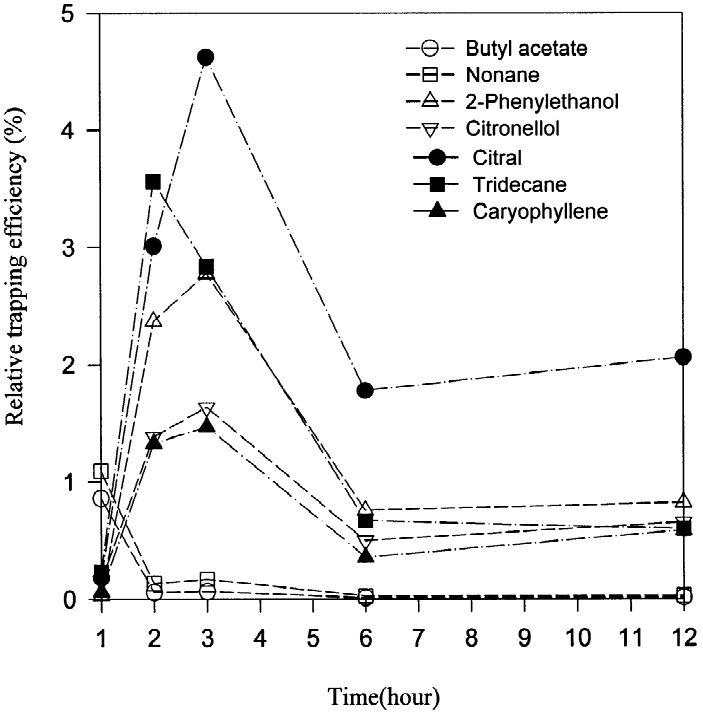

Fig. 3. Relative trapping efficiencies of standard compounds as a function of trapping time by Tenax TA trap.

''Sandra'' collected by Porapak Q and Tenax TA trapping techniques were shown in Fig. 5. The peak numbers in Fig. 5 correspond to the numbers indicated in the first column of Table 1. Components without a peak number were not found as separate peaks in the analysis of rose flower sample.

In Table 4 the relative percentages of the peak area of the all components found in *Rosa hybrida* ''Sandra'' are listed. A total of 41 compounds were identified in the floral fragrances of *Rosa hybrida*. These include 11 alcohols, two aldehydes, five alkanes, six monoterpenes, one sesquiterpene, 10 esters, one ether, and five ketones. Porous trap materials vary considerably in their ability to trap fragrance compounds. Therefore, the predominant components retrieved by different adsorbent traps are variable. In the case of the Tenax-trapped sample citral (18.6%), nonane (12.4%), and butyl acetate (11.0%) were major components, whereas nonane (14.9%), decane (12.7%) and β-phenylethyl acetate (10.4%) in the Porapak Q-trapped sample. Hexadecanol (33.8%) and citral (17.2%) were major fragrance constituents trapped on the Chromsorb P. In the case of Chromosorb W-trapped samples, 2-phenylethanol (14.6%), and hexadecanol (13.0%) were major fragrances but sesquiterpene (caryophyllene) was not found. Very large amount of β-pinene was present on the C_{18} (51.1%) or NH_2 (49.7%). However, small amounts of methyl eugenol and 2-undecanone were detected only by C_{18} trap and Chromosorb P trap, respectively.

Variation in floral fragrances among the three closely related species of *Rosa hybrida* was investigated. Comparison of the identified components of *Rosa hybrida* ''Sandra'', *Rosa hybrida* ''Cardinal'' and *Rosa hybrida* ''Silva'' by Tenax TA trapping method is summarized in Table 5. There are some distinct differences. Three of the species, ''Cardinal'' species contained sesquiterpene caryophyllene, hexadecanol, hexanol, and nonane as the major components. Citral, and β-myrcene were present highly in ''Silva'' species. In contrast to ''Sandra'' species, significantly higher amounts of β-myrcene, limonene, caryophyllene, and small amounts of geranyl acetate, neryl acetate and undecanone found in ''Cardinal'' and ''Silva''. Interestingly, within the

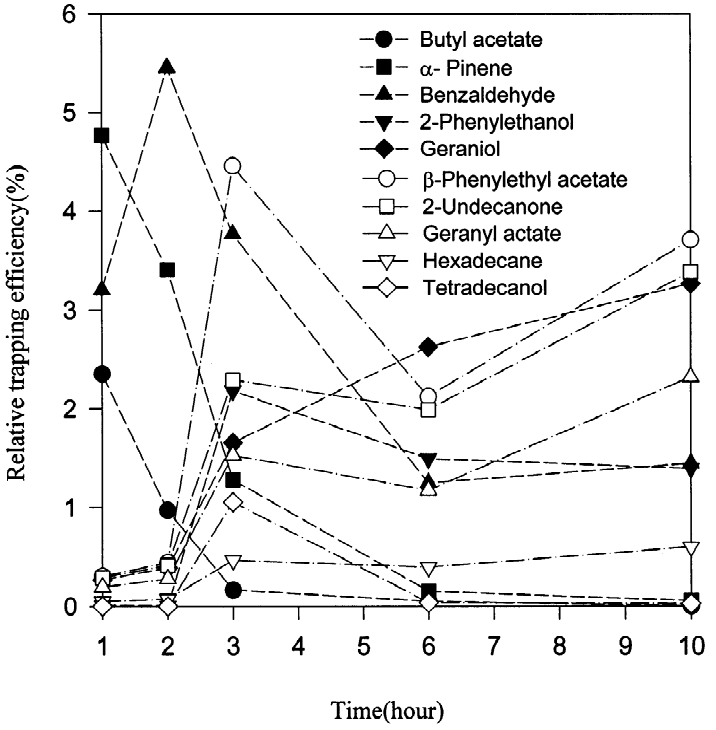

Fig. 4. Relative trapping efficiencies of standard compounds as a function of trapping time by Porapak Q trap.

Rosa hybrida group, pentadecanol was found only in ''Cardinal'' whereas methyl eugenol and β-citronellol were observed only in ''Silva''. However, ''Cardinal'' and ''Silva'' lacked eugenol, α-pinene, methyl benzoate, β-phenylethyl acetate which were present in ''Sandra''. Floral fragrances may differ not only between flower species, but also sample to sample within a single species.

An important finding of the present investigation is that floral fragrance of *Rosa hybrida* contains 2-ethyl hexanol, hexadecanol, *cis*-3-hexen-1-ol, pentadecanol, tetradecanol, benzaldehyde, hexadecane, tetradecane, benzyl acetate, methyl benzoate, methyl salicylate, cineole, 2-cyclohexen-1-one, and isophorone. These components have not earlier been reported as flower fragrances of Rosaceae [1–11]. However, some components such as hexadecanal, tetradecanal, 3-methyl-1-butanol, 3-hexenyl acetate, hexyl acetate, pentyl acetate, tetradecyl acetate, methoxy benzene, geranial, linalyl acetate, linalool oxide and germacrene D were not detected, whereas these components were identified by other researchers [1–11].

Hydrocarbons are known to be produce by flowers from fatty acids by decarboxylation [46–48]. Aliphatic alcohol such as *cis*-3 hexenol (so-called ''leaf alcohol'') is a catabolism product of various unsaturated fatty acids [49]. Benzyl alcohol, benzaldehyde, and bezyl acetate are thought to be phenylpropanoid metabolites formed from the oxidation of cinnamoyl CoA [50]. 2-Phenylethanol is synthesized in rose petals from L-phenylalanine [51,52]. Monoterpenes and aromatic esters such as benzyl acetate and methyl benzoate possess pleasant floral odours. While benzaldehyde possesses a fruit-like odour, benzyl alcohol has a high threshhold of olfactory detection and may contribute significantly to floral odours [50]. The β-phenylethyl acetate is of some interest because it has long been used as a synthetic honey flavor [53]. Caryophyllene has been impli-

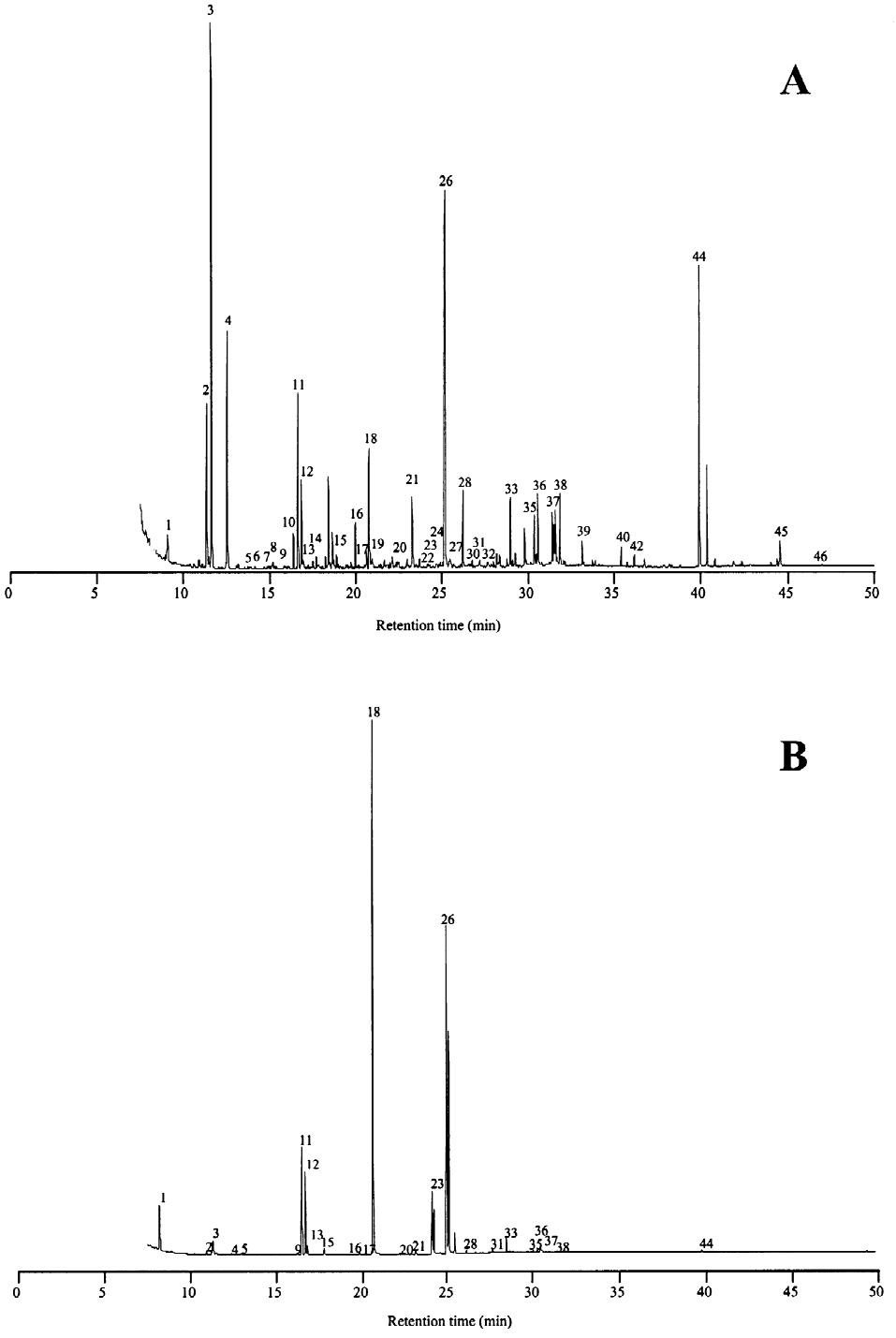

Fig. 5. Total ion chromatograms of floral fragrances collected by (A) Porapak Q trap and (B) Tenax TA trap from *Rosa hybrida* ''*Sandra*''. Peak numbers correspond to the numbers indicated in Table 1. For analytical conditions, see Section 2.

Table 4
Composition of floral fragrances in *Rosa hybrida "Sandra"* collected by different adsorbent traps (mean peak area %)

Group	Compound	Adsorbent[a]						
		A-1	A-2	A-3	A-4	A-5	A-6	A-7
Alcohol	β-Citronellol	nd	0.2	0.2	nd	nd	nd	nd
	2-Ethyl hexanol	3.1	1.9	nd	nd	2.4	3.8	3.1
	Geraniol	0.0	2.9	0.7	nd	nd	0.4	nd
	Hexadecanol	4.0	2.9	33.8	13.0	1.8	5.7	1.8
	Hexanol	3.3	6.1	0.4	2.8	nd	0.9	0.2
	cis-3-Hexen-1-ol	0.2	2.4	nd	nd	0.3	nd	3.1
	Linalool	3.2	0.9	0.5	nd	nd	1.4	nd
	Pentadecanol	nd	nd	nd	nd	nd	0.6	1.4
	2-Phenylethanol	3.0	0.3	1.8	14.6	0.3	6.5	1.7
	Tetradecanol	0.8	nd	nd	9.8	nd	0.5	nd
Aldehyde	Benzaldehyde	5.5	6.8	2.5	7.6	0.3	3.6	0.6
	Citral	18.6	8.3	17.2	5.0	8.2	36.0	8.4
Alkane	Decane	9.1	12.7	0.4	2.2	1.2	0.9	0.6
	Hexadecane	0.8	0.3	1.9	3.4	2.0	0.9	0.8
	Nonane	12.4	14.9	8.8	nd	2.0	14.2	1.4
	Tetradecane	1.0	0.5	nd	1.1	2.4	0.5	0.7
	Tridecane	1.1	0.2	nd	1.0	nd	1.3	nd
Monoterpene	Camphene	1.4	2.2	nd	nd	0.5	nd	2.0
	Limonene	nd	1.5	4.0	4.6	0.3	0.8	nd
	β-Myrcene	nd	6.1	nd	nd	nd	0.4	nd
	α-Pinene	1.4	2.3	0.8	nd	1.0	3.4	4.2
	β-Pinene	1.3	1.5	0.5	nd	51.1	1.8	49.7
Sesquiterpene	Caryophyllene	0.8	0.6	7.3	nd	0.3	1.3	0.8
Ester	Benzylacetate	0.6	0.3	nd	nd	0.6	0.5	nd
	Butyl acetate	11.0	0.5	nd	6.1	8.5	3.8	6.4
	Citronellyl acetate	nd	nd	1.3	nd	0.4	0.4	0.4
	2-Dodecen-1-yl-succinic anhydride	1.0	1.0	7.6	8.6	0.5	1.8	0.4
	Geranyl acetate	nd	0.2	2.1	nd	0.4	0.3	0.2
	Hexyl acetate	4.2	1.8	0.5	1.1	0.2	0.6	0.4
	Methyl benzoate	3.5	1.7	0.6	3.0	1.0	1.3	1.3
	Methyl salicylate	1.2	0.5	0.6	0.0	1.0	1.3	0.4
	Neryl acetate	nd	nd	2.5	nd	0.3	nd	0.5
	β-Phenylethyl acetate	0.9	10.4	nd	nd	nd	nd	nd
Ether	Cineole	1.4	1.7	nd	2.2	0.4	1.3	nd
	Eugenol	0.4	0.2	nd	nd	0.2	nd	nd
	Methyl eugenol	nd	nd	nd	nd	0.2	nd	nd
Ketone	2-Cyclohexan-1-one	1.9	5.2	nd	nd	6.5	nd	7.3
	Isophorone	0.7	0.2	nd	3.2	0.9	1.2	nd
	2-Pentadecanone	0.4	0.3	1.3	2.4	1.5	0.8	0.5
	2-Tridecanone	1.7	0.3	1.8	8.3	3.0	1.8	1.8
	2-Undecanone	nd	nd	1.1	nd	nd	nd	nd

[a] Adsorbent symbols: A-1, Tenax TA; A-2, Porapak Q; A-3, Chromosorb P; A-4, Chromosorb W; A-5, C_{18} cartridge; A-6, CN cartridge; A-7, NH_2 cartridge. $n=3$. nd = not detected.

Table 5
Composition of floral fragrances in different species of *Rosa hybrida* collected by Tenax TA trap

Group	Compound	*Rosa hybrida*		
		''Sandra''	''Cardinal''	''Silva''
Alcohol	β-Citronellol	nd	nd	+
	2-Ethyl hexanol	+++	+	+
	Geraniol	nd	nd	nd
	Hexadecanol	+++	++++	++
	Hexanol	+++	++++	+++
	cis-3-Hexen-1-ol	+	nd	+
	Linalool	++++	+	+
	Pentadecanol	nd	+	nd
	2-Phenylethanol	+++++	nd	+++
	Tetradecanol	+	+++	nd
Aldehyde	Benzaldehyde	++++	++	nd
	Citral	++++	+	+++++
Alkane	Decane	++++	+	++
	Hexadecane	+	++	++
	Nonane	++++	++++	+++
	Tetradecane	++	+++	+++
	Tridecane	+++	+++	nd
Monoterpene	Camphene	+++	+	+
	Limonene	nd	+++	+++
	β-Myrcene	nd	+	+++
	α-Pinene	+++	nd	nd
	β-Pinene	+++	+	+
Sesquiterpene	Caryophyllene	+	+++++	+++
Ester	Benzylacetate	+	++	+
	n-Butyl acetate	++++	+	+
	Citronellyl acetate	nd	+	+
	2-Dodecen-1-yl-succinic anhydride	++	+++	+
	Geranyl acetate	nd	+	+
	Hexyl acetate	+++	nd	+
	Methyl benzoate	+++	nd	nd
	Methyl salicylate	+++	++	++
	Neryl acetate	nd	++	+
	β-Phenylethyl acetate	+	nd	nd
Ether	Cineole	+++	++	+
	Eugenol	+	nd	nd
	Methyl eugenol	nd	nd	+
Ketone	2-Cyclohexan-1-one	+++	nd	+
	Isophorone	+	+++	nd
	2-Pentadecanone	+	++	++
	2-Tridecanone	+++	+	++
	2-Undecanone	nd	+	++

nd = not detected. +, <0.5%; ++, 0.5–1%; +++, 1–5%; ++++, 5–20%; +++++, >20% (peak area %).

cated as an attractant for the green lace wing insect [54]. Many compounds like geraniol, alcohols, ketones, esters, monoterpenes and sesquiterpenes were established in field tests as the most important ''key'' compounds for the attraction and excitation of mate seeking bees [55,56].

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