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Note

Analysis of Fragrance Volatiles of Korean *Rosa hybrida* Using Gas Chromatography-Mass Spectrometry

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Two different extraction procedures, SDE and SPME, were employed to determine a comprehensive and efficient fragrance profile of Korean *Rosa hybrida*. Both extraction methods could compensate for each other, covering compounds with diverse boiling point, polarities, and chemical properties. A total of 46 compounds were identified in *Mi-hyang*. The identified compounds were composed of 17 alcohols, 14 carbonyls, 7 aliphatic hydrocarbons, 2 terpene hydrocarbons, 4 benzenes, 1 ester, and 1 miscellaneous compound. Quantitatively, carbonyls (12.96~21.79% in essential oils of SDE and 2.89~8.44% in SPME headspace) and alcohols (7.98~11.73% in essential oils of SDE and 3.39~17.35% in SPME headspace) were dominant in *Mi-hyang*'s volatiles.

Key words: *fragrance compounds, GC-MS, rose, SDE, SPME*

Many flowers' volatiles are pleasant to the human sensory system and have potential application as components of perfumes, cosmetics, foods, aromatherapy, household products, and many other consumer goods.[1] Especially, rose is one of the most appreciated flowers around the world, the essential oil of which is extensively used in high-grade perfumes and sometimes for the isolation of commercial rhodinol.[2] Several thousand compounds have been identified from various floral fragrances. Most of these compounds are terpenes, esters, alcohols, aldehydes, ketones or alkanes.[3] A good number of reports have appeared on the chemical evaluation of rose oil around the world.[4,5] An excellent review of the useful literature on floral scents was given by Knudsen *et al.*,[6] and there have been some reports on the fragrance compositions of rose flowers. The fragrance signature of rose flowers is a composite of volatile chemicals in specific stoichiometric concentrations.[6] Typically, these compounds are fatty acids derivatives, benzenoids, phenylpropanoids, isoprenoids and nitrogen- or sulfur- containing compounds, and have low polarity, slight water solubility, high vapour pressure and high lipophilicity.[6] Dobson *et al.*[7] found a total of 31 fragrance compounds including 2-phenylethanol, citronellol, benzyl alcohol, methyl eugenol and geraniol from *Rosa rugosa*. Some other workers also reported fragrance components of Rosa species including *Rosa chinensis* and *Rosa damascena*.[3] Various studies on fragrance compositions of various roses cultivated in many countries have been published. However, there have not been adequate studies on the fragrance of rose flower cultivated in Korea.

In this study, volatile compounds in Korean *Rosa hybrida*, were isolated and analyzed to investigate the aroma property. To extract fragrances from *Mi-hyang*, two extraction methods, simultaneous steam distillation and solvent extraction (SDE) and solid phase micro extraction (SPME), were used. SDE provided high extraction efficiency for diverse range of compounds, covering from volatiles to semi-volatiles. However, poor recovery of polar and water-soluble compounds as well as low boiling point compounds, which were eluted earlier or together with solvent used, could not be avoided. On the other hand, efficient extractions of compounds with low boiling points could be achieved by sampling the headspace using SPME. Therefore, both extraction methods could compensate for each other, covering compounds with diverse boiling point, polarities, and chemical properties.[8]

Materials and Methods

Materials. The fresh picked flower samples of *Rosa hybrida* L. cv. *Mi-hyang* grown at a green house farm around the institute in Suwon, Korea were gathered from December (2001) to January (2002) and stored at –70°C until extraction.

Fragrance compounds extraction from Mi-hyang. The fragrance compounds from Mi-hyang were extracted by SDE and SPME. For SDE, the flower samples of Mi-hyang (70 g) were placed in a 2 *l* round flask with 1 *l* of distilled water and heated in a Likens-Nickerson (L-N) apparatus. The volatile compounds were extracted under atmospheric pressure during

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Abbreviations: GCD, gas chromatography-electron ionization detector; GC-MS, gas chromatography-mass spectrometry; SDE, simultaneous steam distillation and solvent extraction; SPME, solid phase micro-extraction.

2 h, using diethyl ether (50 m*l*) as extracting solvent. After running in the L-N apparatus, the solvent extract was divided into two fractions, aqueous and organic, containing non-volatile and volatile components, respectively. The organic fraction was dried over anhydrous sodium sulfate to move any residual water, and concentrated up to 0.1 m*l* by a gentle stream of nitrogen gas.

A 20 g portion of the flower samples in the 60 m*l* headspace glass bottle capped with silicon/teflon septum (Supelco) was maintained at 40°C for 90 min in the water bath to reach equilibrium state. A SPME device obtained from Supelco (Bellefonte, PA) was inserted into the sealed bottle using 20-mm length needle. A 86 m polyacrylate (PA) fiber was used for extracting headspace volatiles. The volatile compounds were absorbed on the fiber for 60 min. Thermal desorption of volatiles from the SPME fiber was carried out at 230°C in the injection port of GC by holding at the splitless mode for 5 min. The fiber used was reconditioned for 10 min in the GC injection port at 230°C to remove any volatiles remaining on the fiber.

Analysis by GCD and GC-MS. GCD and GC-MS analyses were performed using an HP G1800B gas chromatograph-electron ionization detector (GCD) and an HP 6890 series gas chromatograph-5973 mass selective detector (GC-MS, Hewlett-Packard Co., Palo Alto, CA), respectively. DB-5MS in the GCD and Innowax columns (30 m length × 0.25 mm i.d. × 0.25 m film thickness, J & W Scientific, Folsom, CA) in the GC-MS were used to separate the volatile compounds. The carrier gas of GCD and GC-MS was helium with a constant flow rate of 1.0 m*l*/min. One micro liter of the extract obtained using diethyl ether was injected at a split-less mode. In the case of SPME, adsorbent volatiles on fibers were transferred into GDC and GC-MS at the split-less mode with 5 min of initial purge off time. The oven temperature was held for 5 min at 40°C, then raised at 5°C/min to 230°C, and held for 20 min at 230°C. The injector and detector temperatures were 230 and 250°C, respectively. Two mass detectors were operated in electron impact mode with ionization energy of 70eV and the scanning of GCD and GC-MS range of 10-425 amu and 10-720 amu, respectively.

Fragrance compounds identification in *Mi-hyang*. The identification of the volatile compounds was based on comparison of their mass spectra with those of on-computer library (Wiley 275, 1995) (Hewlett-Packard Co., Palo Alto, CA) or by manual interpretation. Also, the linear retention indices (RI) of the compounds, determined by using *n*-paraffins C_8-C_{24} as external references,[9] were compared with those of the published literatures.[10,11] Semi-quantification of the volatile compounds was performed based on the ratios of the peak areas obtained from MS total ion chromatogram.

Results and Discussion

There are numerous methods for isolating fragrance volatiles from a flower. No one individual method is suitable for all purposes because of the complexity of samples, the wide variation of fragrance composition, and different analytical objectives.[12] One of the most popular methods in the fragrance analysis field is SDE, although it has a few disadvantages such as possible artifact formation during the heating process and low recoveries for polar compounds. However, this technique also shows high reproducibility and yield for non-polar and semi-polar compounds such as carbonyl compounds or alcohols.[13] SPME requires a relatively simple sample preparation and is a technique that uses a fused silica fiber, which is coated on the outside with an appropriated stationary phase for the adsorption of volatiles. This method saves sample preparation time, solvent use, and disposal costs. The affinity of the fiber for an analyte depends on the principle of 'like dissolves like', and coating fibers having different properties or thickness are selected in accordance with different compounds. Especially, the more-polar polyacrylate (PA) fiber is preferred for the extraction of more-polar analytes.[14] In this study, two different extraction procedures, SDE and SPME fiber coated with polyacrylate, were employed to determine a comprehensive and efficient fragrance profile of *Mi-hyang*.

Fig. 1 shows the total ion chromatograms of the SDE extracts and SPME headspace of *Mi-hyang* on DB-5MS and Innowax columns. Table 1 lists volatile compounds isolated by SDE and SPME fiber coated with 86 μm polyacrylate, their relative peak areas [(peak area of each compound/total peak areas) × 100 (%)], and RI values of the compounds on DB-5MS and Innowax columns. A total of 46 compounds were identified in *Mi-hyang*. The identified compounds were composed of 17 alcohols, 14 carbonyls, 7 aliphatic hydrocarbons, 2 terpene hydrocarbons, 4 benzenes, 1 ester, and 1 miscellaneous compound. A total of 40 compounds were detected in the extract by SDE, while only 19 compounds were detected by headspace SPME. Some alcohols, such as ethyl alcohol, 1-penten-3-ol, 2-penten-1-ol, *cis*-3-hexenol, and 1-hexanol, present in small amounts in *Mi-hyang*, were found only in SDE extraction, mainly due to the high extraction efficiency of SDE. 2-Pentenal, (*E,E*)-2,4-heptadienal, furfural and 2,4-hexadienal were also detected only in SDE extraction. The formation of these volatiles could also have increased due to the heat treatment during SDE extraction.

Quantitatively, carbonyls (12.96~21.79% in essential oils of SDE and 2.89~8.44% in SPME headspace) and alcohols (7.98 ~11.73%) in essential oils of SDE and 3.39~17.35% in SPME headspace) were dominant in *Mi-hyang*'s volatiles. In SDE extracts, 3,5-dimethyoxy toluene (10.93%), tricosane (9.90%), β-ionone (9.15%), eicosane (6.70%), dihydro-β-ionone (6.39%) and dihydro-β-ionol (6.94%) on DB-5MS column and 2,7-Dihydroxy-4-methylcyclohepta-2,4,6-trien-1-on (6.22%), heneicosane (5.33%), β-ionone (4.43%), and dihydro-β-ionol (2.29%) on Innowax column were the major compounds, quantitatively. In the SPME headspace 3,5-dimethyoxy toluene (9.66%), β-ionone (3.18%) and 2,4-diisocyanto-1-methyl-1-benzene (2.59%) on DB-5MS column and 1,2-butanediol (13.78%), 3,5-dimethyoxy toluene (5.31%), and

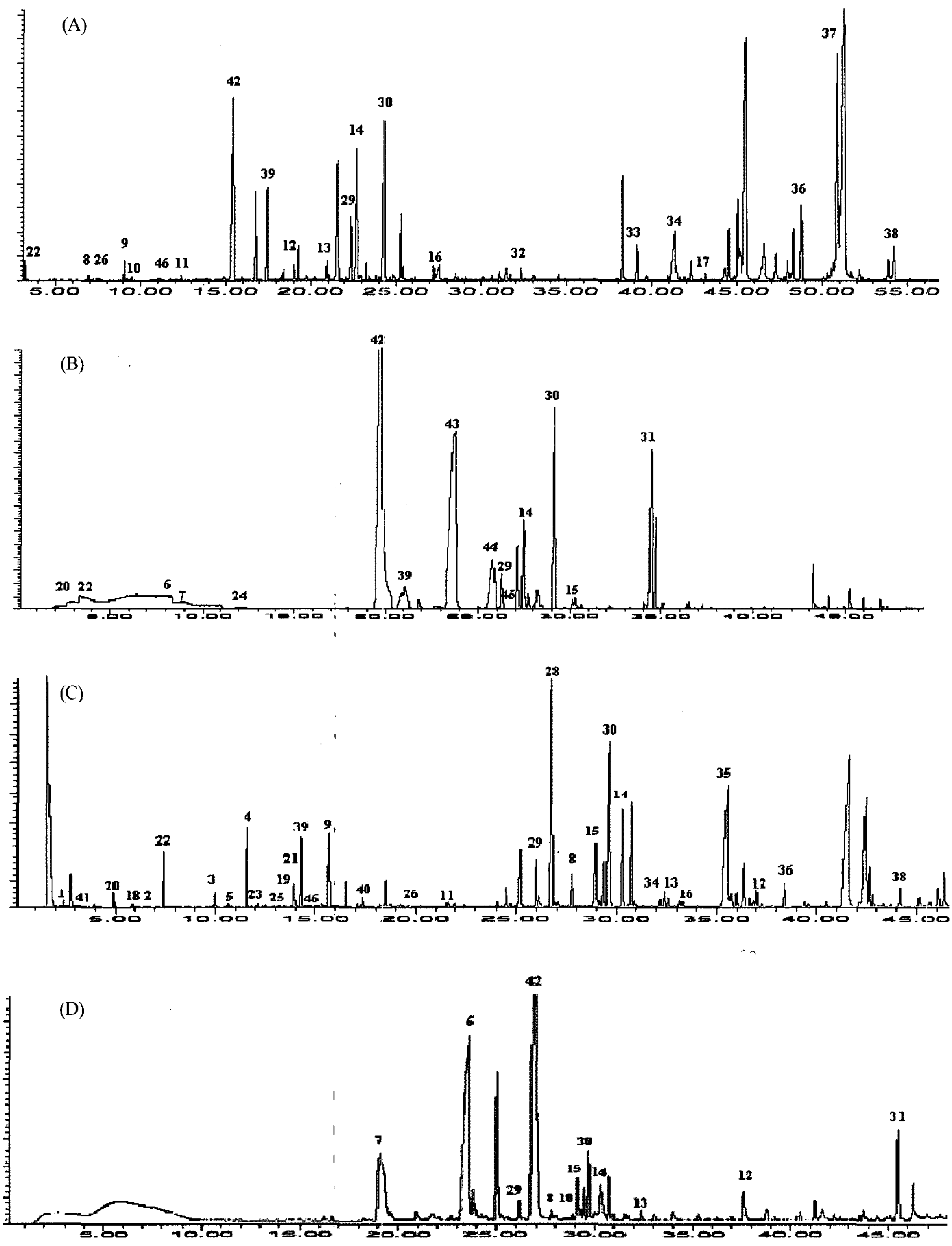

Fig. 1. Total ion chromatogram of Korean *Rosa hybrida* L. cv. *Mi-hyang* volatile isolated by two different extraction methods. (a) SDE extracts on DB-5MS column; (b) SPME headspace on DB-5MS column; (c) SDE extracts on Innowax column; (d) SPME headspace on Innowax column.

diphenyl methanone (1.56%) on Innowax coluumn were the major compounds.

With regard to the characteristic rose fragrance, several compounds with rose odor and other floral notes, such as geranyl acetate (~0.20%), benzyl alcohol (~0.68%), phenyl ethyl alcohol (~0.40%), were identified in this study. Dihydro-β-ionol (~4.94%), nerolidol (~0.44%), 2,4-hexadienal (~0.06%), benzaldehyde (~0.04%), and theaspirane (~4.06%), possessing

Table 1. Volatile compounds of Korean *Rosa hybrida* L. cv. *Mi-hyang* isolated by two different extraction methods

No.	Compounds	DB-5 column			Innowax column			Aroma property	ID[e]
		RI[a]	P.A (%)[b]		RI	P.A (%)			
			SDE[c]	SPME[d]		SDE	SPME		
			Alcohols						
1	ethyl alcohol	ND[f]	-	-	928	0.52	-		MS/RI
2	1-penten-3-ol	ND	-	-	1174	0.04	-	mild green	MS/RI
3	2-penten-1-ol	ND	-	-	1329	0.24	-		MS
4	*cis*-3-hexenol	ND	-	-	1387	1.05	-	green	MS/RI
5	1-hexanol	ND	-	-	1354	0.05	-		MS/RI
6	1,3-butanediol	892	-	0.34	1747	-	13.78		MS
7	2-[2-ethoxyethoxy]-ethanol	1011	-	1.05	1617	-	1.62		MS
8	benzyl alcohol	1034	0.40	-	1867	0.68	0.19	burning	MS
9	linalool	1102	1.42	-	1541	0.39	-	floral	MS/RI
10	phenyl ethyl alcohol	1114	0.40	-	1900	-	0.14		MS
11	α-terpineol	1192	0.34	-	1687	0.15	-	sweety	MS/RI
12	eugenol	1361	0.84	-	2155	0.33	0.08	clove-like	MS/RI
13	methyl eugenol	1372	0.79	-	2006	0.31	0.17	burning	MS/RI
14	dihydro-β-ionol	1405	6.94	1.62	1956	2.29	0.66	woody-flowery	MS/RI
15	2,6-bis[1,1-dimethylethyl]-4-methyl phenol	1508	-	0.38	1904	1.83	0.71		MS
16	nerolidol	1566	0.44	-	2036	0.10	-	rose-like	MS/RI
17	geranyl linalool isomer	2031	0.16	-	ND	-	-		MS/RI
			Carbonyls						
18	2-pentenal	ND	-	-	1131	0.02	-	green	MS/RI
19	(*E,E*)-2,4-heptadienal	ND	-	-	1458	0.03	-		MS/RI
20	hexanal	802	-	0.55	1081	0.19	-	fruity	MS/RI
21	furfural	ND	-	-	1463	0.52	-	penetrating	MS/RI
22	*trans*-2-hexenal	859	6.04	1.91	1214	0.61	-	leafy	MS/RI
23	2,4-hexadienal	ND	-	-	1397	0.01	-	fresh, floral	MS/RI
24	dihydro-2[3H]-furanone	925	-	0.16	1620	-	-		MS
25	benzaldehyde	ND	-	-	1437	0.03	-	bitter almond	MS/RI
26	benzeacetaldehyde	1044	0.15	-	1632	0.06	-		MS/RI
27	nonanal	1106	0.06	-	ND	-	-	citrus-like	MS/RI
28	2,7-dihydroxy-4-ethylcyclohepta-2,3,6-trien-1-one	ND	-	-	1837	6.22	-		MS
29	dihydro-β-ionone	1382	6.39	1.03	1818	0.84	0.28	woody	MS/RI
30	β-ionone	1472	9.15	3.18	1923	4.43	1.05	fruity	MS/RI
31	diphenyl methanone	1622	-	1.61	2451	-	1.56		MS
			Aliphatic Hydrocarbons						
32	heptadecane	1700	0.20	-	1700	0.07	-	alkane	MS/RI
33	nonadecane	1900	0.56	-	1900	-	-	alkane	MS/RI
34	eicosane	2000	6.70	-	2000	0.17	-	alkane	MS/RI
35	heneicosane	2100	-	-	2100	5.33	-	alkane	MS/RI
36	docosane	2200	1.28	-	2200	0.51	-	alkane	MS/RI
37	tricosane	2300	9.90	-	2300	-	-	alkane	MS/RI
38	tetracosane	2400	1.33	-	2400	0.47	-	alkane	MS/RI
			Terpene Hydrocarbons						
39	theaspirane	1321	4.06	0.24	1474	1.26	-	sweety, fruity	MS
40	β-caryophyllene	ND	-	-	1565	0.19	-	clove-like	MS/RI

pleasant floral and fruity odor notes, were also detected in *Mi-hyang*. Some compounds, such as phenyl ethyl alcohol, methyl eugenol and eugenol, that were quantitatively the major fragrance components (~95%) of the rose in a previous study,[15] but were present in only small amounts in *Mi-hyang* fragrances in this study. On the other hand, geraniol, one of the most abundant monoterpene alcohols in essential oil from *Rosa* sp.[16] was not detected in *Mi-hyang*.

Table 1. Continued

No.	Compounds	DB-5 column			Innowax column			Aroma property	ID[e]
		RI[a]	P.A (%)[b]		RI	P.A (%)			
			SDE[c]	SPME[d]		SDE	SPME		
		Benzenes							
41	methyl benzene (toluene)	ND	-	-	1040	0.01	-		MS/RI
42	3,5-dimethoxy toluene	1264	10.93	9.66	1840	-	5.31		MS
43	2,4-diisocyanto-1-methyl-benzene	1336	-	2.59	ND	-	-		MS
44	1,3,5-trimethoxy benzene	1375	1.56	1.17	ND	-	-		MS
		Esters							
45	geranyl acetate	1414	-	0.20	ND	-	-	rose lavendar	MS/RI
		Miscellaneous compounds							
46	linalool oxide	1075	0.06	-	1473	0.03	-	sweety, woody	MS/RI

[a]Retention indices were determined using *n*-paraffins C_8-C_{24} as external references.
[b]Average of percent peak area in MS total ion chromatogram.
[c]Simultaneous steam distillation and solvent extraction (SDE)
[d]Solid phase microextraction (SPME): polyacrylate (PA) fiber.
[e]Tentative identification was performed as follows: MS/RI, mass spectrum was identical with that of Wiley mass spectrum database (1995) (Hewlett Packard Co., Palo Alto, CA, USA), and retention time index was consistent with that of the literatures; MS, mass spectrum was consistent with that of Wiley mass spectrum database.
[f] ND, not detected.

These results differ from volatile compounds of rose flower in previous studies. Kim *et al.*[3] identified a total of 41 compounds in floral fragrances of three species of *Rosa hydrida* and reported that the major components were citral, *n*-nonane, *n*-butyl acetate, *n*-decane, β-phenylethyl acetate and hexadecanol. A total of 35 constituents were identified in the essential oil of *Rosa Brunonii* flowers in which the major compounds were eugenol, citronellol, geraniol and terpinen-4-ol, quantitatively.[5] Volatile compounds of flower are known to be different according to flower species, sample to sample within single species and extraction condition.[3] β-phenyl ethanol which have been described as the major component of rose flower essential oil, is a common compound in *R. damascena* and *R. centifolia* but was found to be absent in *R. brunonii*.[5]

The present study is an analysis of volatile compounds of *Rosa hydrida* L. cv. *Mi-hyang* originating from Korea and no such studies have been reported in the literature until now. The volatile compound of *Mi-hyang* is of academic interest due to minimal commercial value. However, further studies are required on quality evaluation in order to determine their commercial application.

References

1. Rees, J. D. (1991) Reverse enzyme synthesis in microemulsion-based organogels. *Biochim. Biophys. Acta* **1073**, 493-501.
2. Rajeswara Rao, B. R., Kaul, P. N., Mallavarapu, G. R. and Ramesh, S. (2000) First observation of little leaf disease and its impact on the yield and composition of essential oil of rose-scented geranium (*Pelargonium sp.*) *Flavour Fragr. J.* **15**, 137-140.
3. Kim, H. J., Kim, K., Kim, N. S. and Lee, D. S. (2000) Determination of floral fragrances of *Rosa hybrida* using solid- phase trapping-solvent extraction and gas chromatography-mass spectrometry. *J. Chromatogr. A.* **902**, 389-404.
4. Sood, R. P., Singh, B. and Singh, V. *J.* (1992) Constituents of rose oil from Kangra Valley, H.P. (India). *Essent. Oil Res.* **4**, 425-426.
5. Kaul, V. K., Gujral, R. K. and Singh, B. (1999) Volatile constituents of the essential oil flowers of *Rosa brunonii* Lind. *Flavour Fragr. J.* **14**, 9-11.
6. Knudsen, J. T., Tollsten, L. and Bergstrom, G. (1993) Floral scents-a checklist volatile compounds isolated by head-space techniques. *Phytochemistry* **33**, 253-280.
7. Dobson, H. E. M., Bergstrom, J., Bergstrom, G. and Groth, I. (1987) Pollen and flower volatiles in two *Rosa* species. *Phytochemistry* **26**, 3171-3173.
8. Cho, I. H., Kim, T. H., Cho, S. K., Lee, H. J. and Kim, Y. S. (2002) Analysis of volatile compounds in bulgogi. *Food Sci. Biotechnol.* **11**, 303-309.
9. van den Dool, H. and Kratz, P. D. (1963) A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. *J. Chromatogr.* A. **11**, 463-471.
10. Kondjoyan, N. and Berdague, J. L. (1996) Compilation of relative retention indices for the analysis of aromatic compounds: Laboratoire Faveur, Station de Recherches sur la Viande. Clermont-Ferrand, France.
11. Adams, R. P. (2001) In *Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy.* Allured Publishing Co., Carol Stream, USA.

12. Yang, X. and Peppard, T. (1999) Food and flavor application, In *Solid Phase Microextraction. A Practical Guide*, Wercinski SAS. (ed.) pp. 177-201, Marcel Dekker, New York, USA.
13. Siegmund, B. and Pfannhauser, W. Z. (1999) Changes of the volatile fraction of cooked chicken meat during chill storing: Results obtained by the electronic nose in comparison to GC-MS and GC olfactometry. *Z. Lebensm. Unters. F. A.* **208**, 336-341.
14. Kataoka, H., Lord, H. L. and Pawliszyn, J. (2000) Application of solid-phase micro extraction in food analysis. *J. Chromatogr. A.* **880**, 35-62.
15. Aydinli, M. and Tutas, M. (2003) Production of rose absolute from rose concrete. *Flavour Fragr. J.* **18**, 26-31.
16. Rohloff, J. (2002) Volatiles from rhizomes of *Rhodiola rosea* L. *Phytochemistry.* **59**, 655-661.