Comparative Analyses of the Flavors from Hallabong (*Citrus sphaerocarpa*) with Lemon, Orange and Grapefruit by SPTE and HS-SPME Combined with GC-MS

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The aroma component of Hallabong peel has been characterized by GC-MS with two different extraction techniques: solid-phase trapping solvent extraction (SPTE) and headspace solid-phase microextraction (HS-SPME). Aroma components emitted from Hallabong peel were compared with those of other citrus varieties: lemon, orange and grapefruit by SPTE and GC-MS. *d*-Limonene (96.98%) in Hallabong was the main component, and relatively higher peaks of *cis*-β-ocimene, valencene and -farnesene were observed. Other volatile aromas, such as sabinene, isothujol and δ-elemene were observed as small peaks. Also, principal components analysis was employed to distinguish citrus aromas based on their chromatographic data. For HS-SPME, the fiber efficiency was evaluated by comparing the partition coefficient (K_{gs}) between the HS gaseous phase and HS-SPME fiber coating, and the relative concentration factors (CF) of the five characteristic compounds of the four citrus varieties. 50/30 μm DVB/CAR/PDMS fiber was verified as the best choice among the four fibers evaluated for all the samples.

Key Words : Hallabong, Citrus flavor, Solid phase trapping solvent extraction, Headspace solid-phase microextraction, GC-MS

Introduction

Hallabong (*Citrus sphaerocarpa Tan.*, Rutaceae family, Citrus genus) shown in Figure 1 is a hybrid variety of tangerine and belongs to a crossbreeding between Cheonggyeon (Kiyomi tangor) and Ponggang (Ponkan). Introduced to Jeju Island in the early 1990's, the Hallabong is grown as the representative citrus fruits produced in Korea.[1,2] This loose skin fruit has a pleasant and comforting aroma and sweet taste, with more than 15 Brix (equal to percent) of high sugar content. Several compounds in the citrus oil extracted from the Hallabong's peel have various therapeutic effects.[3-7]

Citrus fruits, such as lemon, orange and grapefruit, are of great importance in foods, flavor and the cosmetics industry. Especially, their essential oils are primary byproducts of citrus processing along with juice production. Botanical insecticides that include citrus oil are an alternative to synthetic chemical formulations for controlling ants, roaches, and fleas. In some industrial settings, citrus-based products have been substituted for toxic solvents to clean metals. Essential oils are often found on the market adulterated with similar essential oils, chemicals and synthetics, as well as extenders, such as dipropylene glycol.

Detailed analyses of the aroma components of citrus fruits are important for citrus industries to ensure the production of quality foods with consistent flavors from batch to batch, and to detect adulteration. Citrus oils are highly susceptible to oxidation, resulting in significant changes in the odor and flavor profile of food. They are also important in plant breeding for the selection of superior cultivars and in other agriculture related issues.

Gas chromatography (GC), GC-mass spectrometry (MS) and GC-olfactometry (GC-O) are widely used to study the composition of citrus aroma.[8-18] Traditionally, steam distillation and solvent extraction are the common methods to extract citrus aroma oils.[8,14-17] The main drawbacks of these conventional processes are their low yields, time and labor requirements, concentration step, the formation of thermally degraded undesirable byproducts, and solvent contamination. Novel techniques of sample preparation may mitigate these problems and provide a more convenient procedure.

Cold-pressing, also known as expression, is used exclusively for citrus oils. This is a mild and gentle pressing treatment in which the rind of the citrus fruit is removed, the outer layer of the peel is ruptured and the oil is then pressed out. Alternatively, supercritical fluid extraction (SFE) methods also are reported.[19,20] Most recently, Arce *et al.* evaluated the solvent of propanediol for the separation of the citrus oil components, limonene and linalool.[21]

Because of the convenience of extraction, headspace (HS) sampling and HS-GC-MS methods have been attractive for

Figure 1. Photograph of Hallabong (*Citrus sphaerocarpa Tan.*) and its cross-sectional view.

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both food and flavor applications. Previously, we studied the volatile fragrances and flavors from rose, lavender, thyme, rosemary and garlic by GC-MS with solid-phase trapping solvent extraction (SPTE) or headspace solid-phase microextraction (HS-SPME).[22-27] SPME developed by Pawliszyn and coworkers in 1989, is a solvent-free extraction technique widely used in extractions of natural products, food, biological and environmental samples.[28-31] SPTE and HS-SPME techniques arealternatives to conventional extraction techniques in analytical scale. Both techniques allow analytical sampling, avoiding losses or decomposition of the components sought and contamination of fruit tissues constituents. They also minimize the activity of enzymes. In addition, these extraction techniques allow obtaining real aroma profiles of fruits.

Detail aroma compositions emitted from Hallabong have not been studied until now. In the present study, we characterize the aroma components of Hallabong peel by GC-MS with two different extraction techniques, SPTE and HS-SPME. Aroma components emitted from Hallabong peel were compared with those of lemon, orange and grapefruit by SPTE and GC-MS. Principal components analysis (PCA) was employed to distinguish citrus aromas based on their chromatographic data. For HS-SPME, the fiber efficiency was evaluated by comparing the partition coefficient (K_{fs}) between the HS gaseous phase and HS-SPME fiber coating, and the relative concentration factors (CF) of the five characteristic compounds of the four citrus varieties.

Experimental Section

Plant material and reagents. Hallabong (*Citrus sphaerocarpa Tan.*) grown in Jeju Island in Korea was supplied by a local farm. Lemon (*Citrus limonum*), orange (*Citrus aurantium* (Linn.) *var. dulcis*) and grapefruit (*Citrus paradisi*) were purchased from a local market in Korea. Only the peel of the fruits was used to collect aroma components. All reference standards were of analytical grade and were purchased from Sigma-Aldrich (St. Louis, MO, USA), or Tokyo Kasei (Nihonbashi, Tokyo, Japan). A working reference standards mixture was prepared using hexane as a solvent with a concentration of 2 mg/mL for each compound. Organic solvents of chromatographic grade were obtained from Sigma-Aldrich.

Solid-phase trapping solvent extraction (SPTE). Flavor compounds were collected from the four citrus varieties by using a SPTE apparatus (Fig. 2) designed in our laboratory. First, 100 grams of citrus peels was chopped manually with a knife into about 10 mm × 10 mm pieces and sealed in a 250 mL round bottom flask. Volatile aroma compounds were collected for 2 hours at ambient temperature by a SPTE device, using the ethylvinyl benzene divinyl benzene copolymer (Porapak-Q, Supelco, 149-125 μm) as an adsorbent, which is identical to the procedure in our previous reports.[22-27] Before use, Porapak-Q particles were pre-rinsed with organic solvent to remove impurities. The inlet of the Pasteur pipet packed with Porapak-Q was attached to the flask containing the citrus samples. An oil-free electric vacuum pump (Vacuubrand GMBH, 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 via glass-manifold. Purified nitrogen gas (purity, 99.99%) flowing at *ca.* 400 mL/min was passed into the flask and out through the adsorbent trap under reduced pressure. After one run, the captured compounds were eluted with 2 mL of petroleum ether. The eluate was concentrated to final volume of approximately 200 μL in a water bath at 40 °C. The experiments were carried out in triplicate.

Headspace solid-phase microextraction (HS-SPME).

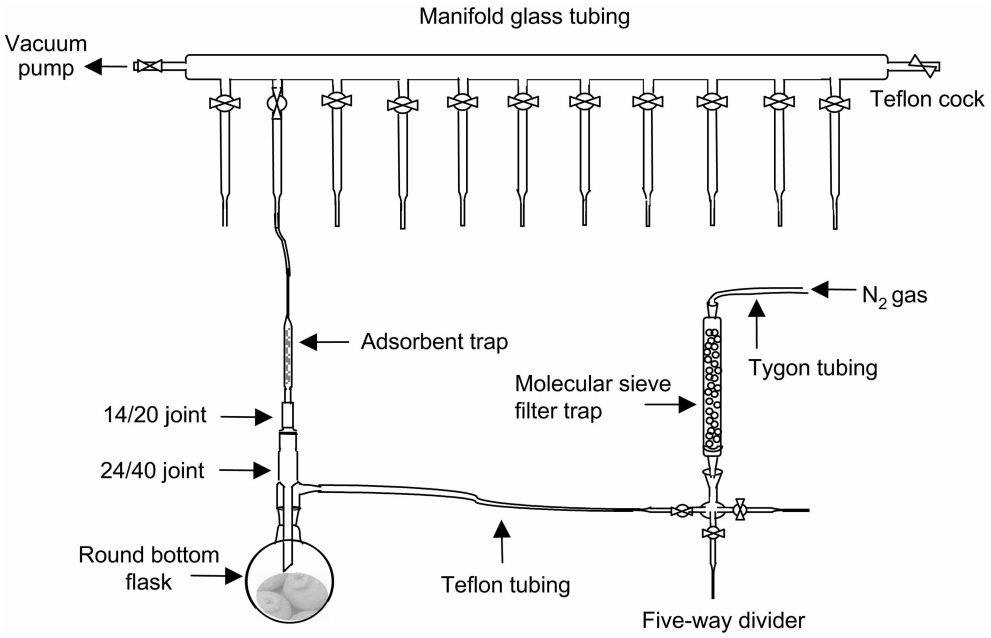

Figure 2. Illustration of solid-phase trapping solvent extraction (SPTE) apparatus.

All SPME holders and coating fibers were obtained from Supelco (Bellefonte, PA, USA). For the HS-SPME sampling, four SPME devices, the 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS), 100 μm polydimethylsiloxane (PDMS), 30 μm PDMS, and 75 μm carboxen-polydimethylsiloxane (CAR/PDMS) phases, were used. Before and after use, SPME fibers were conditioned by heating them in the hot injection port of a gas chromatograph at 250-320 °C for 30-240 min to remove contaminants. Also, before an analysis, a fiber blank was run to confirm no contamination peak.

A series of experiments was performed using working standards mixture to optimize conditions of HS-SPME, such as the most suitable temperature and equilibration time to obtain a significant headspace fraction. The fiber adsorption profile for each compound was determined by varying the exposure time of the fiber to the working standards mixture (every 10 min from 10 min to 60 min). Moreover, comparative experiments for the adsorption temperature were carried at 20 °C and also 40 °C. The same conditions were applied to all HS-SPME experiments to standardize sample preparation procedure.

About 7 g of the citrus peel samples was placed in a 100 mL vial, and the vial was capped tightly with a Teflon cap. The vial was left to equilibrate for at least 1 h at ambient temperature before HS-SPME and static HS sampling. The SPME fiber was exposed to the headspace above the sample for 60 min at 20 °C or 40 °C. After adsorption, the SPME fiber was removed from the sample vial and immediately inserted into the injection port of the GC-MS system, where the thermal desorption occurs at 250 °C for 60 sec.

A 5000 μL Hamilton TLL gastight syringe (Supelco) was used to inject a part of the sample headspace (5000 μL) into the injection port of the GC. Static HS was used for the determination of each K_{fg} value.

Gas chromatography-mass spectrometry. GC-MS analyses, using a Trace GC 2000 and a GC-Q Plus ion trap MS^n (Thermoquest-Finnigan, Austin, TX, USA) with electron impact ionization mode were carried out. Chromatographic separations were performed on a cross-linked 5% phenyl polydimethylsiloxane (SPB-5, Supelco, 60 m × 0.25 mm × 0.25 μm film thickness) column. The oven temperature program was 50 °C (3 min)-5 °C/min-240 °C (10 min). Injector and transfer line temperatures were 250 °C and 275 °C, respectively. The flow rate of the carrier gas (He, 99.9995%) was 1.0 mL/min. A split injection with a ratio of 1 : 30 was used. The electron impact ionization mass spectrometer was operated as follows: ionization voltage, 70 eV; ion source temperature, 200 °C; scan mode, 50.0-500.0 (mass range). The volatile aroma compounds were identified by linear retention indices of a series of *n*-alkane (C_8 to C_{23}) on SPB-5 column and by comparison of the mass spectra of each component with the NIST (National Institute of Standards and Technology, Gaithersburg, MD, USA) mass spectral library as well as the Wiley (Wiley, New York, NY, USA) mass spectral library.

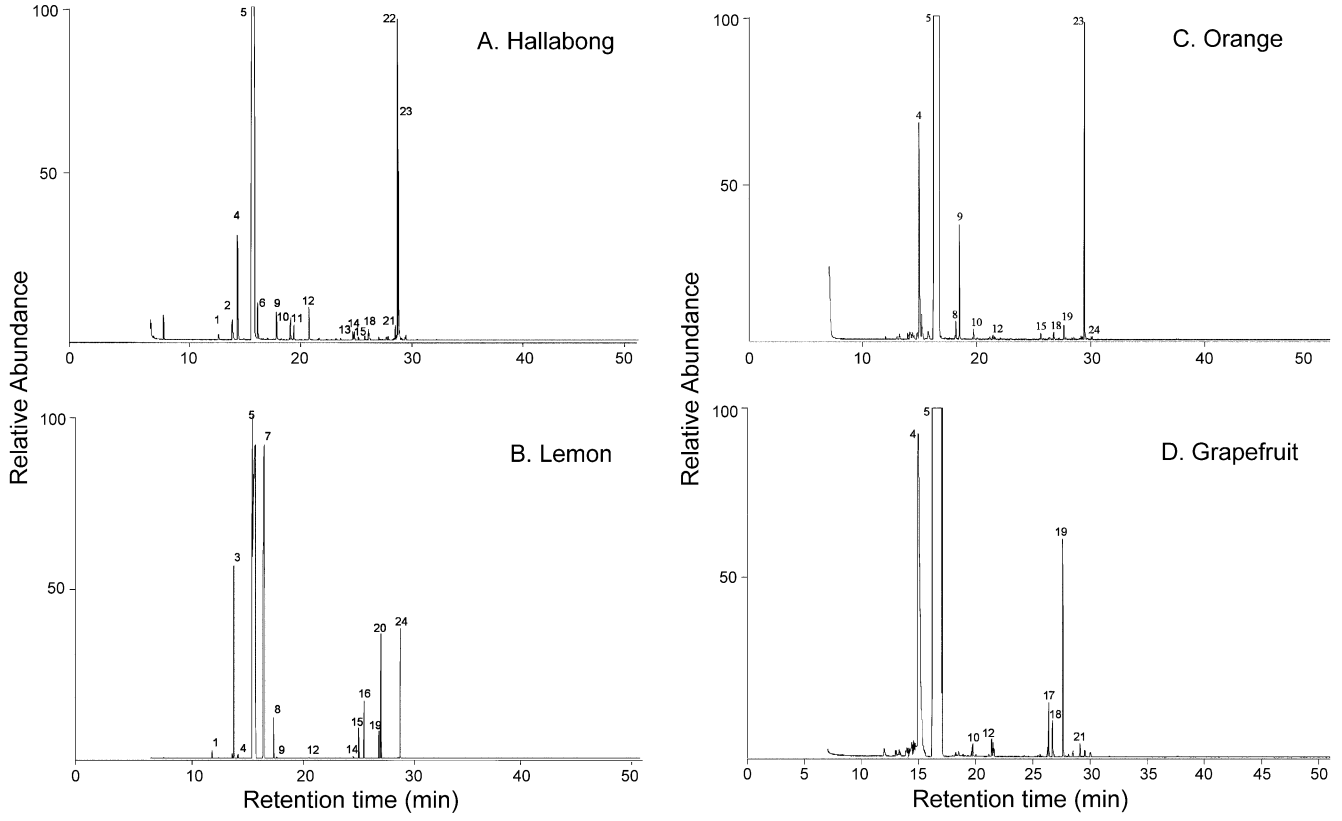

Figure 3. Total ion chromatogram on a SPB-5 column of citrus peels collected by SPTE. (A) Hallabong; (B) Lemon; (C) Orange; (D) Grapefruit. Peak numbers correspond to the numbers in first column of Table 1. For analytical conditions, see experimental section.

A GC-14B gas chromatograph with FID (Shimadzu, Japan) was also used for the measurement of analyte partition coefficient (K_{fg}) and the relative concentration factor (CF). GC-FID conditions were maintained as follows: temperature program, 50 °C (3 min)-5 °C/min-240 °C (10 min) on a cross-linked 5% phenyl polydimethylsiloxane (SPB-5, Supelco, 60 m × 0.25 mm × 0.25 mm film thickness) column; injector temperature, 250 °C; FID temperature, 250 °C; flow rate of carrier gas (N_2, 99.99% purity), 1.0 mL/min; split ratio, 1 : 2; flow rate of hydrogen, 35 mL/min; flow rate of air, 500 mL/min.

Principal components analysis (PCA). Chemometric analyses were accomplished with multivariate statistical analysis program (MVSAP, version 4.0) software developed in our laboratory and pre-validated by using known values and data sets in the literature.[32-36] From a multivariate data matrix having p variables and n samples, principal component scores were computed, using MVSAP.

Results and Discussion

Aroma components from peels of Hallabong and other citrus species by SPTE and GC-MS. Typical total ion chromatograms (TIC) on a SPB-5 column of the aroma components collected from fruit peels of four citrus species, which were analyzed by SPTE and GC-MS are shown in Figure 3. Table 1 gives a list of 24 aroma components found for fruit peels of four citrus species analyzed by GC-MS. The retention indices and characteristic mass spectral ions of each peak from citrus varieties used in the present study are also given. Comparison of aroma components found in Hallabong peel with those found in peels of lemon, orange and grapefruit are summarized in Table 2. *d*-Limonene was the main component in all samples with concentration (normalized peak area %) of 96.98% for Hallabong, 64.82% for lemon, 99.59% for orange, 98.38% for grapefruit. Relatively higher peaks of *cis*-β-ocimene, valencene and α-farnesene in Hallabong are observed in Figure 3. Other volatile aromas found at small peaks may be also important in the contributions to the aroma activity. Particularly, sabinene, isothujol and δ-elemene were found only in Hallabong. However, a previous study involving steam distillation and cold-pressing indicates that sabinene was also found in lemon and orange, and δ-elemene in orange.[16] In Table 2, lemon shows a quite different aroma composition compared with the others. Of fourteen components in lemon peel, the contribution of γ-terpinene (18.64%), β-pinene (4.21%) and *cis*-β-ocimene (3.21%) is significant.

The sample amount of citrus peel required is as much as 2.0-2.5 kg to obtain an analytical sample by the conventional cold-pressing method.[16] In contrast, the amount of peel sample could be reduced to less than 100 g with the SPTE method.

Principal components analysis (PCA). Principal compo-

Table 1. Characteristic mass spectral ions of volatile compounds assigned from the flavors of the four citrus varieties using a 5% phenylpoly(dimethylsiloxane) column (Supelco SPB-5 60 m × 0.32 mm × 0.25 mm)

Peak No.	Compound	I	k	M_r	Base Peak m/z (100%)	Characteristic mass spectral ions (EI)
1	α-Pinene	954	1.70	136	91	77(84), 51(79), 93(45), 65(28), 136(M, 17)
2	Sabinene	991	1.96	136	91	77(90), 51(62), 65(28), 136(M, 8)
3	β-Pinene	994	1.97	136	91	65(51), 75(50), 93(48), 107(8), 136(M,8)
4	*cis*-β-Ocimene	1005	2.04	136	91	91(88), 77(58), 65(52), 106(10), 136(M, 12)
5	*d*-Limonene	1038	2.27	136	67	120(63), 77(49), 63(39), 91(26), 136(M, 2)
6	Santrolina triene	1061	2.42	136	91	77(90), 51(85), 65(36), 105(10), 136(M, 6)
7	γ-Terpinene	1077	2.52	136	91	77(40), 121(10), 51(25), 136(M, 42)
8	Terpinolene	1104	2.70	136	91	77(84), 51(60), 121(12), 136(M, 40)
9	Linalool	1114	2.76	154	91	41(75), 84(27), 94(22), 53(15), 109(12), 154(M, 5)
10	IsoPulegol	1156	3.02	154	67	53(62), 95(31), 81(24), 121(10), 137(6)
11	Isothujol	1165	3.07	154	67	53(62), 95(31), 81(24), 121(8), 137(6), 111(5), 154(M, 2)
12	α-Terpineol	1213	3.36	154	67	53(60), 81(28), 137(2), 157(M+3, 6)
13	δ-Elemene	1331	4.16	204	67	91(72), 67(50), 54(30), 105(18), 121(16), 204(M, 1)
14	4-*p*-Menthene	1334	4.19	138	67	81(40), 58(20), 95(14), 123(2), 138(M, 1)
15	*cis*-Geraniol	1342	4.27	154	67	80(24), 91(19), 52(25), 137(10)
16	Geranyl acetate	1352	4.36	196	67	81(28), 93(26), 121(4), 137(6), 138(2)
17	Geranial	1355	4.39	152	79	91(100), 105(72), 77(58), 119(40), 133(6)
18	β-Elemene	1362	4.46	204	67	79(54), 91(54), 93(32), 54(28), 119(16), 133(8), 204(M, 2)
19	β-Caryophyllene	1381	4.63	204	91	77(80), 67(62), 105(32), 107(10), 133(10), 161(8), 204(M, 5)
20	α-Bergamotene	1385	4.67	204	91	77(63), 119(44), 67(20), 161(4), 204(M, 4)
21	α-Farnesene	1502	4.94	204	91	77(80), 105(62), 65(44), 119(22), 161(16), 133(10), 204(M, 10), 204(M+1, 2)
22	α-Farnesene(isomer)	1511	4.99	204	91	91(98), 55(42), 68(36), 119(28), 135(6), 204(M, 12)
23	Valencene	1515	5.01	204	67	91(96), 77(89), 105(52), 119(27), 133(13), 161(12), 204(M, 30)
24	α-Farnesene(isomer)	1520	5.03	204	91	77(99), 51(65), 67(40), 105(26), 123(24), 204(M, 2)

Table 2. Flavor composition identified by GC-MS of four citrus varieties collected by SPTE method

Peak No.	Compound	Hallabong	Lemon	Orange	Grapefruit
1	α-Pinene	0.03 ± 0.14	0.79 ± 0.14	–	–
2	Sabinene	0.13 ± 0.11	–	–	–
3	β-Pinene	–	4.21 ± 0.08	–	–
4	cis-β-Ocimene	0.60 ± 0.11	3.21 ± 0.18	0.17 ± 0.10	1.34 ± 0.10
5	*d*-Limonene	96.98 ± 0.08	64.82 ± 0.28	99.59 ± 0.18	98.38 ± 0.09
6	Santrolina triene	0.14 ± 0.20	–	–	–
7	γ-Terpinene	–	18.64 ± 0.18	–	–
8	Terpinolene	–	0.54 ± 0.18	0.01 ± 0.14	–
9	Linalool	0.13 ± 0.04	0.03 ± 0.10	0.04 ± 0.16	–
10	IsoPulegol	0.12 ± 0.26	–	0.01 ± 0.20	0.02 ± 0.30
11	Isothujol	0.04 ± 0.08	–	–	–
12	α-Terpineol	0.12 ± 0.14	0.75 ± 0.15	0.01 ± 0.16	0.01 ± 0.19
13	δ-Elemene	0.05 ± 0.08	–	–	–
14	4-*p*-Menthene	0.04 ± 0.21	0.33 ± 0.29	–	–
15	*cis*-Geraniol	0.01 ± 0.11	0.36 ± 0.22	–	–
16	Geranyl acetate	–	0.71 ± 0.10	–	–
17	Geranial	0.06 ± 0.10	–	0.01 ± 0.31	0.05 ± 0.17
18	β-Elemene	0.02 ± 0.13	–	0.01 ± 0.17	0.03 ± 0.07
19	β-Caryophyllene	–	0.42 ± 0.07	0.01 ± 0.16	0.16 ± 0.05
20	α-Bergamotene		2.44 ± 0.12	–	–
21	α-Farnesene	0.06 ± 0.11	–	–	0.01 ± 0.29
22	α-Farnesene(isomer)	0.92 ± 0.03	–	–	–
23	Valencene	0.51 ± 0.09	–	0.13 ± 0.09	–
24	α-Farnesene(isomer)	0.04 ± 0.22	2.75 ± 0.21	0.01 ± 0.06	–

Unit: mean peak area percentage + RSD, n = 3. –, Not detected

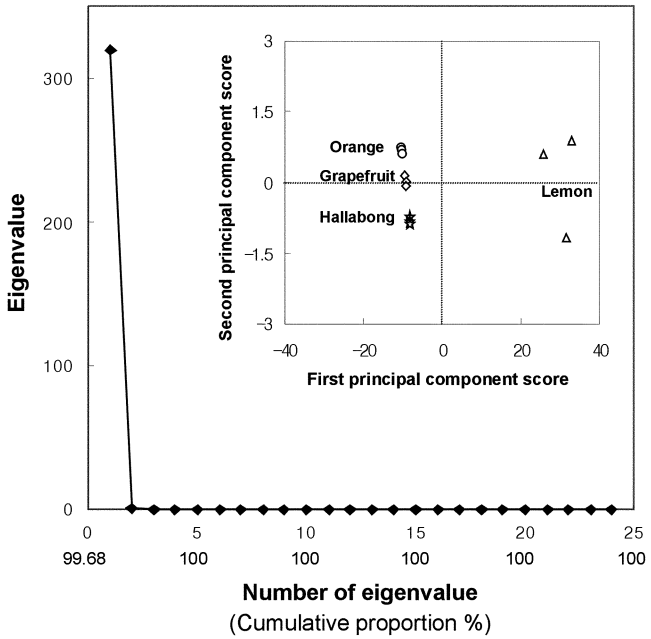

Figure 4. Scree graph and principal components score plot (inset) for flavor composition of selected citrus peels listed in Table 3.

nent analysis (PCA) was employed to provide an overview of capacity to distinguish citrus aroma based on GC data set (variables of 24 aroma components × each triplicate samples of four citrus species) as tabulated in Table 2. The scree graph, a plot of eigenvalue as a function of eigenvalue number, was utilized to decide how many principal components should be retained.[32,35] The scree graph (Fig. 4) for the data of Table 2 exhibits an ideal pattern, with the inset illustrating the first two principal components scores as a dimension reduction device. Data points of Hallabong samples are closer to its parent fruit of orange. Grapefruit samples also show a pattern similar to that of its parent fruit of orange. These results agree with a previous report on the similarity of aroma composition in hybrid fruit with its parent fruit.[17]

Comparison of different SPME fibers for analysis of aroma from Hallabong peels. HS-SPME is considered complete when the analyte concentration has reached equilibrium between the sample matrix and the fiber coating. During HS-SPME, the volatile compounds present in the gas phase are adsorbed in the fiber coating at a much faster rate than their release from the matrix. This is because of the large diffusion coefficients of analytes in the gas phase; thus, sufficient time is required to reach equilibrium.[37] To optimize the adsorption equilibrium time for each fiber used in this study, five standard compounds detected in citrus varieties, β-pinene, *d*-limonene, γ-terpinene, terpinolene and linalool, were tested. 50/30 μm DVB/CAR/PDMS, 100 μm PDMS, 30 μm PDMS and 75 μm CAR/PDMS fibers were used and adsorption times were varied at 10 min interval from 10 min to 60 min at 20 °C. The extraction time profiles were estab-

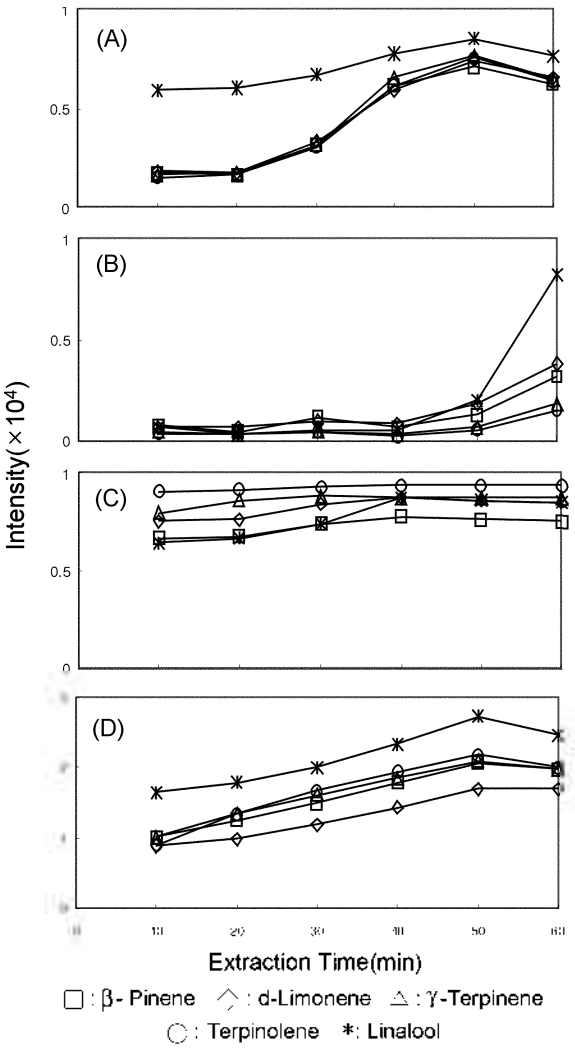

Figure 5. Effects of extraction time on HS-SPME-GC-FID of the five flavor standards with different fiber coatings: (A) PDMS 30 mm; (B) CAR/PDMS 75 μm; (C) PDMS 100 μm; (D) DVB/CAR/PDMS 50/30 μm. Analytical conditions: a cross-linked 5% phenyl polydimethylsiloxane (SPB-5, Supelco, 60 m × 0.25 μm × 0.25 μm film thickness) column temperature program, 50 °C (3 min) - 5 °C/min - 240 °C (10 min); injector temperature, 250 °C; FID temperature, 250 °C; flow rate of carrier gas (N_2, 99.99% purity), 1.0 mL/min; split ratio, 1 : 2.

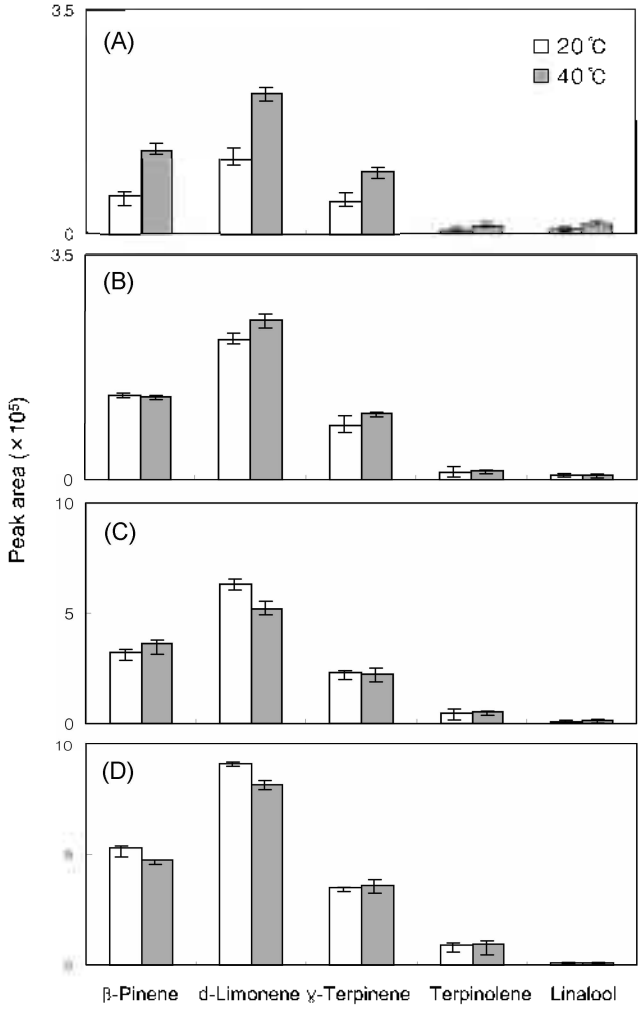

Figure 6. Effects of temperature on HS-SPME-GC-FID of five flavor standards using different fiber coatings: (A) PDMS 30 μm; (B) CAR/PDMS 75 μm; (C) PDMS 100 μm; (D) DVB/CAR/PDMS 50/30 μm.

lished by plotting the detector response versus the extraction time as shown in Figure 5. Equilibrium times were reached after 40 min for 30 μm PDMS and for 50/30 μm DVB/CAR/PDMS, and within 10 min for 100 μm PDMS. All compounds did not reach equilibrium within 40 min for 75 μm CAR/PDMS. Although it took almost 40 min to reach equilibrium for the adsorption of most analytes, 60 min of sampling time was finally decided on to ensure complete equilibrium.

The effect of the extraction temperature on HS-SPME efficiency was also investigated at 20 and 40 °C. At this evaluation stage, the extraction time was set at 60min to obtain equilibrium. GC-FID peak areas for the five compounds mentioned above are shown in Figure 6. The peak areas of most compounds at 40 °C were a little larger than those at 20 °C, however, those of limonene in the cases of 50/30 μm DVB/CAR/PDMS and 100 μm PDMS at 40 °C were a little smaller than those of the 20 °C case. This phenomenon seems to be caused by degradation caused by heat, air in headspace and light to form a small amount of oxidation products.[38] The lower temperature is preferred for prevention of the degradation of thermally labile components, and 20 °C is better for the observation of flavor composition emitted from citrus fruits at ambient temperature. For sample analysis, extraction for 1 h at 20 °C was finally used.

In HS-SPME, the transfer of analyte into a fiber is related two equilibria, K_{gs} (the analyte partition coefficient between headspace and sample matrix) and K_{fg} (the analyte partition coefficient between the SPME fiber coating and the headspace gas phase). The definition and calculation of both values can be found elsewhere.[25,27,31] In addition, the relative concentration factor (CF), which is achieved by the ratio between the peak area of the analyte obtained by HS-SPME-GC-FID and the corresponding area obtained by static HS GC-FID, is also considered according to different fiber

Table 3. Partition coefficient (K_{fg}) between fiber coating and headspace gas phase, and concentration factors (CF) of characteristic components of Hallabong peel samples

Fiber coating	V_f (μL)	β-Pinene		d-Limonene		γ-Terpinene		Terpinolene		Linalool	
		K_{fg}	CF	K_{fg}	CF	K_{fg}	CF	K_{fg}	CF	K_{fg}	CF
DVB/CAR/PDMS 50/30-μm (2 cm)	1.000	5.66×10^{4}	11.32	8.41×10^{4}	16.81	9.19×10^{4}	18.37	1.30×10^{5}	26.10	1.54×10^{4}	3.07
PDMS 100-μm (1 cm)	0.612	1.37×10^{5}	16.75	1.44×10^{5}	17.60	1.90×10^{5}	23.24	1.60×10^{5}	19.63	4.52×10^{4}	5.53
PDMS 30-μm (1 cm)	0.132	4.00×10^{5}	10.56	8.11×10^{4}	2.14	9.89×10^{4}	2.61	2.01×10^{4}	0.53	–	–
CAR/PDMS 75-μm (1 cm)	0.436	1.28×10^{4}	1.12	2.63×10^{4}	2.29	2.71×10^{4}	2.36	1.27×10^{5}	11.07	–	–

types. Both K_{fg} and CF values can be criterion of relative fiber efficiency of HS-SPME for the analyte. The determinations of the experimental K_{fg} and CF values were carried out using real citrus samples instead of standards to conserve matrix effects that appeared in the actual sampling.[25,30] The same sampling conditions were applied to static HS and HS-SPME, although all conditions were probably not the most effective for each fiber. HS-SPME followed by S-HS was applied successively to the same sample. The relatively large amount (7 g) of citrus peel samples was chopped and placed in a relatively large capping vial (100 mL) to ensure that the depletion of the headspace by HS-SPME sampling before S-HS would be negligible and, therefore, the effect of decreasing volume for the S-HS was not affected. Table 3 shows K_{fg} and CF values for the characteristic compounds in Hallabong peel samples obtained with each SPME fiber investigated. CF value is the relative evaluation parameter of fiber recovery efficiency, depending on physical properties and preparation conditions of analyte. HS-SPME showed better recovery than static SPME, because K_{fg} values ranged in order of magnitude from 10^{4} to 10^{5}, indicating larger mass transfer of analyte into fiber coating than in headspace and CF values, using the 50/30 μm DVB/CAR/PDMS or 100 μm PDMS fiber in the 3.07-26.10 ranges. Based on these experimental data, the 50/30 μm DVB/CAR/PDMS fiber was most efficient among the four fibers evaluated for all the samples.

Allowing for the greatest recoveries of analytes examined, the percent normalization of peak areas for each characteristic components was achieved, standardizing the corresponding peak areas obtained with the 50/30 μm DVB/CAR/PDMS fiber as equal to 100% in accordance with Bicchi *et al.*.[31] The HS-SPME-GC-FID normalized intensity of a characteristic compounds of Hallabong obtained with different fibers versus 50/30 μm DVB/CAR/PDMS fiber is shown in Figure 7. It can be seen that the 50/30 DVB/CAR/PDMS fiber has the greatest response among the four fiber types for all the compounds investigated. This elucidates higher K_{fg} and CF values for the characteristic components in citrus peel samples as shown in Table 3. Such higher affinity of 50/30 μm DVB/CAR/PDMS fiber for the analyte can be explained by its thickness and nature of coating materials. In the mixed phases with CAR-PDMS and DVB-PDMS fibers,

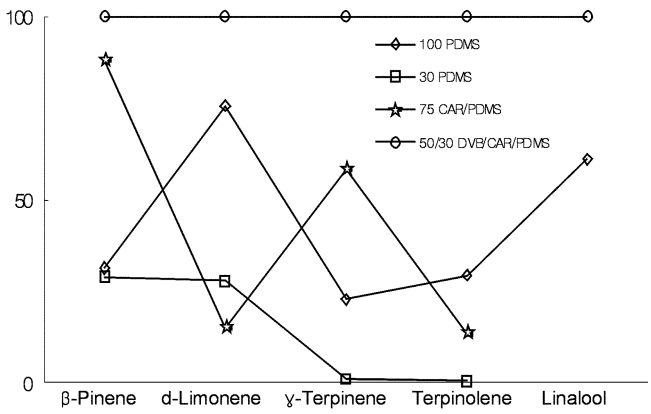

Figure 7. HS-SPME-GC-FID normalized peak areas of five characteristic flavor components of citrus peels obtained with the fiber relative to the other fiber coatings.

porous carbon (CAR, total porosity 0.78 mL/g) and microspheres of the DVB polymer are immobilized onto the fiber by using PDMS coating. The combination of DVB with CAR phase increases both the porosity distribution and the polarity of the fiber and provides better retention of analytes than PDMS alone (non-polar phase), although bare PDMS fiber has a recommendable efficiency to the non-polar analytes and a tolerance for the high injection temperature. The thicker layer of these mixed phases compared with other

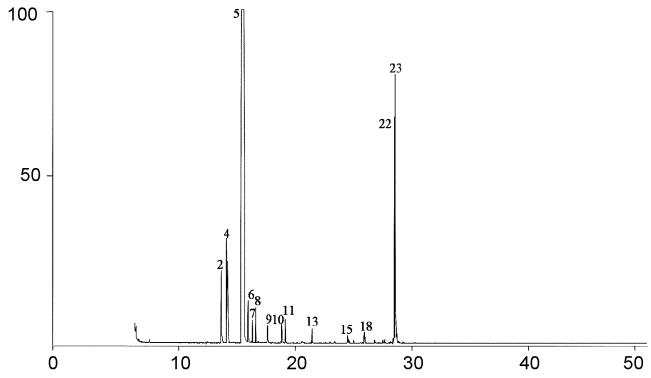

Figure 8. A typical total ion chromatogram on a SPB-5 column collected by HS-SPME with DVB/CAR/PDMS 50/30 μm fiber from Hallabong peel.

Table 4. Flavor composition identified by GC-MS of four citrus varieties collected by HS-SPME method using DVB/CAR/PDMS

Peak No.	Compound	Hallabong	Lemon	Orange	Grapefruit
1	α-Pinene	–	5.79 ± 0.12	3.65 ± 0.24	3.62 ± 0.13
2	Sabinene	5.30 ± 0.26	–	1.73 ± 0.20	4.89 ± 0.15
3	β-Pinene	–	18.01 ± 0.27	–	–
4	*cis*-β-Ocimene	5.62 ± 0.04	–	2.82 ± 0.08	9.16 ± 0.05
5	*d*-Limonene	83.39 ± 0.24	47.66 ± 0.09	88.03 ± 0.08	76.36 ± 0.06
6	Santrolina triene	0.91 ± 0.09	–	0.14 ± 0.29	0.45 ± 0.11
7	γ-Terpinene	0.47 ± 0.23	13.54 ± 0.06	0.62 ± 0.09	0.64 ± 0.03
8	Terpinolene	0.82 ± 0.21	3.77 ± 0.12	1.77 ± 0.13	0.48 ± 0.17
9	Linalool	0.31 ± 0.14	0.39 ± 0.44	0.24 ± 0.14	–
10	IsoPulegol	0.51 ± 0.31	–	0.19 ± 0.33	0.33 ± 0.04
11	Isothujol	0.49 ± 0.19	–	0.40 ± 0.11	0.39 ± 0.09
12	α-Terpineol	–	0.55 ± 0.36	–	–
13	δ-Elemene	0.18 ± 0.22	–	–	0.26 ± 0.13
14	4-*p*-Menthene	–	0.23 ± 0.25	–	–
15	*cis*-Geraniol	–	2.58 ± 0.13	–	–
16	Geranyl acetate	–	–	–	–
17	Geranial	–	–	–	–
18	β-Elemene	–	–	–	0.94 ± 0.03
19	β-Caryophyllene	–	2.89 ± 0.19	–	1.89 ± 0.08
20	α-Bergamotene		2.20 ± 0.19	–	–
21	α-Farnesene	–	–	–	–
22	α-Farnesene(isomer)	0.91 ± 0.27	–	0.41 ± 0.14	–
23	Valencene	1.09 ± 0.16	–	–	0.59 ± 0.15
24	α-Farnesene(isomer)	–	2.39 ± 0.32	–	–

Unit : mean peak area percentage ± RSD. n = 3. –, Not detected.

fibers also increases the capacity to extract analyte.

Aroma compositions of different citrus varieties by HS-SPME combined with GC-MS. A typical TIC on a SPB-5 column of the flavor constituents from Hallabong extracted by HS-SPME, using a DVB/CAR/PDMS fiber is shown in Figure 8. The peak numbers shown in Figure 8 correspond to those given in the first column in Table 4. Higher sharp peaks of *cis*-β-ocimene, *d*-limonene, α-farnesene and valencene were observed. Flavor composition of four citrus varieties collected by HS-SPME, using a DVB/CAR/PDMS fiber is summarized in Table 4. *d*-Limonene was the most abundant compound in all samples with the concentration (normalized peak area %) of 83.39% for Hallabong, 47.66% for lemon, 88.03% for orange, 76.36% for grapefruit.

The composition of volatile flavors found was dependant on the extraction methods involved. When compared with SPTE, the use of HS-SPME by DVB/CAR/PDMS fiber provides several differences in flavor compositions. For example, the relatively small molecules γ-terpinene and terpinolene were observed by HS-SPME from Hallabong but not by SPTE in the same sample (Fig. 3A, Fig. 8). Higher molecules such as geranial and β-elemene were not detected by HS-SPME but detected by SPTE (Table 2 and Table 4). These differences between two extraction techniques are possibly related to various effects, including not only extraction time and sample amount, but also the nature, polarity, surface area and porosity of Porapak Q by SPTE and CAR/DVB/PDMS by HS-SPME. SPTE seems to be a complementary sampling technique to HS-SPME, and *vice versa*.

Conclusions

The flavor components of Hallabong peel were characterized by GC-MS with two different extraction techniques. Both SPTE and HS-SPME could be used for this purpose with satisfactory results. We observed that these techniques had several advantages, including no apparent thermal degradation, lower sample and solvent requirements, and investigation of flavor composition emitted from citrus fruits at ambient temperature. When K_{fs} and CF values were determined to select suitable fiber for HS-SPME, the 50/30 μm DVB/CAR/PDMS fiber was most efficient among the four fibers evaluated. Aroma components emitted from Hallabong peel were compared with those of the other citrus varieties, lemon, orange and grapefruit. *d*-Limonene was the main component in all samples. When compared with SPTE, the use of HS-SPME by DVB/CAR/PDMS fiber provides several differences of flavor compositions. SPTE seems to be a sampling technique complementary to HS-SPME, and *vice versa*. These two sampling techniques could also be applicable to the collection of volatile flavors from fruits peel.

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