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Aroma dilution method using GC injector split ratio for volatile compounds extracted by headspace solid phase microextraction

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Abstract

Dilution methods are widely used for the screening of aroma-active compounds in gas chromatography-olfactometry (GC-O). An aroma dilution method for the samples extracted by headspace solid phase microextraction (HS–SPME) was developed using GC injector split ratio. In an aqueous model system containing seven authentic standards, the relationship between logarithmic peak area and logarithmic split ratio showed a high linearity as indicated by the good agreement between calculated and experimental data. This result suggests that the split ratio is a suitable and reliable tool for the successive dilution of volatiles in HS-SPME–GC-O. When this method was applied to yuzu (*Citrus junos*) tea, linalool (floral/lemon) and decanal (orange/waxy) were identified as the most intense aroma-active compounds.

Keywords: Split ratio; Gas chromatography-olfactometry; Headspace solid phase microextraction; Aroma dilution; *Citrus junos*

1. Introduction

During the past decade, solid phase microextraction (SPME) has been widely practiced for extracting volatile and semivolatile compounds from biological, environmental, and food samples (Berlardi & Pawliszyn, 1989; Kataoka, Lord, & Pawliszyn, 2000). SPME is a simple, rapid, and solvent-free sampling procedure, in which samples taken from either liquid phase (direct immersion sampling) or vapor phase (headspace sampling) are absorbed onto a phase-coated fused silica fiber. Headspace SPME (HS-SPME), in particular, has been largely applied to flavor analyses in combination with gas chromatography (GC) and GC–mass spectrometry (GC–MS).

GC-olfactometry (GC-O) is a valuable analytical procedure for the detection of aroma-active compounds among various volatiles extracted from food samples (Grosch, 1993). The methodology of GC-O can be classified into dilution, intensity, and detection frequency methods (van Ruth & O'Connor, 2001), among which dilution methods such as CharmAnalysis (Acree, Barnard, & Cunningham, 1984) and aroma extract dilution analysis (AEDA) (Ullrich & Grosch, 1987) have been generally used as quantitative GC-O. In AEDA, stepwise dilutions of an aroma extract are perceived to provide flavor dilution (FD) factors, which are proportional to the relative aroma potency of each compound.

When SPME was first used in combination with GC-O by Ulrich, Krumbein, Schonhof, and Hoberg (1998), the absorption time was consecutively reduced by half to achieve the effect of stepwise dilution. The concentrations of volatiles, however, did not decrease proportionally to the absorption time. Deibler, Acree, and Lavin (1999) combined SPME with CharmAnalysis by varying the thickness and length of polydimethylsiloxane (PDMS) fiber to obtain various absorbent volumes. They showed that the concentration of an absorbed compound is proportional to the volume of the exposed fiber, and applied this procedure to HS-SPME–

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GC-O of commercial brewed coffee. Nevertheless, this procedure has disadvantages that the fiber length cannot be accurately controlled and the dilution range is limited to approximately 50-fold. In addition, not all fiber coatings are available in various fiber thicknesses, which affect the extraction time of compounds (Shirey, 1999). Bazemore, Goodner, and Rouseff (1999) studied aroma-active compounds of orange juice by HS–SPME-Osme. Despite the advantage of Osme that aroma extract at only a single concentration can be used for GC-O without dilution (Miranda-Lopez, Libbey, Watson, & McDaniel, 1992), the measurement of accurate aroma intensity is difficult without well-trained panelists.

The split and splitless injections are representative sample introduction systems available for GC analysis (Grob, 2001; Kitson, Larsen, & McEwen, 1996). In the split mode, a portion of the sample vaporized at high temperature is transferred into a GC column, and the remaining sample is vented out. The split ratio is a critical factor for determining the amount of sample introduced into the column. Among the different definitions of split ratio (Grob, 2001; Kitson et al., 1996), the following was used in this study:

$$\text{split ratio} = \frac{\text{column flow rate} + \text{split vent flow rate}}{\text{column flow rate}}$$

In the splitless mode with the split vent closed, all samples are transferred into the column. Therefore, we can obtain the effect of serial dilution through successive increases of the split ratio. In addition, split ratios can be easily modulated in GC by rotating a split vent flow controller or by an electronic system without additional equipment.

Yuzu (*Citrus junos* Tanaka) is a citrus fruit native to the northern east Asian regions including Korea, China, and Japan (Shinoda, Shiga, & Nishimura, 1970). Particularly in Korea, yuzu has been commonly used as a raw material for beverages and herbal medicines due to its unique flavor and cold-effectiveness (Jeong, Lee, Lee, Kim, & Lee, 1998; Song, Sawamura, Ito, & Ukeda, 1999). The volatile components of yuzu oil have been extensively investigated, among which monoterpene hydrocarbons such as limonene, γ-terpinene, and myrcene were reported as the predominant ones (Jeong et al., 1998; Njoroge, Ukeda, & Sawamura, 1996; Shinoda et al., 1970; Song et al., 1999; Song, Sawamura, Ito, Kawashimo, & Ukeda, 2000). Song et al. (2000) identified 6-methyl-5-hepten-2-ol and dimethyl trisulfide as the characteristic aroma-active compounds of yuzu peel oil.

In this study, we demonstrated that serial dilution of the flavor compounds in HS-SPME–GC-O can be achieved through the successive increase of GC split ratio using an aqueous model system and yuzu tea.

2. Materials and methods

2.1. Sample preparations

Authentic flavor standards were obtained from Aldrich Chemical (Milwaukee, WI, USA), Seoul Aromatics (Seoul, Korea), and Borak Corporation (Seoul, Korea).

Ethyl 2-methylbutanoate, 2-ethylthiophene, (+)-limonene, linalool, citronellal, (−)-carvone, and (*E*,*E*)-2,4-decadienal were diluted in ethanol at 20 μl/ml. A 100-μl portion of the ethanol stock solution was then added into 100 ml distilled water kept in a 250-ml headspace glass bottle with a Teflon/silicon screw cap and a magnetic stir bar (25.4×9.5 mm). Therefore, the final concentration of each compound was 20 ppm (v/v) in standard solution.

Commercial yuzu paste was purchased from National Agricultural Cooperative Federation (Tongyoung, Korea). The yuzu paste (200 g) containing sliced yuzu (40%, w/w) with flesh and peel, and sucrose (60%, w/w) was ground using a mixer and added into 800 ml of distilled water. After vigorously shaking, 20 ml of the fresh sample was transferred into a 40-ml screw-capped headspace vial containing a stir bar (10×6 mm).

2.2. SPME

The SPME device and fibers were obtained from Supelco (Bellefonte, PA, USA). All fibers were conditioned before use as recommended by the manufacturer.

The standard solution with seven flavor compounds was preheated at 45 °C for 1 h with stirring at 300 rpm, and the volatiles were absorbed onto a 100 μm PDMS fiber. Detailed absorption conditions were as follows: SPME needle length, 20 mm; exposure fiber length, 10 mm; temperature, 45 °C; stirring speed, 300 rpm. To determine the optimal absorption time, the PDMS fiber was exposed to the headspace of the standard solution for 1, 5, 10, 20, and 40 min. The desorption of volatiles was conducted in a GC injector with a SPME inlet liner (0.75 mm i.d., Supelco) for 2 min at 200 °C with 40 mm of needle length.

For HS–SPME of yuzu tea, the sample was preheated with stirring at 300 rpm for 30 min at 75 °C, which is the normal temperature of serving the tea. In the same conditions, volatile compounds were extracted for 30 min by 100 μm PDMS, 75 μm Carboxen (CAR)/PDMS, 65 μm PDMS/divinylbenzene (DVB), and 50/30 μm DVB/CAR/PDMS fibers. To investigate the time-course extraction, the volatiles of yuzu tea were absorbed by a 100 μm PDMS fiber for 1, 2, 4, 8, 15, 30, 60, and 120 min. The desorption conditions were the same as those of the standard solution.

2.3. GC–MS

GC–MS was employed using an HP 5890 Series II GC/5972 mass selective detector (Hewlett-Packard, Palo Alto, CA, USA) equipped with an electronic pressure controller. A nonpolar DB-5ms column (30 m length×0.25 mm i.d.×0.25 μm film thickness, J & W Scientific, Folsom, CA, USA) was used for analysis, and carrier gas was helium at a constant flow rate of 0.8 ml/min. The oven temperature was programmed from 40 to 160 °C at 4 °C/min and from 160 to 240 °C at 8 °C/min, with initial and final hold times of 5 and 15 min, respectively. Other conditions were as follows: injector temperature, 200 °C; septum purge flow rate, 2.7 ml/min; detector temperature, 250 °C; scanning range, 33–450 a.m.u.; electron ionization energy, 70 eV. The retention indices (RIs) of volatile compounds were determined using *n*-paraffins C5–C22 as external references (van den Dool & Kratz, 1963).

The volatiles extracted from the standard solution were diluted stepwise by varying the injector split ratio as follows: 1 (splitless), 8, 16, 32, 64, and 128. The split vent flow rate was changed by rotating a flow controller and measured using a digital flowmeter (Agilent Technologies, Palo Alto, CA, USA) prior to sample injection. At the split ratios of 8, 16, 32, 64, and 128, the split vent flow rates were 5.6, 12.0, 24.8, 50.4, and 102 ml/min, respectively. In the splitless injection, the injection purge was off during the initial 5 min.

2.4. GC-O

GC-O was conducted on a Varian 3800 GC (Varian Instrument Group, Walnut Creek, CA, USA) equipped with a flame ionization detector (FID) and a sniffing port (Alltech Associates, Deerfield, IL, USA) using a DB-5ms column (30 m length×0.25 mm i.d.×0.25 μm film thickness). Helium was used as a carrier gas at a constant flow rate of 1.0 ml/min. Effluent from the end of GC column was split 1:1 between FID and the sniffing port. The oven temperature was held at 40 °C for 5 min, raised to 200 °C at 8 °C/min, and held at 200 °C for 20 min. Injector and detector temperatures were 200 and 250 °C, respectively.

The volatiles were stepwise diluted by controlling the split ratio through an electronic flow control system. The split ratios used for aroma dilution were 1 (splitless), 9, 27, 81, 243, and 729. The split vent flow rate was also checked using a digital flowmeter at each split ratio. The injection purge was off during the initial 5 min for the splitless injection. FD factor was defined as the maximum split ratio at which a compound could be perceived, and FD chromatogram was based on the evaluation of one experienced panelist.

To check whether the HS-SPME sample represented the aroma of yuzu tea, the extracted sample was injected (splitless mode) into a deactivated column (0.5 m length×0.25 mm i.d.) at 200 °C and evaluated at the sniffing port by four panelists familiar with yuzu tea aroma.

2.5. Identification

Volatile compounds were identified by comparing the mass spectra and RIs of unknowns with those of authentic standards. When authentic compounds were not available, compounds were identified using the Wiley 275 mass spectral database (Hewlett-Packard Co., 1995) and reported RIs in the literatures (Acree & Ahn, 1997; Adams, 2001; Kondjoyan & Berdagué, 1996; Rychlik, Schieberle, & Grosch, 1998). For the identification of aroma-active compounds, mass spectra, RIs, and aroma properties of unknowns were compared with those of authentic standards.

3. Results and discussion

3.1. HS–SPME-GC–MS of standard solution

Fig. 1 illustrates the amounts of seven standard compounds absorbed on a PDMS 100 μm fiber depending on the extraction time. (+)-Limonene was the most abundant compound in GC–MS. This compound is a nonpolar terpene hydrocarbon, which can be extracted efficiently by a nonpolar PDMS fiber (Shirey, 1999), and is practically insoluble in water, which leads to a high partition coefficient (=concentration in gas phase/concentration in aqueous phase). Although the total

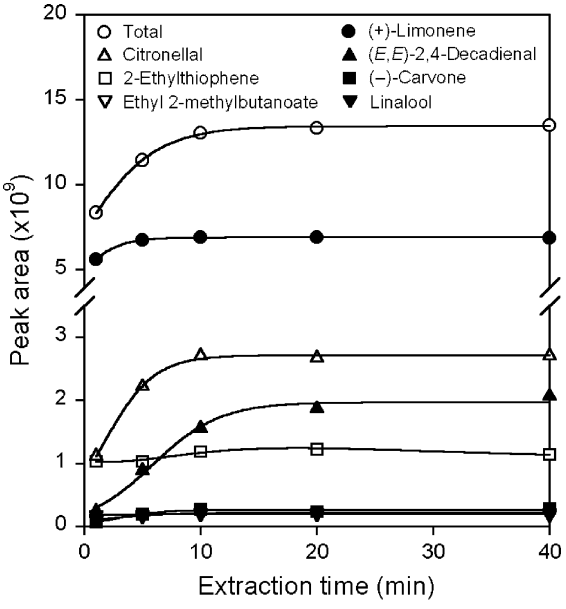

Fig. 1. Effect of extraction time on the absorption of volatile compounds in the standard solution. Concentration of each compound was 20 μl/l, and a 100 μm PDMS fiber was used for HS–SPME.

amount of volatile compounds gradually increased during the 40-min extraction period, over 95% of each compound except (*E*,*E*)-2,4-decadienal was extracted within 10 min. Therefore, 10 min was selected as the optimum extraction time for the standard solution.

Regression plots of $\log_2$ (peak area) versus $\log_2$ (split ratio) in HS-SPME–GC–MS of the standard solution are shown in Fig. 2 with slopes ranging from −1.07 to −0.93 (Table 1). The theoretical value of these slopes is −1 if the peak area is inversely proportional to the split ratio. R^2 values of all plots were over 0.998 (Table 1), and the standard deviations of peak areas at all split ratios were less than 10% (data not shown). Split ratios as low as 2 or 4 and higher than 128 could not be obtained due to a mechanical limitation in the GC, which is related to the unstable flow of the carrier gas.

A high linearity of the log–log plot between peak area and split ratio revealed in our results could not be obtained by syringe-type split injection (Grob, 2001),

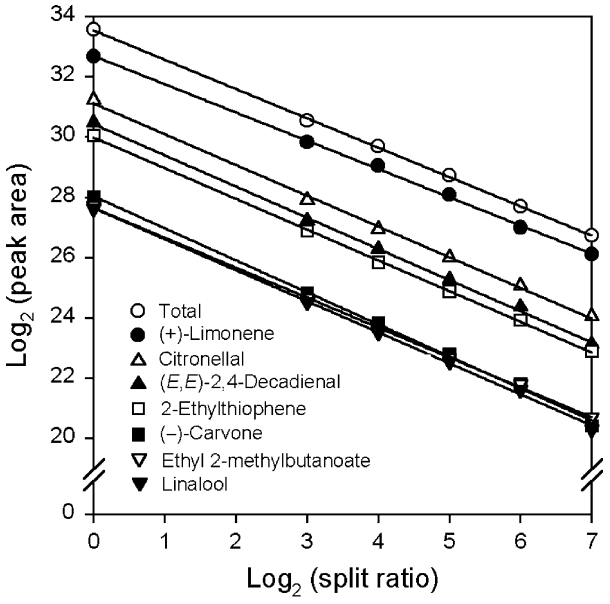

Fig. 2. Regression plots of $\log_2$ (peak area) versus $\log_2$ (split ratio) for flavor compounds in HS-SPME-GC–MS of the standard solution. Regression equations and R^2s of the plots are presented in Table 1. The results are the averages of triplicate data.

Table 1
Regression equations and R^2s of the plots in Fig. 1

Compound	Regression equation[a]	R^2
Total	$Y=33.55-0.972X$	0.9997
(+)-Limonene	$Y=32.70-0.937X$	0.9991
Citronellal	$Y=31.12-1.019X$	0.9981
(*E*,*E*)-2,4-Decadienal	$Y=30.44-1.034X$	0.9988
2-Ethylthiophene	$Y=30.00-1.019X$	0.9995
(−)-Carvone	$Y=28.05-1.063X$	0.9980
Ethyl 2-methylbutanoate	$Y=27.67-0.993X$	0.9992
Linalool	$Y=27.65-1.029X$	0.9988

[a] $Y=\log_2$ (peak area). $X=\log_2$ (split ratio).

where deviation and discrimination were observed. The pressure wave is an important factor that causes a difference between true and pre-set split ratios. For example, two microliters of liquid sample injected with a syringe produce a vapor cloud with a volume as low as 0.3 to over 2 ml (Grob, 2001). Therefore, the injection of a sample is presumed to cause fluctuations in pressure and flow rate of the injector. Another possible reason is the volumetric contraction resulting from the recondensation of sample vapor in the column inlet at a low oven temperature, which leads to the unstable entrance of sample vapor into the column (Grob, 2001). In addition, the incomplete evaporation of less volatile components with high boiling points may cause a disturbance in the split injection (Grob, 2001).

On the other hand, the injection pattern of volatiles extracted by HS-SPME is different from that of the liquid sample described above. HS-SPME is a solvent-free extraction procedure, whereas most liquid samples injected using a syringe contain large amounts of solvents. Therefore, SPME is preferred to minimize the pressure wave and the recondensation, which are mainly caused by the solvent (Grob, 2001; Grob & Neukom, 1979). Furthermore, the sample extracted by HS-SPME shows a much smaller volumetric change, which prevents the pressure wave of the sample vapor, than the liquid sample in a hot injector (Grob, 2001). Finally, most of the volatiles extracted by HS-SPME can completely vaporize in the injector due to their low boiling points.

Based on the high linearity of the log–log plot of peak area versus split ratio, which has been experimentally verified in this study, we conclude that aroma dilution by changing GC split ratio is a reliable method for HS-SPME–GC-O.

3.2. HS-SPME-GC–MS-O of yuzu tea

The aroma dilution method using GC split ratio was also applied to an actual sample, yuzu tea. Table 2 lists the volatile flavor compounds detected in HS–SPME-GC–MS of yuzu tea with their RIs and peak areas using four different SPME fiber coatings. A total 44 compounds were found, 40 of which were positively identified. The volatile profile of yuzu tea in HS–SPME was similar to that of yuzu oil (Jeong et al., 1998; Njoroge et al., 1996; Song et al., 1999, 2000). Limonene (No. 10) was the most abundant compound in GC–MS, followed by γ-terpinene (No. 12), bicyclogermacrene (No. 33), and (*E*)-β-farnesene (No. 31).

Polarity, porosity, and volume of the fiber-coating materials are significant factors determining the absorption efficiency in SPME (Shirey, 1999). Among the four different fiber coatings (Table 2), nonpolar PDMS fiber was the most effective for the extraction of yuzu volatiles, in which nonpolar terpene hydrocarbons

Table 2
Volatile compounds detected in HS–SPME-GC-MS of yuzu tea

No.[a]	Compound	RI[b]	ID[c]	Peak area ($\times 10^4$)[d]			
				PDMS[e]	CAR/PDMS[f]	PDMS/DVB[g]	DVB/CAR/PDMS[h]
1	Ethanol	<500	A	41±4	238±36	52±4	229±30
2	Hexanal	801	A	24±5	304±11	22±2	42±1
3	(*E*)-2-Hexenal	857	A	–[i]	533±64	–[i]	54±1
4	α-Thujene	923	B	1093±14	481±65	465±24	513±105
5	α-Pinene	929	A	4637±82	2077±223	2086±89	2265±438
6	β-Pinene	971	A	3431±94	1575±203	1586±62	1737±316
7	Myrcene	989	A	7920±238	7826±503	4148±131	4897±656
8	α-Phellandrene	1000	A	3201±96	2071±138	1681±36	1922±277
9	α-Terpinene	1013	B	1977±54	1202±89	1041±20	1162±157
10	Limonene	1030	A	338 123±6804	235 415±10 946	209 729±4717	227 158±21 706
11	(*E*)-β-Ocimene	1048	B	1739±156	2622±325	926±47	1168±125
12	γ-Terpinene	1058	A	76 191±1362	46 862±3477	45 186±1238	50 353±5548
13	Terpinolene	1086	A	4596±85	2865±154	2720±92	2984±341
14	p-Cymenene	1090	B	581±17	4168±373	473±34	952±101
15	Linalool	1103	A	8435±343	4918±551	8152±758	9962±187
16	Menthatriene	1141	C	226±19	579±56	151±14	216±19
17	1-Terpinen-4-ol	1180	A	1254±73	727±65	1202±78	1449±66
18	α-Terpineol	1195	A	1008±63	772±72	1275±53	1495±97
19	Decanal	1206	A	383±9	790±59	304±18	384±21
20	Thymol	1290	B	506±33	813±62	1122±136	1706±70
21	δ-Elemene	1339	B	1953±165	670±66	317±30	631±50
22	α-Cubebene	1351	B	91±16	99±11	79±9	77±7
23	α-Copaene	1378	B	560±43	322±26	245±20	298±15
24	β-Cubebene	1392	B	332±44	143±23	35±7	100±12
25	β-Elemene	1395	B	848±68	342±25	137±24	300±23
26	(*Z*)-α-Bergamotene	1407	B	156±26	99±8	92±9	103±9
27	Dodecanal	1411	B	173±34	236±14	149±22	163±16
28	β-Caryophyllene	1423	A	4532±328	2672±193	2513±151	2962±180
29	γ-Elemene	1437	B	315±38	267±18	232±29	253±34
30	Aromadendrene	1443	B	187±34	738±98	1733±264	1088±194
31	(*E*)-β-Farnesene	1459	B	14 525±941	10 123±356	8698±580	10 804±831
32	Germacrene D	1486	B	5852±458	2834±228	2552±220	3539±398
33	Bicyclogermacrene	1501	B	24 396±1325	15 493±712	12 481±631	16 865±1009
34	(*E*,*E*)-α-Farnesene	1510	A	1240±93	850±45	599±49	866±33
35	δ-Cadinene	1528	B	4460±270	3128±154	2971±125	3634±381
36	Unknown	1538		1412±114	1086±53	1358±96	1464±73
37	Germacrene B	1562	B	4647±223	3130±156	2702±155	3488±294
38	Nerolidol	1569	A	185±42	141±10	247±22	237±39
39	Unknown	1575		305±57	173±23	315±12	345±48
40	Globulol	1592	B	554±82	436±31	669±17	710±75
41	Viridiflorol	1600	B	1861±203	1277±74	1969±47	2203±257
42	Unknown	1612		262±58	163±14	303±6	328±49
43	α-Muurolol	1654	B	1105±190	848±52	1176±57	1325±77
44	α-Cadinol	1668	B	1655±264	1462±76	1995±98	2177±104
	Total			531 669±3580	368 952±18 210	332 150±9823	370 225±27 375

[a] Peak numbers. Numbers correspond to those in Table 3 and Fig. 5.
[b] Retention indices on DB-5ms column.
[c] Identification: A, mass spectrum and retention index (or retention time) were consistent with those of an authentic standard; B, mass spectrum was identical with that of Wiley mass spectral database (Hewlett-Packard Co., 1995) and retention index was consistent with that of literatures (Acree & Ahn, 1997; Adams, 2001; Kondjoyan & Berdagué, 1996; Rychlik et al., 1998); C, mass spectrum was consistent with that of Wiley mass spectrum database (tentative identification).
[d] The results are the averages±standard deviations ($n=3$).
[e] PDMS 100 μm fiber.
[f] CAR/PDMS 75 μm fiber.
[g] PDMS/DVB 65 μm fiber.
[h] DVB/CAR/PDMS 50/30 μm fiber.
[i] Not detected.

were the main constituents, and was thus used for further experiments. In addition, the volume of 100 μm PDMS fiber (0.612 mm^3) is larger than those of 75 μm CAR/PDMS (0.436 mm^3), 65 μm PDMS/DVB (0.357 mm^3), and 50/30 μm DVB/CAR/PDMS (0.500 mm^3) fibers (Bicchi, Cordero, Iori, Rubiolo, & Sandra, 2000; Shirey, 1999). However, PDMS fiber was not effective for the extraction of low molecular weight polar compounds such as (*E*)-2-hexenal (No. 3). CAR/PDMS and PDMS/DVB fibers showed high efficiency for the extraction of aliphatic aldehydes (Nos. 2, 3, 19, and 27) and terpene alcohols (Nos. 15, 17, 18, 20, 38, 40, 41, 43, and 44), respectively. The absorption pattern of DVB/CAR/PDMS fiber was similar to that of PDMS/DVB fiber.

Time course absorption pattern of yuzu volatiles on the PDMS fiber is shown in Fig. 3. The absorbed amounts of monoterpene hydrocarbons such as limonene gradually increased with increasing absorption time, whereas those of sesquiterpene hydrocarbons such as bicyclogermacrene decreased after 30 min. Moreover, monoterpene and sesquiterpene hydrocarbons showed considerably different extraction patterns during the initial absorption period. The peak area ratios of 1 to 8 min were 0.87 and 0.35 for monoterpenes and sesquiterpenes, respectively. This result indicates that the absorbed amount of each aroma compound does not always decrease at the same rate by successive reduction of absorption time in HS–SPME–GC-O. For example, in the dilution analysis of headspace broccoli aroma by Ulrich et al. (1998), relative concentration of (*Z*)-3-hexenol to hexanal at the initial absorption time (7.5 min) changed considerably as the absorption time was successively shortened by half. In general, high molecular weight compounds require longer equilibrium time than low ones (Bartelt, 1997; Matich, Rowan, & Banks, 1996).

Prior to GC-O of yuzu tea, a preliminary experiment was performed using a deactivated column to check whether the HS-SPME sample represented the overall aroma of yuzu tea. All of four panelists participating in the test determined that the aroma property of HS-SPME sample extracted by the 100 μm PDMS fiber was virtually the same as that of yuzu tea (data not shown). Based on these results, we concluded that HS-SPME was suitable for GC-O of yuzu tea.

The split ratio stepwise increased three times to dilute yuzu volatiles extracted by HS-SPME. In this study, GC-O had a wider dilution range (1–729) than GC–MS (1–128). Fig. 4 shows the regression plots of $\log_3$ (peak area) versus $\log_3$ (split ratio) for the major yuzu volatiles [limonene, γ-terpinene, bicyclogermacrene, and (*E*)-β-farnesene] detected in HS-SPME–GC–FID. All plots show high linearity ($R^2 > 0.97$), their slopes ranging from −1.07 to −0.90, similarly to the results of the standard solution. However, the split injection could not be carried out at split ratio 3 due to the unstable carrier gas flow under a low split flow rate. At split ratios 243 and 729, the signals of bicyclogermacrene and (*E*)-β-farnesene were too weak to detect.

Among the volatiles extracted by the PDMS fiber in HS-SPME, a total of 11 aroma-active compounds were found in GC-O. Table 3 lists these aroma-active compounds with their RIs on DB-5ms column, aroma properties, and threshold values published in the literatures (Rychlik et al., 1998; van Gemert, 1999). FD chromatogram (Fig. 5) shows the relative aroma potencies of the

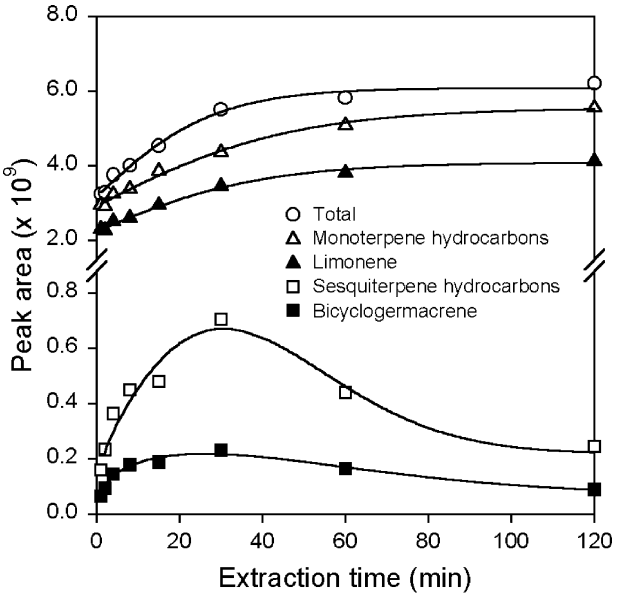

Fig. 3. Extraction patterns of major volatile compounds in yuzu tea according to different absorption times in HS-SPME–GC–MS. A 100 μm PDMS fiber was used for the extraction.

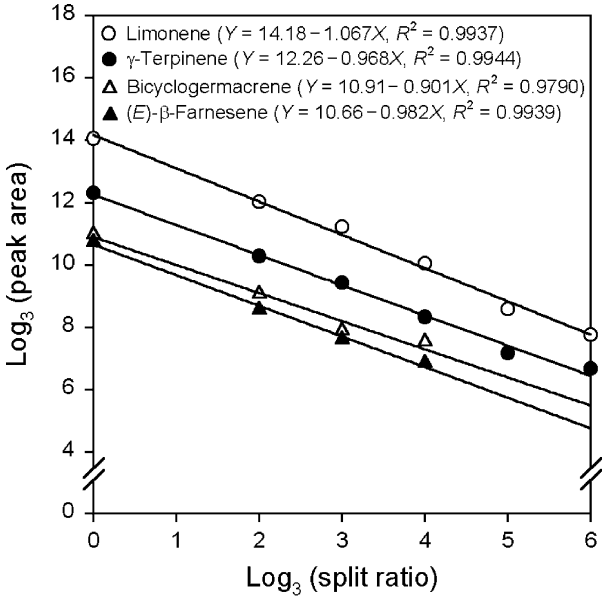

Fig. 4. Regression plots of $\log_3$ (peak area) versus $\log_3$ (split ratio) of major volatile compounds detected in HS-SPME–GC–FID of yuzu tea. The results are the averages of triplicate data.

Table 3
Aroma-active compounds detected in HS-SPME–GC-O of yuzu tea using a PDMS 100 μm fiber

No.[a]	Compound	RI[b]	Aroma property	ID[c]	Threshold ($\mu g/m^3$)[d]
2	Hexanal	802	Green, leafy	A	30–53[e]
3	(*E*)-2-Hexenal	853	Green, apple	A	50–200[e]
I	Unknown	930	Grassy, metallic		
7	Myrcene	987	Plastic, fresh	A	41[e]
10	(+)-Limonene	1035	Lemon, orange, sweet	A	135[e]
15	Linalool	1102	Floral, lemon	A	0.4–0.8[e]
17	1-Terpinen-4-ol	1179	Jasmin, burnt wood, earthy	A	295–1180[e]
19	Decanal	1202	Orange, waxy	A	1[e]
II	Geraniol	1256	Floral, orange	B	0.71[f]
III	Skatole	1388	Mothball, floral	B	0.35–0.78[f]
IV	Unknown	1512	Lemon, floral		

[a] Numbers correspond to those in Table 2 and Fig. 5. Roman numerals represent compounds not detected by GC–MS.
[b] Retention indices on DB-5ms column.
[c] Identification: A, mass spectrum, retention index, and aroma property were consistent with those of an authentic standard; B, retention index and aroma property were consistent with those of an authentic standard (tentative identification).
[d] Aroma threshold in air.
[e] Rychlik et al. (1998).
[f] van Gamert (1999).

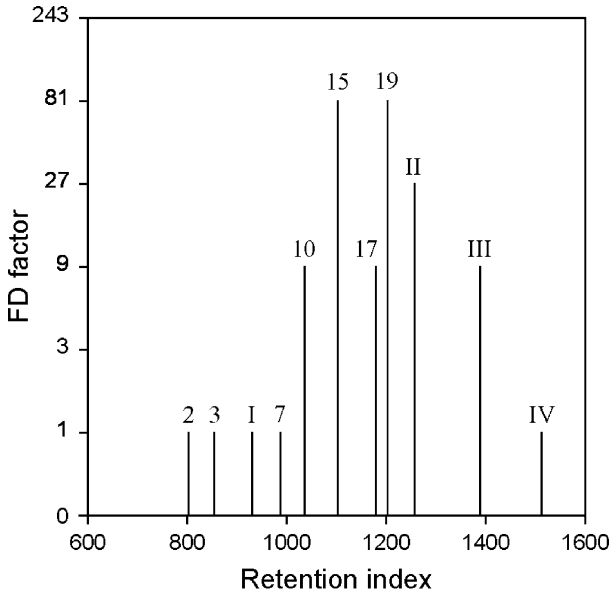

Fig. 5. FD chromatogram of yuzu tea in HS-SPME–GC-O. FD factor is the highest split ratio at which a compound can be perceived. Numbers correspond to those listed in Tables 2 and 3.

compound. Except myrcene (No. 7), (+)-limonene (No. 10), and linalool (No. 15), the potent aroma-active compounds of yuzu peel oil (Song et al., 2000), including 6-methyl-5-hepten-2-ol and dimethyl trisulfide, were not detected in our study probably due to sample variations and/or differences in extraction methods. However, most aroma-active compounds (Nos. 2, 3, 7, 10, 15, 17, 19, and II) identified in our study have been reported in other citrus fruits such as orange (Bazemore et al., 1999), lemon (Schieberle & Grosch, 1988), grapefruit (Rouseff, Jella, Bazemore, & Yang, 2001), and lime (Chisholm, Wilson, Gaskey, Jell, & Cass, 2001).

Linalool (No. 15) and decanal (No. 19) are considered to play important roles in the aroma of yuzu tea due to their high FD factors (81) and characteristic aroma notes. These compounds were reported to have relatively low threshold values of 0.4–0.8 and 1 $\mu g/m^3$ in air, respectively (Rychlik et al., 1998). Geraniol (No. II) and skatole (3-methylindole) (No. III) were tentatively identified based on their RIs and aroma properties although they were not detectable by GC–MS (Table 2). This result can be explained by their low threshold values (<1 $\mu g/m^3$ in air) (van Gemert, 1999). Limonene (No. 10) showed a relatively low FD factor despite its high content, which could have resulted from its high threshold value (Rychlik et al., 1998). We identified this compound as (+)-limonene due to its lemon, orange-like aroma note, whereas its enantiomer, (−)-limonene, was reported to exhibit a turpentine-like aroma (Berger, 1995).

4. Conclusions

In this study, the aroma dilution method for HS-SPME–GC-O using GC split ratio, which can be conveniently used in a wide dilution range regardless of the SPME fiber type, was developed. Control of the carrier gas flow between the column and the split vent is a key factor for determining the accuracy of the split ratio. Most of the recently manufactured GCs have electronic flow control systems, which ensure the accurate control of split ratio. Although split injection at certain split ranges was not possible in this study, this limitation could be overcome by using the electronic flow control system with a wide split range or by changing the fiber exposure length (Deibler et al., 1999).

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