Identification of Potent Odorants in Japanese Green Tea (Sen-cha)

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Application of aroma extract dilution analysis using the volatile fraction of a Japanese green tea (Sen-cha) sample resulted in the detection of 36 odor-active peaks with flavor dilution (FD) factors between 10 and 5000. Thirty-six potent odorants were identified from 36 odor-active peaks by gas chromatography/mass spectrometry (GC/MS) and/or the multidimensional GC/MS (MDGC/MS) system. Among these components, 4-methoxy-2-methyl-2-butanethiol (meaty), (*Z*)-1,5-octadien-3-one (metallic), 4-mercapto-4-methyl-2-pentanone (meaty), (*E*,*E*)-2,4-decadienal (fatty), *β*-damascone (honey-like), *β*-damascenone (honey-like), (*Z*)-methyl jasmonate (floral), and indole (animal-like) showed the highest FD factors. Therefore, these odorants were the most important components of the Japanese green tea odor. In addition, 4-methoxy-2-methyl-2-butanethiol, 4-mercapto-4-methyl-2-pentanone, methional, 2-ethyl-3,5-dimethylpyrazine, (*Z*)-4-decenal, *β*-damascone, maltol, 5-octanolide, 2-methoxy-4-vinylphenol, and 2-aminoacetophenone were newly identified compounds in the green tea.

Keywords: *Green tea; aroma extract dilution analysis; gas chromatography/olfactometry; multidimensional GC/MS; odorant; Sen-cha*

INTRODUCTION

Green tea is the most widely consumed beverage in Japan. Among green tea products, Sen-cha is the most popular one, representing >80% of the total production of green tea. The high acceptability of green tea is due to many factors, especially one of its most contributory factors, its odor. Volatile compounds of green tea have been investigated by many researchers (Takei et al., 1976; Kosuge et al., 1978; Kawakami and Yamanishi, 1981; Yamaguchi and Shibamoto, 1981; Shimoda et al., 1995a,b), and >270 volatile compounds have been reported (Nijssen et al., 1996). However, the actual significance of these volatile compounds to the odor of Japanese green tea has not yet been determined. The current investigations are concerned with the volatile compounds that contribute to the flavor using aroma extract dilution analysis (AEDA) (Grosch, 1993). Guth and Grosch (1993) have reported the application of AEDA to the odorants of Chinese green tea powder. On the basis of this result, (*Z*)-1,5-octadien-3-one, 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone, and 3-methylnonane-2,4-dione were identified as the most important odorants of the Chinese green tea powder. However, Japanese green tea (Sen-cha), which is manufactured according to a process different from that used for Chinese green tea, has not been analyzed using the AEDA technique.

In the present paper, we reported that the potent odorants in the characteristic odor of Japanese green tea (Sen-cha) infusion were screened by AEDA and identified using gas chromatography/mass spectrometry (GC/MS) and/or multidimensional gas chromatography/mass spectrometry (MDGC/MS) techniques (Nishimura, 1995a,b; Kumazawa et al., 1998).

MATERIALS AND METHODS

Materials. *Tea Sample.* Green tea (Sen-cha) products (*Camellia sinensis* L. var. Yabukita) of high grade, based on market price, were produced in Shizuoka prefecture in 1997.

Chemicals. The following compounds were synthesized according to the literature: (*Z*)-1,5-octadien-3-one and 1-octen-3-one (Swoboda and Peers, 1977); 4-methoxy-2-methyl-2-butanethiol (Guth and Grosch, 1991); 4-mercapto-4-methyl-2-pentanone (Guth, 1997a); and 3-methylnonane-2,4-dione (Guth and Grosch, 1989).

Isolation of the Volatiles. Deionized hot water (70 °C, 4 L) was added to 200 g of green tea, and the leaves were filtered using a coarse filter paper after standing for 3 min. The filtrate (3 L) was immediately cooled to ∼30 °C in tap water, and steam distillation was performed under reduced pressure (40 °C, 20 mmHg). The steam distillate (1 L) was passed through a column packed with 10 g of Porapak Q (Waters). Adsorbed compounds were eluted with methylene chloride (50 mL). The eluate was dried over anhydrous sodium sulfate, and the solvent was removed by a rotary evaporator to ∼5 mL in volume. Further concentration was conducted with a nitrogen stream to ∼100 *μ*L. The concentrate was used as the AEDA sample. For an identification sample, these procedures were repeated 10 times and concentrated to ∼50 *μ*L (total: 40 L of deionized water was added to 2 kg of green tea).

GC/Olfactometry (GC/O). A Hewlett-Packard (HP) Model 5890A gas chromatograph equipped with a thermal conductivity detector (TCD) was used. A fused silica column [30 m × 0.53 mm i.d.; coated with a 1 *μ*m film of DB-Wax (J&W Scientific) or 30 m × 0.53 mm i.d.; coated with a 1.5 *μ*m film of DB-1 (J&W Scientific)] was used without splitting. The column temperature was programmed from 40 to 210 °C at a rate of 5 °C/min for all runs. The injector and detector temperatures were 250 and 230 °C, respectively. Helium was used as the carrier gas at a flow rate of 4.4 mL/min. A glass sniffing port was connected to the outlet of the TCD and was heated by a ribbon heater. Moist air was pumped into the sniffing port at ∼100 mL/min to quickly remove the odorant eluted from the TCD out of the sniffing port.

AEDA. The original odor concentrate of the green tea infusion was stepwise diluted with methylene chloride 1:10, 1:100, 1:500, 1:1000, and 1:5000, and aliquots (0.5 *μ*L) of each fraction were analyzed by capillary GC on the DB-Wax column. The odor active compounds were detected by GC eluate sniffing (GC/O). The flavor dilution (FD) factors of the odorants were

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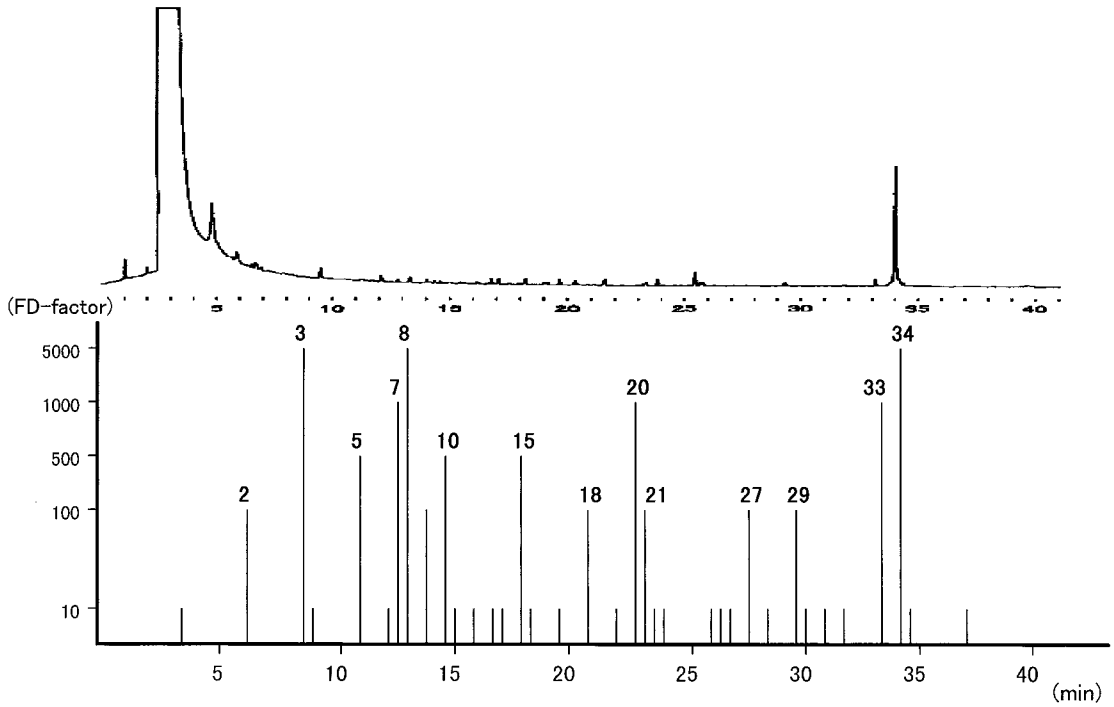

Figure 1. Gas chromatogram (DB-Wax stationary phase column) monitored by TCD (top) and FD chromatogram (bottom) of the volatile fraction of Japanese green tea (Sen-cha). The numbers indicate the positions at which an odor was perceived at the sniffing port.

determined by AEDA, and an FD chromatogram (plot of the FD factor of each odorant versus its retention time) was prepared.

GC/MS. An HP5890 series II gas chromatograph coupled with an HP Model 5972 series mass spectrometer was used. The column was a 60 m × 0.25 mm i.d. DB-Wax, film thickness = 0.25 μm, fused silica capillary column (J&W Scientific). The column temperature was programmed from 80 to 210 °C at the rate of 3 °C/min in all runs. The injector temperature was 250 °C. The flow rate of the helium carrier gas was 1 mL/min, and the split ratio was 1:50. The mass spectrometer was used under the following conditions: ionization voltage, 70 eV; ion source temperature, 150 °C.

MDGC/MS System. The gas chromatograph used for preseparation of the MDGC was an HP Model 5890A gas chromatograph (GC1) equipped with a TCD. A fused silica column (30 m × 0.53 mm i.d.; coated with a 1.5 μm film of DB-1; J&W Scientific) was used without splitting as the preparative column. The outlet of the TCD (GC1) was connected to a 6 mm o.d. glass tube, which was packed with Tenax GC (GL Sciences, Inc.) adsorbent. The column temperature was programmed in the same way as the GC/O. The GC/MS was used as an analytical gas chromatograph (GC2), and the thermal desorption cold trap injector (TCT; supplied from Chrompack) was mounted on this instrument. The column, oven, and MS conditions were as described above for the GC/MS analysis. The TCT was operated under the following conditions in all runs: precooling, the cold trap was cooled to −100 °C; thermal desorption, 180 °C, 5 min, cold trap was maintained at −100 °C; and injection, the cold trap was heated to 220 °C. The purge gas flow rate in the TCT was 10 mL/min.

The analysis was carried out as follows: the volatile heart-cut compounds after separation by the GC1 column were adsorbed onto the Tenax GC column directly out of the GC1 detector. This Tenax GC column was then fitted to the TCT. The volatile compounds adsorbed on the Tenax GC were thermally desorbed, cold trapped, and injected onto the analytical column in the GC/MS (GC2).

Identification of Components. The identification of the components was made by comparison of their Kovats GC retention indices, mass spectra, and odor quality to those of authentic compounds.

RESULTS AND DISCUSSION

Japanese green tea (Sen-cha) infusion has a characteristic fresh green odor. The components causing this characteristic odor were separated by steam distillation under reduced pressure, and the steam distillate was concentrated by the adsorptive column method. AEDA was used for the objective determination of the components that contribute to the characteristic odor of Japanese green tea (Sen-cha) infusion. The AEDA of this odor concentrate revealed 36 odor-active peaks with FD factors of ≥10 (Figure 1). Components responsible for the 36 peaks were identified by comparison of their Kovats indices, mass spectra, and odor quality to those of authentic compounds. The odors of these peaks were sniffed by the GC/O, and the results are summarized in Table 1. Twenty-three compounds were identified from 17 peaks by GC/MS (peaks 1, 4, 6, 10, 12, 17, 21−24, 29, 30, and 32−36). However, the remaining 19 peaks were not identified because they were complex mixtures and/or in too small amounts. For the identification of the unknown compounds in these peaks, the MDGC/MS system was used. This system was a very effective tool for the separation of complex mixtures and identification of compounds of low concentration. Each fraction (A−G, Figure 2) on the DB-1 column was heart-cut, and the volatiles in these fractions were separately adsorbed on Tenax GC. To concentrate the compounds found in small amounts, these procedures were repeated five times. Each volatile in the fractions was separately injected into a GC/MS equipped with a DB-Wax column using the TCT instrument, and 12 peaks (peaks 3, 5, 7, 8, 11, 14, 15, 18, 20, 25, 27, and 31) were identified. A total of 36 potent odorants were identified from the 36 odor-active peaks on the basis of the GC/MS and MDGC/MS results. A comparison with the data from the

Table 1. Potent Odorants in the Volatile Fraction of Japanese Green Tea (Sen-cha)

peak[a]	RI[b]	RI[c]	fraction[d]	compound	odor quality[f]	FD factor
1	972	975		2,3-butanedione	buttery	10
2	1112			unknown	green	100
3	1216	1213	A	4-methoxy-2-methyl-2-butanethiol[e]	meaty	5000
4	1247	1253		(*Z*)-4-heptenal	hay-like	10
5	1309	1307	B	1-octen-3-one	mushroom-like	500
6	1349	1352		2-ethylpyrazine	nutty	10
7	1379	1379	B	(*Z*)-1,5-octadien-3-one	metallic	1000
8	1389	1387	A	4-mercapto-4-methyl-2-pentanone[e]	meaty	5000
9	1436			unknown	nutty	100
10	1456	1465		methional[e]	potato-like	500
11	1476	1475	C	2-ethyl-3,5-dimethylpyrazine[e]	nutty	10
12	1505	1507		(*E*,*E*)-2,4-heptadienal	fatty	10
13	1535			unknown	earthy, musty	10
14a	1550	1544	E	(*Z*)-4-decenal[e]	green	10
14b	1550	1550		linalool	floral, green	10
15	1598	1605	D	(*E*,*Z*)-2,6-nonadienal	green, cucumber-like	500
16	1626			unknown	roasty	10
17	1648	1653		phenylacetaldehyde	honey-like	10
18a	1715	1719	G	(*Z*)-3-hexenyl (*Z*)-3-hexenoate	green	100
18b	1715	1722		3-methylnonane-2,4-dione	green	100
19	1762			unknown	roasty	10
20a	1824	1820		(*E*,*E*)-2,4-decadienal	fatty	1000
20b	1824	1822	G	β-damascone[e]	honey-like	1000
20c	1824	1823	G	β-damascenone	honey-like	1000
21	1849	1852		hexanoic acid	green, acid	100
22	1858	1855		geraniol	floral	10
23a	1868	1862		geranyl acetone	hay-like	10
23b	1868	1869		guaiacol	burnt	10
24a	1959	1954		β-ionone	woody, violet-like	10
24b	1959	1961		(*Z*)-jasmone	green	10
25a	1980	1981		maltol[e]	sweet	10
25b	1980	1986	F	5-octanolide[e]	sweet	10
26	1994			unknown	sweet	10
27	2047	2045	G	4-nonanolide	sweet	100
28	2090			unknown	spicy	10
29	2167	2176		eugenol	spicy	100
30	2195	2203		2-methoxy-4-vinylphenol[e]	spicy	10
31	2223	2228	F	2-aminoacetophenone[e]	grape-like	10
32	2274	2278		jasmine lactone	sweet	10
33	2400	2407		(*Z*)-methyl jasmonate	floral	1000
34	2444	2450		indole	animal-like	5000
35	2467	2467		coumarin	sweet, camphoraceous	10
36	2588	2588		vanillin	vanilla-like	10

[a] Numbering refers to Figure 1. [b] Retention index on DB-Wax column (30 m × 0.53 mm i.d.; coated with a 1 μm film) observed for GC/O. [c] Retention index on DB-Wax column (60 m × 0.25 mm i.d.; coated with a 0.25 μm film) observed for GC/MS. [d] Fraction obtained by preseparation of MDGC system (GC1), refers to Figure 2. [e] Newly identified compounds of green tea. [f] Odor quality assigned during AEDA.

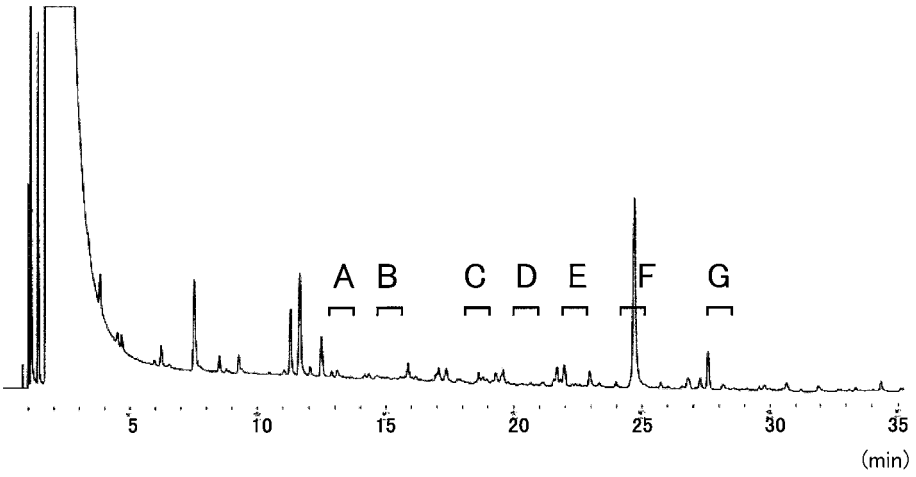

Figure 2. Gas chromatogram (DB-1 stationary phase column) of the volatile fraction of Japanese green tea (Sen-cha) on preseparation of MDGC system (GC1). The letters A (meaty), B (metallic, mushroom-like), C (nutty), D (cucumber-like), E (green), F (sweet, grape-like), and G (sweet, honey-like) are the heart-cut fractions by the MDGC system.

literature revealed that 4-methoxy-2-methyl-2-butanethiol (peak 3), 4-mercapto-4-methyl-2-pentanone (peak 8), methional (peak 10), 2-ethyl-3,5-dimethylpyrazine (peak 11), (*Z*)-4-decenal (peak 14a), β-damascone (peak 20b), maltol (peak 25a), 5-octanolide (peak 25b), 2-methoxy-4-vinylphenol (peak 30), and 2-aminoacetophenone

(peak 31) are reported here for the first time as components of the green tea.

Stepwise dilution of the odor concentrate, followed by GC/O, resulted in an FD chromatogram indicating six peaks with the highest odor potencies (displayed by their FD factors in the range 1000−5000). Potent odorants in these peaks showed meaty (peaks 3 and 8), metallic (peak 7), fatty and honey-like (peak 20), floral (peak 33), and animal-like (peak 34) odor qualities. The eight compounds from these peaks with the highest odor potencies could be identified as 4-methoxy-2-methyl-2-butanethiol (peak 3), (*Z*)-1,5-octadien-3-one (peak 7), 4-mercapto-4-methyl-2-pentanone (peak 8), (*E*,*E*)-2,4-decadienal (peak 20a), *β*-damascone (peak 20b), *β*-damascenone (peak 20c), (*Z*)-methyl jasmonate (peak 33), and indole (peak 34). Among these potent odor-producing compounds, 4-methoxy-2-methyl-2-butanethiol and 4-mercapto-4-methyl-2-pentanone have not been identified as volatile component of teas (for example, green, oolong, and black teas). On the basis of this suggestion, these thiols were involved as key odorants of the Japanese green tea (Sen-cha). These thiols have recently been identified as character impact odorants of black currant (Rigaud et al., 1986; Quere and Latrasse, 1990), olive oil (Guth and Grosch, 1991), wine (Darriet et al., 1995; Guth, 1997a,b; Tominaga et al., 1998), and box tree and broom (Tominaga and Dubourdien, 1997). According to the literature, the odor threshold values of these thiols were very low and showed 0.1 ng/L in water (Rigaud et al., 1986; Darriet et al., 1995), respectively. Therefore, these thiols may contribute to the odor of the Japanese green tea (Sen-cha) infusion in trace amounts.

In this investigation, 36 potent odorants were identified as contributors to Japanese green tea (Sen-cha) odor. Among them, 10 odorants are reported here for the first time as components of the green tea. Especially, 4-methoxy-2-methyl-2-butanethiol, 4-mercapto-4-methyl-2-pentanone, and indole were characteristic components of Japanese green tea (Sen-cha).

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