Identification of Volatile Compounds which Enhance Odor Notes in Japanese Green Tea using the OASIS (Original Aroma Simultaneously Input to the Sniffing port) method

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We evaluated the volatile compounds of Japanese green tea using GC/O with the original aroma simultaneously input to the sniffing port (OASIS) method in order to determine the relationship between the volatile compounds and the green tea aroma. Application of OASIS to the volatile fractions of Japanese green tea infusions revealed 11 odor-active peaks. Those of \( \text{cis-1,5-Octadien-3-one} \), \( \text{4-mercapto-4-methyl-2-pentanone} \), \( \text{3-methylxnonane} \), \( \text{2,4-dione} \), geraniol, decanal and \( \text{cis-3-hexenol} \) emphasized the green note of the tea. \( \text{2-Acetylpyrrolo} \), \( \text{2-ethyl-3,5-dimethylpyrazine} \) and \( \text{2-acetyl-3,5-dimethylpyrazine} \) emphasized the roast note. Indole strengthened the overall green tea odor. \( \beta \)-Ionone emphasized the sweet note in the green tea. Of these odorants, \( \text{2-acetyl-3,5-dimethylpyrazine} \) was identified for the first time as a volatile compound in tea.

Keywords: Japanese green tea, \( \text{Camellia sinensis} \) cv. Yutakamidori, GC/O with original aroma simultaneously input to the sniffing port, Gas chromatography Olfactometry

Introduction

Green tea is the most popular beverage in Japan. This great popularity may be due to many factors, but one of the reasons is its characteristic and complex odor. Volatile compounds of green tea have been investigated by many researchers (Hara and Kubota, 1982; Hara and Horita, 1987; Kawakami and Kobayashi, 1991; Kawakami and Yamanishi, 1981; Kobayashi, 1995; Kosuge et al., 1978; Nijssen et al., 1996; Shimoda et al., 1995a, b; Takei et al., 1976; Takeo, 1996, 2000; Yamaguchi, 2000; Kumazawa and Masuda, 1999, 2002), and over 400 volatile compounds have been reported. These reports only identified the odor compounds but did not show how those compounds affect the odor notes of green tea. As described in our previous paper (Hattori et al., 2003), we developed the OASIS method in order to identify potent odors in original aromas; this method is not based only on the odor threshold.

These days, consumers desire varieties of green tea and enjoy drinking those which suit their varied tastes. It is important to produce bottled green tea products which target the varied consumer needs in the market. In this study, we report that volatile compounds enhanced the roast and green notes in Japanese green tea infusions as screened by OASIS.

Materials and Methods

Green Tea  Japanese green tea (\( \text{Camellia sinensis} \) cv. Yutakamidori, which has roast and green attributes) was produced in Kagoshima prefecture in May 2003.

Isolation of the Volatile Compounds  Deionized water was prepared using a TORAYPURE LV-10T (Toray Industries Inc., Tokyo, Japan). Three liters of deionized hot water at 60°C was added to 180 g of Japanese green tea, and the leaves were filtered through a coarse filter paper after standing for 5 min. The filtrate (2.1 L) was immediately cooled to about 30°C in tap water, and steam distillation was performed under reduced pressure at 40°C and 20 mmHg. 1.5 L of the steam distillate was passed through a column packed with 20 g of Porapak Q (Waters Co.: Milford, Massachusetts). Adsorbed compounds were eluted with 100 mL of diethyl ether, the eluate was dried over anhydrous magnesium sulfate, and the solvent was removed by a rotary evaporator to about 2 mL. Further concentration was carried out under a nitrogen stream to about 10 \( \mu L \). These procedures were repeated five times (total: 15 L = 3 L × 5 of deionized water was added to 0.9 kg = 0.18 kg × 5 of Japanese green tea) to finally obtain 50 \( \mu L \) of aroma concentrate. This concentrate was used as the OASIS sample.

Enrichment of Odorants for Identification  For the identification experiments, the green tea volatiles were isolated as described above. These procedures were repeated twenty-five times and the isolates were concentrated to about 250 \( \mu L \) (total, 75 L = 3 L × 25 of deionized water was added to 4.5 kg = 0.18 kg × 25 of Japanese green tea). To divide the green tea volatiles into a basic fraction and a neutral/acidic fraction, liquid-liquid extractions were performed as follows. First, the green tea volatiles mentioned above were dissolved in 20 mL of diethyl ether and mixed with 10 mL of 1 M hydrochloric acid in a separatory funnel and shaken. The acidic aqueous layer including the basic fraction was collected and the remaining organic layer was extracted twice with 10 mL of 1 M
hydrochloric acid. The organic solution containing the neutral and acidic fractions, was washed twice with 5 mL of 26% sodium chloride solution in the funnel, dried over anhydrous magnesium sulfate and finally concentrated to about 50µL (neutral/acidic fraction). The collected acidic aqueous solution with the basic fraction was washed twice with 5 mL of diethyl ether in a separatory funnel. This aqueous solution was adjusted to pH 8 with 6M aqueous sodium hydroxide, and extracted three times with 10 mL of diethyl ether. Addition of sodium hydroxide changes the pH from 2 to 8, so the basic compounds are expected to leave the aqueous layer, and return to the organic layer. This organic layer was then washed twice with 5 mL of 26% sodium chloride solution in a funnel, dried over anhydrous magnesium sulfate, and finally concentrated to about 50µL (basic fraction).

**OASIS** We screened volatile compounds which enhanced the roast and green notes in Japanese green tea infusions using the OASIS method. An Agilent Model 5890A gas chromatograph (Agilent Technologies, Inc.: Palo Alto, California) equipped with a thermal conductivity detector (TCD) was used. A fused silica column 30 m × 0.53 mm i.d.; coated with a 1µm film of INNOWax (Agilent) was used without splitting. The column temperature was programmed from 40 to 230°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 4.4mL/min. A glass sniffing port was connected to the outlet of the TCD and was heated by a ribbon heater at 200°C. Although the sensitivity of the TCD is inferior to the flame ionization detector (FID), the TCD is more convenient for GC/O because there is no need for a branching detector and sniffing port, and no time delay between detection and sniffing. This TCD method has been used for evaluation of green tea aroma (Kumazawa and Masuda, 1999, 2002) and strawberry guava aroma (Jorge et al., 2001). Two hundred milliliters of deionized hot water (60°C) was added to 12g of Japanese green tea, and the leaves were filtered through a coarse filter paper after standing for 5 min. The filtrate (140 mL) was placed in glass vessels at 60°C and the green tea odor was input to the sniffing port by air flow at 150 mL/min. The original odor concentrate of the green tea infusion was stepwise diluted with diethyl ether 1: 3, 1: 9, 1: 27, 1: 81, 1: 243, 1: 729, 1: 2187 and 0.5µL aliquots of each fraction were analyzed by capillary GC on the INNOWax column. A GC eluted compound was mixed with the green tea odor, and the mixed odors were sniffed. These procedures were carried out by a panel of 7 well-trained flavorists, 5 males and 2 females, ranging from 20-40 yr. Immediately after sniffing, the flavorists were asked to write their opinion on the mixed odors. When the panel was in complete agreement as to the odor note of each compound, the flavor dilution (FD) factors of the odorants were determined, also using the OASIS method, and an aromagram (plot of the FD factor of each odorant versus its retention time) was prepared.

**GC and GC/MS** To solve the problem of the low sensitivity of the TCD, an Agilent 6890 gas chromatograph equipped with an FID was used for calculation of GC retention indices. The column was a TC-Wax fused silica capillary column (30 m × 0.25 mm i.d., film thickness =0.25µm; GL-Sciences, Inc., Tokyo, Japan). The column temperature was programmed from 65 to 250°C at a rate of 5°C/min in all runs. The injector and detector temperatures were 250°C and 280°C, respectively. The flow rate of the helium carrier gas was 1 mL/min, and the split ratio was 1:100. GC/MS conditions were identical to those of GC. The mass spectrometer was operated under the following conditions: ionization voltage, 70 eV; ion source temperature, 150°C.

**Identification of Components** The GC Kovats index and MS fragmentation pattern of each component were compared to those of the authentic compound.

**Results and Discussion** An infusion of Japanese green tea has characteristic and complex odors. The complex odors are thought to originate from ten aroma groups as follows. 1) young leaf type: fresh green note (derived from cis-3-hexenol, hexanol, etc.); 2) lily of the valley type: light sweet flowery note (derived from linalool, etc.); 3) rose type: flowery note (derived from phenylethylalcohol, etc.); 4) jasmine and gardenia type: heavy sweet flowery note (derived from cis-jasmone, methyl jasmonate, indole, etc.); 5) fruit note (derived from some esters, some lactones, etc.); 6) lumber note (derived from sesquiterpenoids, 4-vinylphenol, etc.); 7) heavy green note (unknown compounds from green tea); 8) roast note (derived from some pyrazines, etc.); 9) off-flavor notes during preservation (derived from trans-2-cis-4-heptadienial, 2,3-epoxy-β-ionone, etc.); and 10) other notes (complex and full-bodied are added to the whole) (Yamanishi, 1977). In the present research, there were 34 odorants with FD factors of >3; the 11 odorants which enhanced the odor notes in the green tea were screened by OASIS (Fig. 1). The other 23 have non-enhancing effects. Fig. 2 indicates how those 11 compounds influence the odor notes of green tea with FD factors in the range of 3 to 2187. Geraniol, cis-3-hexenol and decanal emphasize the green note. β-Ionone emphasizes the sweet note. Indole strengthens the overall green tea odor. These 5 compounds were reported in our previous report of a different cultivar.

Of the remaining compounds, cis-1,5-octadien-3-one is known to have a metallic odor (Swoboda and Perris, 1977). Using the OASIS method, enhancement of the green note for this compound in the green tea odor was detected in the FD range from 9 to 81. 4-Mercapto-4-methyl-2-pentanone is known to have a meaty odor (Kumazawa and Masuda, 1999). Enhancement of the green note was detected for this compound in the FD range from 243 to 729. 3-Methylnonane-2,4-dione is known to have a green odor (Guth and Grosch, 1989). There was enhancement of the green note for this compound in the FD range from 243 to 729. 2-Acetylpyrrole has a nutty odor. There was enhancement of the roast note for this compound in the FD range from 3 to 9. 2-Ethyl-3,5-dimethylpyrazine has a nutty odor (Yamanishi et al., 1973). There was enhance-
ment of the roast for this compound in the FD range from 3 to 2+.

Peak 0 had a nutty odor but usual GC/MS analysis could not identify the compound. For identification of peak 0, the volatile fraction was obtained from a large volume of green tea infusion (about 1/2 L), which was separated into basic and neutral fractions. The mass spectrum of -acetyl-, -dimethylpyrazine was obtained with the same retention index and the same enhancement as described above. This compound had not previously been detected in teas and had only been reported as one of the nitrogen-containing heterocycles in coffee (Vitzthum and Werkhoff, 1974). Using OASIS, in the range 9 to 81 there was enhancement of the roast note in the green tea odor.

Application of the OASIS method using the volatile fraction of Japanese green tea infusions revealed 11 odorants which enhanced odor notes in Japanese green tea. -Acetyl-3,5-dimethylpyrazine was identified for the first time among the tea volatiles. cis-1,5-Octadien-3-one, 4-mercapto-4-methyl-2-pentanone, 3-methylnonane-2,4-dione, geraniol, decanal and cis-3-hexenol emphasized the green note; and 2-Acetylpyrrole, 2-ethyl-3,5-dimethylpyrazine and 2-acetyl-3,5-dimethylpyrazine emphasized the roast note. This suggests that the enhancement effect has a specific character and that it can be clearly detected by OASIS. OASIS enabled us to determine that even high odor threshold compounds affect the overall aroma of Japanese green tea.
References


