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Methyl Anthranilate is the Cause of Cultivar-Specific Aroma in the Japanese Tea Cultivar 'Sofu'

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Abstract

Volatile constituents of the new Japanese tea (*Camellia sinensis*) clonal cultivar 'Sofu', which has a characteristic aroma, were extracted by means of a simultaneous distillation extraction method and analyzed by means of gas chromatography. A peak found in 'Sofu', and not in any other tea cultivar that we analyzed, was identified as methyl anthranilate. 'Sofu' is a hybrid of 'Yabukita' (var. *sinensis*) and 'Shizu-Inzatsu 131', which is derived from a cross between var. *assamica* and var. *sinensis*. 'Shizu-Inzatsu 131', the pollen parent of 'Sofu', also contained methyl anthranilate. Furthermore, 'Fujikaori', the hybrid of 'Shizu-Inzatsu 131' crossed with 'Yabukita' as the pollen parent, contained methyl anthranilate. 'Shizu-Inzatsu 131' was selected from a natural cross population of the clonal strain introduced from Assam. These suggest that the origin of tea which contains methyl anthranilate is in var. *assamica*. This is the first report of methyl anthranilate in Japanese cultivars or native varieties.

Discipline: Tea industry
Additional key words: simultaneous distillation extraction (SDE), gas chromatography (GC), 'Shizu-Inzatsu 131', 'Fujikaori'

Introduction

Many clonal cultivars of tea (*Camellia sinensis*) are cultivated in Japan. However, just one popular cultivar, 'Yabukita', currently accounts for 85% of all clonal cultivars grown in Japan. Varietal differences in the volatile compounds of each tea have been investigated[1,6,13,16] but most studies have concluded that the differences were due to differences in the concentrations of common components. In only a few instances has a specific key compound been newly found. One of the reasons that almost all Japanese tea cultivars are similar in aroma to 'Yabukita' is that they have been bred with 'Yabukita', which serves as the standard for aroma. However, a few cultivars have different aromas. The new Japanese cultivar 'Sofu', bred at our institute, is one such cultivar. It has a specific aroma reminiscent of incense or jasmine that most people can easily distinguish. This paper describes the volatile cultivar-specific key compound contained in 'Sofu'.

Materials and methods

1. Plant materials

Shoots of nine tea cultivars ('Yabukita, Asatsuyu, Shunmei, Yamanami, Izumi, Karabeni, Sofu, Shizu-Inzatsu 131', and 'Fujikaori') were harvested at the Kanaya Tea Research Station, National Institute of Vegetable and Tea Science, and were processed separately as green tea. 'Shizu-Inzatsu 131' was also processed as oolong tea.

2. Extraction of volatile compounds

Green or oolong tea (50 g) and 0.5 mL of 100 ppm ethyl decanoate (internal standard) were placed in a 2-L round-bottom flask containing 400 mL of boiling water. The volatiles were steam-distilled and extracted into 50 mL of diethyl ether for 10 min using a modified Likens-Nickerson apparatus (simultaneous distillation extraction, SDE, method)[4,11]. The extracts were dried over anhydrous sodium sulfate and filtered. The solvent was

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then removed to obtain a concentrate of the volatiles as the "SDE extract". These samples were then analyzed by means of gas chromatography (GC) and gas chromatography-mass spectroscopy (GC-MS).

3. GC

GC analyses were performed with a Shimadzu 17A gas chromatograph (Shimadzu Corp., Kyoto, Japan) equipped with a flame ionization detector and a 60 m × 0.25 mm (I. D.) DB-WAX (J & W Scientific, Folsom, CA) fused-silica capillary column. The operating conditions were as follows: oven temperature, 40ºC, 3ºC min^{-1} rise to 220ºC; carrier gas, helium; and flow rate, 1.0 mL min^{-1}. The retention times and peak areas of the eluted volatile components were integrated with a Shimadzu C-R7A Chromatopack integrator (Shimadzu).

4. GC-MS

GC-MS analyses were conducted with an HP5890 gas chromatograph (Hewlett-Packard, Avondale, PA) coupled directly to a JEOL JMS-SX102A mass spectrometer and a MS-MP7000 mass data system (JEOL Ltd., Tokyo, Japan). All conditions for GC operation were the same as those for the GC analyses as described in section 3 of this paper.

5. Identification of compounds

Individual peaks were characterized by comparing GC retention data with those of the authentic compounds and by matching the mass spectra with those of authentic compounds.

6. The concentration of methyl anthranilate in tea infusion

Sixty grams of the green tea of 'Sofu' was brewed in 400 mL of boiling water for 5 min. The infusion with leaves removed was placed in a flask and the volatiles were extracted by SDE method as described above. The extract was analyzed by GC and the concentration of methyl anthranilate in tea infusion was calculated by comparing the areas of its peak to that of the authentic compound.

7. Calculation of human olfactory thresholds of methyl anthranilate

The threshold level of detection of methyl anthranilate in water was estimated by sensory evaluation. A panel of taste-testers was given water samples containing various amounts of methyl anthranilate or dummy, and distinguished the aroma.

Results and discussion

The aroma constituents of the new tea cultivar 'Sofu', which have a distinctive aroma, were extracted with an SDE method and analyzed with GC. The identified compounds and their quantities, as calculated from the ratio of the areas of each peak to that of an internal standard, are shown in Table 1. The profile was compared with that of six other Japanese cultivars ('Yabukita, Asatsuyu, Shunmei, Yamanami, Izumi', and 'Karabeni') that lack the 'Sofu' cultivar's distinctive aroma (only the results for 'Yabukita' are shown in Table 1). Most of the compounds were common to all cultivars. However, one distinct peak was found in the aroma extract from 'Sofu'. The aroma extract was subjected to GC-MS analysis and the compound was tentatively identified as predicted to be methyl anthranilate (M. W. = 151) by comparison with the spectral library of the GC-MS. The compound's identity was subsequently confirmed by matching its GC retention data to that of authentic methyl anthranilate (Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan).

Methyl anthranilate is a characteristic volatile component of grapes[7–9,12], citrus[3,14,15] and jasmine[5]. The highly fragrant, flower-like aroma of 'Sofu' is similar. Sixty grams of the green tea of 'Sofu' was brewed in 400 mL of boiling water for 5 min as a model infusion of general tea beverage. The concentration of methyl anthranilate was calculated as 194 ppb. On the other hand, the threshold level of detection of methyl anthranilate in water was estimated by sensory evaluation. A panel of taste-testers was given water samples containing various amounts of methyl anthranilate. Samples containing more than 10 ppb were successfully distinguished. This low taste threshold, which is much lower than the amount of methyl anthranilate present in tea made from 'Sofu', suggests that methyl anthranilate contributes to the characteristic aroma of 'Sofu'.

The presence of methyl anthranilate in black tea made in Kenya, Darjeeling, Ceylon, and Assam was reported by Cazenave and Horman[2]. We developed 'Sofu' by selecting from the F_1 progeny of crosses between 'Yabukita' (var. *sinensis*) as the seed parent and 'Shizu-Inzatsu 131' (var. *assamica* × var. *sinensis*) as the pollen parent. Green tea of 'Shizu-Inzatsu 131' was analyzed by GC in the same way as the other teas. A similar level of methyl anthranilate was detected as in 'Sofu' (Table 1). 'Shizu-Inzatsu 131' was selected from a natural cross population of the clonal strain 'Manipur No. 5' introduced from Manipur, Assam.

It is interesting that methyl anthranilate has not been detected in extracts of Japanese tea cultivars or of native Japanese varieties[6,13,16]. This suggests the hypothesis that

Table 1. The contents of volatile components in teas

Compound	Sofu	Yabukita	Shizu-Inzatsu 131			Fujikaori
			Non-fermentation (N)	Semi-fermentation (S)	(S)/(N)	
Heptanal	0.53	0.84	0.72	2.72	3.79	1.52
(*E*)-2-Hexenal	0.04	0.03	0.05	0.32	6.66	0.34
Pentanol	0.10	0.15	0.11	1.07	9.94	0.29
Hexanol	0.25	0.16	0.13	0.58	4.30	0.48
(*Z*)-3-Hexenol	0.15	0.06	0.07	3.08	41.53	trace
Nonanal	0.48	1.10	0.70	2.18	3.13	2.44
Linalool oxide (*cis*-furanoid)	trace	0.03	0.09	0.19	1.99	trace
Linalool oxide (*trans*-furanoid)	0.02	0.04	0.08	0.09	1.15	trace
Linalool	0.43	0.79	0.41	2.00	4.81	0.27
Octanol	0.16	0.27	0.19	0.44	2.33	0.27
(*Z*)-3-Hexenyl hexanoate	0.37	0.13	0.08	1.64	20.08	0.17
Linalool oxide (*trans*-pyranoid)	0.05	0.06	0.10	0.30	2.86	trace
Geraniol	0.12	0.16	0.11	0.44	3.92	0.14
Benzylalcohol	0.15	0.05	0.08	0.19	2.26	trace
β-Ionone	0.12	0.06	0.08	0.40	5.01	1.53
(*Z*)-Jasmone	0.34	0.16	trace	0.43	–	0.07
Nerolidol	1.38	0.90	0.11	0.83	7.27	0.40
Methyl anthranilate	**0.13**	**trace**	**0.11**	**0.19**	**1.68**	**0.13**
Indole	0.37	0.36	0.01	0.45	31.85	trace

The content of each component is expressed by the ratio of each peak area to the peak area of the internal standard on the gas chromatogram.

the origin of tea which contains methyl anthranilate is in var. *assamica*. It may be possible to classify all of the tea plants in the world by containing methyl anthranilate or not.

Extracts of green (non-fermented) and oolong (semi-fermented) teas prepared from 'Shizu-Inzatsu 131' were compared by means of GC (Table 1). The amounts of almost all the volatile components, including alcoholic aroma constituents hydrolyzed by enzyme[10], increased as a result of semi-fermentation. However, the amount of methyl anthranilate did not increase as much. This suggests that methyl anthranilate exists naturally in the shoots of 'Sofu' and 'Shizu-Inzatsu 131' and does not increase after harvest due to enzyme activities. This is a cultivar-specific volatile compound.

'Fujikaori' is another cultivar that is derived from a cross between 'Shizu-Inzatsu 131' as the seed parent and 'Yabukita' as the pollen parent, and also contained methyl anthranilate (Table 1). Thus, the phenotype responsible for the presence of methyl anthranilate appears to have a high heritability; it is clearly inherited from 'Shizu-Inzatsu 131' whether the latter cultivar is the seed parent or the pollen parent. We should also mention that not all individuals in the progenies of 'Shizu-Inzatsu 131' necessarily contain methyl anthranilate (data not shown) but those which have the characteristic flower-like aroma always contain methyl anthranilate.

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