Effect of Clonal Specificity of the Monoterpene Alcohol Composition of Tea Shoots on Black Tea Aroma Profile

By TADAKAZU TAKEO

Tea Technology Division, National Research Institute of Tea
(Kanaya, Haibara, Shizuoka, 428 Japan)

There are many flavory black teas in the world. These teas have typical different aromas, respectively. Sri Lanka tea has a sweet fruity and flower-like flavor. Darjeeling tea is very famous by its characteristic heavy-rosy flavor. Keemun tea (a historical black tea in China), has also a rosy and thick flavor.

It was assumed that the difference in black tea aromas may be originated from the genetic peculiarities of tea cultivars from which black teas were made.

Recently, the analysis technique of volatile compounds has made a marked advance with the progress of GC–MS method. Many reports on the aroma composition of black tea have appeared in the world. These reports give also many interesting suggestions concerning the characteristics of black tea aromas of cultivars from different geographical locations.

The author reported that the amounts of linalool and geraniol which are typical aromatic compounds influencing black tea aroma were different among tea cultivars.[3,4,5] It has been known that the both compounds were produced during black tea fermentation.[2,6,7]

The author has investigated the genetical relation on the variations of the amounts of linalool and geraniol produced in tea shoots and also on the aroma characteristics of black teas made from different varieties.

Experimental methods

1) Preparation of volatiles in triturated tea shoots

Two g of fresh shoots, consisting of a bud and two leaves, (*Camellia sinensis* L.), was blended in a 100 *ml* flask with 1 g of Polyclar AT and 20 *ml* of 0.2 M acetate buffer, pH 4.5, for 2 min at 2°C. The triturated material was incubated for 30 min at 40°C. At the end of this period, the flask was cooled in an ice-water bath and n-pentane (3 *ml*) was added to the homogenate. The mixture was shaken for 1 min and centrifuged. The clear pentane layer was sampled by an air-tight micro-syringe and used for the assay of volatile compounds.

2) Preparation of volatile concentrate of black tea

The aroma concentrate from black tea was prepared by steam distillation as follows; 200 g of tea and 1.2 *l* of H_2O were put into 5 *l* of flask. After steam distillation at 45°C under reduced pressure for 2 hr, the distillate condensed in four cold traps arranged in order of an ice-water cooled trap, an ice-NaCl cooled trap, and two dry ice-acetone cooled traps was transferred with ether. The ether layer was dried with Na_2SO_4 anhydrid and concentrated to 200 mg.

3) Gas-chromatograph and peak identification

Each aroma preparation was analysed by a gas-chromatography with a FID. GC conditions were as follows; column; 2 m×3 mm of glass column, column packing; 5% Carbowax 20 M on chromosorb G, He flow rate; 30 *ml*/min, column temp. program; 60–180°C at the rate of 2°C/min.

The amount of each volatile compound was estimated from the peak area (cm^2). The aroma pattern was compared by the area of individual peak expressed as a percentage of the total area of all peaks.

The identities of the volatile compounds were established by a GC-MS technique.

Results

1) Cultivar variation in monoterpene alcohol composition

The pentane-soluble volatiles produced immediately after blending tea shoots are shown in Fig. 1. The main volatiles present are trans-2-hexenal, linalool and geraniol. Trans-2-hexenal is produced from linolenic acid,[1] and linalool and geraniol are liberated from the bound-type non-volatile compounds, which are considered as glucosides.[3]

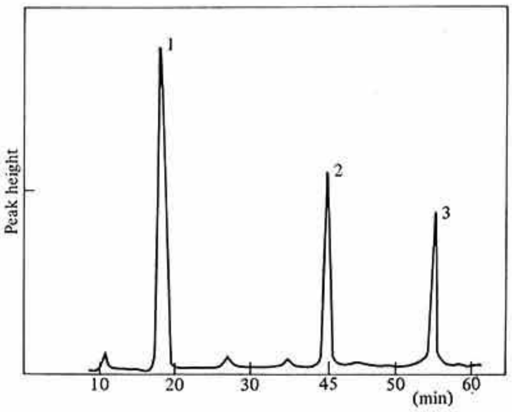

Fig. 1. GC analysis of volatiles in injured tea shoots
1. Trans-2-hexenal, 2. Linalool, 3. Geraniol.

The ratios of linalool content to the total monoterpene alcohol content (Terpene index) are shown for different cultivars in Table 1. The clones of var. *assamica* cultivars, which were imported from Malaysia and Sri Lanka and cultured in a greenhouse, have a terpene index of near 1. In contrast, clones of var. *sinensis* forma bohea, which were imported to Taiwan from Fuchien one hundred years ago and selected as clones for Oolong tea, (Shin-shin-Dār-Pan, Dâr-Yei-Wu-Lon, etc.)

Table 1. Clonal property in the production of monoterpene alcohol in tea shoots

Location	Variety	Clone	Terpene index
India	v. *assamica*	TV-2	1.00
	v. *assamica*	TV-17	1.00
	?	I-1	0.25
Sri Lanka	v. *assamica*	S-1	1.00
	v. *assamica*	S-21	1.00
	?	S-64	0.52
Malaysia	v. *assamica*	B-11	1.00
	v. *assamica*	B-17	1.00
	v. *assamica*	B-44	1.00
	v. *assamica*	B-45	1.00
	v. *assamica*	B-50	1.00
Japan Clones for black tea	Hybrid of v. *assamica* & *sinensis*	Benihomare	0.25
		I-131	0.35
		Izumi	0.35
		Benifuji	0.75
		Karabeni	0.43
		Tadanishiki	0.60
		Indo	0.35
		Satsumabeni	0.60
		Benikaori	0.65
		Hatsumomiji	0.75
Japan Clones for green tea	v. *sinensis*	Fujimidori	0.25
		Kurasawa	0.30
		Yaeho	0.30
		Asahi	0.45
		Tamamidori	0.46
		Asatsuyu	0.50
		Yabukita	0.55
		Yamakai	0.69
		Z-1	0.65
		Sayamamidori	0.70
China Chianghsi-Ningchou (Cn)	v. *sinensis*	Cn-6	0.16
		Cn-10	0.49
		Cn-14	0.47
Chechiang-Pingsuey (C, Cp)	v. *sinensis*	C-3	0.15
		C-7	0.10
		Cp-3	0.60
		Cp-13	0.44
		Cp-26	0.19
Anhwei-Keemun (Ck)	v. *sinensis*	Ck-19	0.83
		Ck-23	0.90
		Ck-24	0.48
Fuchien	v. *sinensis*	Fuchoow	0.23
		Ti-Kuan-Yien	0.80
Taiwan Clones for semi-fermented tea	v. *sinensis*	Chin-Shin Dār-Pan	0.05
		Hwan-Kuan	0.05
		Shi-Ran	0.05
		Dâr-Yei-Wu-Lon	0.10
		Teki-Shin	0.15

Terpene index:
Linalool ($\mu g/g$)/linalool+geraniol ($\mu g/g$)

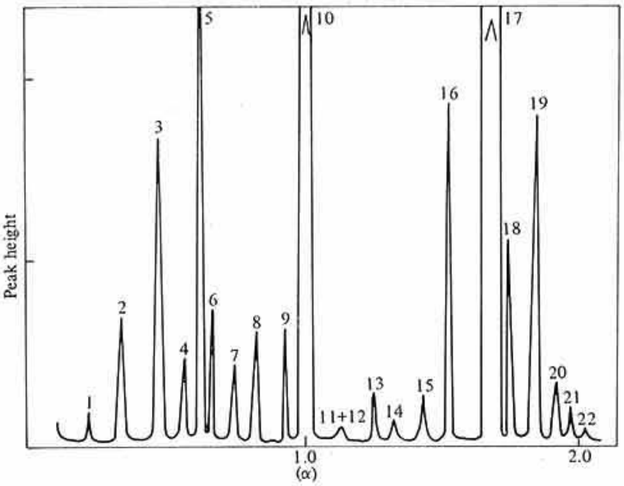

Fig. 2. Gas chromatograms of the aroma concentrate from black tea. (*Izumi*, hybrid of var. *assamica* and var. *sinensis*)
α: Ratio of retention time of each compound divided by the t_R of linalool.
Peak number: 1. 1-penten-3-ol, 2. trans-2-hexenal, 3. cis-2-penten-1-ol, 4. hexanol, 5. cis-3-hexenol, 6. trans-2-hexenyl formate, 7. linalool oxide furanoid-cis, 8. linalool oxide furanoid-trans, 9. benzaldehyde, 10. linalool, 11. t-3, t-5-octarien-3-ol, 12. unknown, 13. cis-3-hexenyl hexanoate, 14. unknown, 15. linalool oxide pyranoid-trans, 16. methylsalcylate, 17. geraniol, 18. benzylalcohol 19. 2-phenylethanol, 20. β-ionone + cis-jasmone, 21. unknown, 22. nerolidole.

have values of near 0.1. The domestic clones of Japan and the hybrids of var. *assamica* and var. *sinensis*, which were selected as clones for black tea in Japan, have intermediate values between these two extremes. Furthermore, the clones of var. *sinensis* cultivars in China mainland, which are planted in the National Research Institute of Tea, showed the wider distribution of terpene index.

From these results, it is thought that the terpene index shows the genetic characteristic of each tea cultivar.

2) Comparison of the black tea aromas

A typical volatile composition of black tea aroma is shown in Fig. 2. Among about 20 compounds, linalool and its oxides, geraniol, 2-phenylethanol, benzylalcohol and methyl salcylate are the important aromatic compounds in making flavory black tea aroma.

Of these compounds, the aroma pattern of linalool and its oxides and geraniol showed typical differences among black teas made from cultivars of different geographical locations, as shown in Table 2.

In black tea aromas made from cultivars of var. *assamica*, such as Assam teas, Uva and Dembura teas, the dominant high ratios of linalool and its oxides in the total aroma areas were recognized. On the other hand, Keemun tea made from a cultivar of var. *sinensis* forma bohea contained higher ratio of geraniol. Darjeeling tea and Yunnan tea showed intermediate profiles regarding the ratios of linalool and geraniol.

These results coincided with the monoterpene alcohol compositions found in blended tea shoots of different varieties.

Generally, black teas made from cultivars of var. *assamica* have sweet flowery aroma. This characteristic of the aroma may be related to the high ratio of linalool and its oxides to the total aroma pattern. On the contrary, Keemun tea has a heavy and rosy aroma. This is a peculiarity of China black tea which has a high ratio of geraniol in

Table 2. Genetic characteristics found on the aroma pattern of black teas

Country	Place	Variety	Clone	Linalool[a] & oxides	Geraniol[a]
Sri Lanka	Uva	var. *assamica*		38	1.3
	Dembura			28	2.2
India	Assam 1	var. *assamica*		30	3.3
	Assam 2		T V-17[b]	48	1.6
	Darjeeling 1	Hybrid of *assamica*		41	7
	Darjeeling 2	& *sinensis*		30	16
China	Yunnan 1	var. *sinensis*		44	12
	Yunnan 2	forma macrophlly		47	11
	Keemun	var. *sinensis* forma bohea		15	35
Japan	Shizuoka 1	var. *sinensis* forma bohea	C-7[c]	1.3	31
	Shizuoka 2	hybrid of *assamica* & *sinensis*	Benihomare[c]	26	22

a) Area (%) of linalool + its oxides and geraniol in total area.
b) TV-17: clone of Tocklai Experimental Station in Assam of India.
c) C-7 & Benihomare: clones of National Research Institute of Tea in Shizuoka of Japan.

the aroma pattern.

Yunnan tea made from cultivars of var. *sinensis* forma macrophylla showed a very similar flavor to Darjeeling tea. The both teas had intermediate ratios of terpene alcohols to the total aroma patterns as compared with black teas of var. *assamica* and var. *sinensis* forma bohea. These results may explain the similarity of tea aromas of Darjeeling tea and Yunnan tea.

These results on the aroma pattern of black tea show that the aroma profile of black tea relates to the genetic characteristic of tea plants.

Conclusion

In tea shoots injured by mechanical means, linalool and geraniol were liberated from the non-volatile compounds. The variations observed in the amounts of linalool and geraniol produced in the tea shoots were found to be genetically determined. The *assamica* variety produced mainly linalool while some special clones of the *sinensis* cultivars in Taiwan and Fuchien produced higher amounts of geraniol.

Typical differences in the ratios of linalool, its oxide and geraniol were also observed in the aroma patterns of volatile compositions prepared from black teas made from different cultivars of var. *assamica* and var. *sinensis*. The differences on aroma pattern were related to the genetical peculiarity which is responsible for the characteristic aroma profile of black tea.

References

1) Hatanaka, A. et al.: Biosynthesis of trans-2-hexenal in chloroplasts from *Thea sinensis*. *Phytochem.*, **15**, 1125–1126 (1976).
2) Saijô, R. & Takeo, T.: Volatile and non-volatile forms of aroma compounds in tea leaves and their changes due to injury. *Agr. Biol. Chem.*, 37, 1367–1373 (1973).
3) Takeo, T.: Production of linalool and geraniol by hydrolytic breakdown of bound forms in disrupted tea shoots. *Phytochem.*, **20**, 2145–2147 (1981).
4) Takeo, T.: Variation in amounts of linalool and geraniol produced in tea shoots by mechanical injury. *Phytochem.*, **20**, 2149–2151 (1981).

5) Takeo, T.: Clonal specificity of the monoterpene alcohol composition of tea shoots. *Nippon Nôgeikagaku Kaishi*, 56, 455–457 (1982) [In Japanese with English summary].
6) Takeo, T.: Variations in aromatic compound content in non-fermented and semifermented tea. *Nippon Nôgeikagaku Kaishi*, 56, 799–801 (1982) [In Japanese with English summary].
7) Takeo, T. & Mahanta, P. K.: Comparison of the black tea aromas between orthodox tea and CTC tea and among black teas made from different varieties. *J. Sci. Fd. Agr.*, 34, 307–310 (1983).

(Received for publication, January 25, 1983)