Mechanisms of Developing Black Tea Aroma with Special Reference to Alcoholic Compounds

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Tea aroma is one of the most important factors determining the quality of black tea. As comprehensive reviews on black tea flavor were already made in an excellent way by other authors[1-3], the present author intends to describe here the problem how the black tea aroma is developed. It is well known from the results of organoleptic test that the aroma of black tea is developed during the process of manufacturing and that the quality of the aroma is attributed to manufacturing methods and to the quality of fresh tea leaves. Therefore, good manufacture is necessary to obtain good quality of black tea. In the previous review[4] the author explained the mechanisms of producing the volatile carbonyl compounds having their origins from amino acids and fatty acids. However, the mechanisms in the formation of alcoholic compounds remained to be solved. In this paper the formation of volatile alcoholic compounds will be presented by referring the papers published already[5-7].

Preparation of volatile fraction

In estimating the quantity and quality of volatile compounds the method to prepare volatile fraction is most important. It was reported in the previous paper[5] that two methods, i.e., "chloroform-methanol extraction method" and "ether extraction method" were compared and that the former was more excellent than the latter. Therefore the "chloroform-methanol extraction method" was adopted in the following experiments. This method is described briefly as follows. Tea leaves were homogenized with chloroform-methanol mixture (1 : 1, v/v) in a Warling Blender and then filtrated. Some water was added to the extraction until the combination of water and methanol reached 40% of the whole solution, and the chloroform-soluble fraction was separated. After the chloroform fraction was concentrated, the concentrate was subjected to steam distillation in *vacuo* at 50°C of water bath. The volatile constituents were obtained in the distillate and extracted with diethyl ether, which was then concentrated to a small amount to be analyzed by gas chromatography.

Rapid increases of aliphatic, aromatic and terpenoid alcohols during black tea manufacture[5]

Aliphatic, aromatic and terpenoid alcohols were compared among fresh leaves, withered leaves, fermented leaves and stored leaves. The result is shown in Table 1. The amounts of aliphatic alcohols, such as *cis*-2-pentenol, hexanol, *cis*-3-hexenol and *trans*-2-hexenol, aromatic alcohols, such as phenylmethanol and 2-phenylethanol, and terpenoid alcohols, such as linalool, linalool oxide (*cis*, 5-membered), linalool oxide (*trans*, 5-membered), linalool oxide (*trans*, 6-membered), linalool oxide (*cis*, 6-membered), nerol and geraniol markedly increased during 16 hr withering process. The amounts of these compounds except hexanol, *cis*-3-hexenol and *trans*-2-hexenol continued to increase during 1 hr rolling and 1.5 hr fermentation processes. Such drastic changes in the amounts of these alcohols were not observed during the storage of tea leaves for 6 days at

Table 1. Changes in amounts of aroma compounds during black tea manufacture and storage

Peak No.	Aroma compound	Stage of manufacture			
		Fresh leaves[a]	Withered leaves[b]	Fermented leaves[c]	Stored leaves[d]
1	*cis*-2-Pentenol	0.4	20.0	50.7	4.2
2	Hexanol	0.2	89.0	24.0	3.3
3	*cis*-3-Hexenol	7.6	146.0	79.0	1.9
4	*trans*-2-Hexenol	trace	144.0	51.7	0.8
5	Linalool oxide (*cis*, 5-membered)	6.8	53.5	115.0	4.7
6	Linalool oxide (*trans*, 5-membered)	7.1	65.5	146.5	28.4
7	Linalool	16.1	38.0	66.0	11.0
8	Linalool oxide (*trans*, 6-membered)	9.2	37.0	42.3	12.2
9	Linalool oxide (*cis*, 6-membered)	3.1	11.2	17.8	2.2
10	Nerol	0.5	8.0	28.9	0.9
11	Geraniol	1.1 (0.1 μg)[e]	50.0 (4.2 μg)	227.5 (18.9 μg)	13.7 (1.1 μg)
12	Phenylmethanol	23.1	155.0	371.0	21.4
13	2-Phenylethanol	16.9	355.0	1,540.0	52.2
14	Unidentified	18.3	6.6	trace	10.3
15	Nerolidol	trace	trace	trace	0
16	Unidentified	89.0	125.0	60.0	56.0
17	Unidentified	123.0	24.5	18.3	24.0

Notes (1) Each figure indicates the peak area (cm^2) on gas chromatogram which is obtained from the concentrated ether solution corresponding to 10 g fresh tea leaves.

(2) a) The clone used was *Benihomare* harvested in September.

b) The leaves were allowed to wither on a net for 16 hr at room temperature (average, 21°C). The weight of withered leaves was 57% that of the original leaves.

c) The withered leaves were rolled for 1 hr with a rolling machine and allowed to ferment for 1.5 hr at room temperature (23°C) after spreading to a depth of about 2 cm.

d) The storage condition was at 5°C for 6 days in a polyethylene bag.

e) Calculated weight from the calibration curves of geraniol.

5°C. These results indicate that the increase of the alcohols is ascribed to the process of black tea manufacture.

Influences of mechanical and chemical injuries on the amounts of the alcohols[5)]

Tea leaves were macerated for 1.5 hr by a mortar machine, or they were treated with monoiodoacetate or malonate for 2 days, and then the volatile alcohols were checked by the same way. As shown in Table 2 amounts of most of the aliphatic, aromatic and terpenoid alcohols with some exceptions increased remarkably by the treatments causing mechanical and chemical injuries. In the experiment, the extent of injury caused by these treatments was different each other, so that the extent of the increase of the alcohols differed with the method of treatment. However, it might be concluded, at least, that these alcohols were produced when fresh tea leaves suffered from any kinds of stimulations or injuries.

Time course of the increases of the aliphatic, aromatic and terpenoid alcohols after mechanical injury[5)]

Time course of the increases of the alcohols was investigated for 5 hr after maceration.

Table 2. Changes in amounts of aroma compounds by various treatments

Aroma compound	Fresh leaves[a]	Normally fermented leaves[b]	Mechanically injured leaves[c]	Chemically injured leaves[d]	
				Monoiodo-acetate	Malonate
cis-2-Pentenol	1. 0	29. 3	9. 7	5. 0	3. 5
Hexanol	trace	43. 1	8. 7	22. 7	5. 4
cis-3-Hexenol	6. 3	220. 5	51. 0	18. 9	6. 3
trans-2-Hexenol	0. 2	94. 7	2. 0	1. 1	0. 5
Linalool oxide (*cis*, 5-membered)	2. 8	126. 0	72. 0	61. 0	37. 5
Linalool oxide (*trans*, 5-membered)	7. 7	312. 0	231. 0	136. 0	108. 0
Linalool	10. 8	95. 6	49. 0	23. 4	11. 7
Linalool oxide (*trans*, 6-membered)	1. 7	12. 8	16. 8	12. 8	8. 8
Linalool oxide (*cis*, 6-membered)	14. 2	41. 7	61. 4	35. 7	24. 3
Nerol	0. 6	8. 0	20. 2	2. 7	1. 8
Geraniol	5. 9 (0. 5 μg)	215. 6 (17. 9 μg)	155. 8 (12. 9 μg)	33. 2 (2. 8 μg)	23. 5 (2. 0 μg)
Phenlymethanol	14. 1	197. 5	495. 0	59. 7	33. 0
2-Phenylethanol	37. 5	891. 0	1, 106. 0	126. 8	109. 3
Nerolidol	5. 2	9. 0	4. 2	trace	trace

Notes (1) Each figure is expressed by the same way as in Table 1.

(2) a) The clone used was *Benifuji* harvested in October.

b) The leaves were withered for 16. 5 hr, rolled for 1. 0 hr, and fermented for 1. 5 hr at room temperature (average, 17°C).

c) The leaves were macerated for 1. 5 hr by using a mortar machine and allowed to ferment after spreading to a depth of about 2 cm.

d) 10^{-2} M Monoiodoacetate (or 10^{-1} M malonate) solution was absorbed from stem into the leaves under fluorescent light and wind produced by a fan. During 2 days absorption, the leaves gradually changed in color from green to brown.

Table 3. Comparison of radioactivities in lipids and volatile fractions between intact and injured seedlings

	radioactivity incorporated (dpm)		
	$CHCl_3$-soluble Fraction 1	MeOH-H_2O-soluble Fraction 2	Volatile fraction in (1)
Intact seedlings	13.1×10^6	27.1×10^6	3, 430
Injured seedlings	9.9×10^6	16.6×10^6	51, 240

The results are shown in Fig. 1. *cis*-2-Pentenol, hexanol, *cis*-3-hexenol and linalool increased markedly at an earlier stage after maceration, and followed by a slight decrease. Other aromatic and terpenoid alcohols, such as linalool oxide (*cis*, 5-membered), linalool oxide (*trans*, 5-membered), geranoil, phenylmethanol and 2-phenylethanol continued to increase during the period tested.

Experiments using radioactive compounds[7]

Etiolated tea seedlings grown for 2 months were exposed to radioactive $^{14}CO_2$ for 4 days in the light, and thereafter grown for 1 month under 10,000 lux light intensity in order to

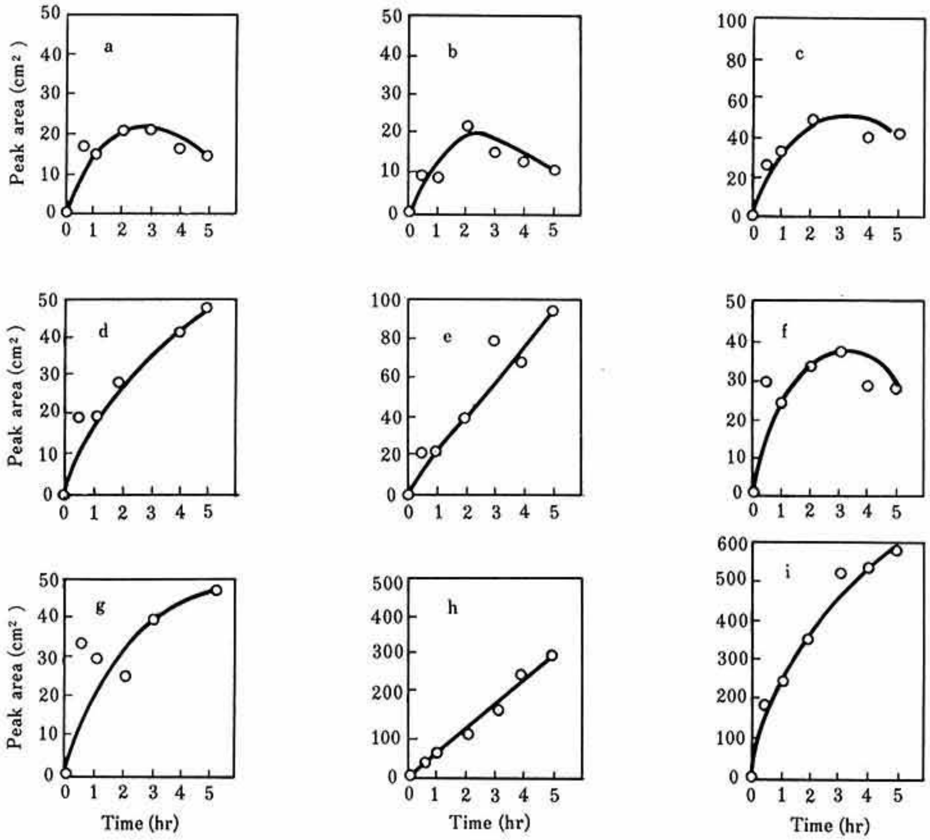

Fig. 1. Time course of the increases of aroma compounds in tea leaves aftere maceration

The clone used was *Benifuji* harvested in February. Tea leaves were macerated for 0.5 hr by using a mortar machine at room temperature (6°C), and then allowed to undergo fermentation at 12°C.

a, *cis*-2-pentenol; b, hexanol; c, *cis*-3-hexenol; d, linalool oxide (*cis*, 5-membered); e, linalool oxide (*trans*, 5-membered); f, linalool; g, geraniol; h, phenylmethanol; i, 2-phenylethanol. In the leaves only a small amount of *trans*-2-hexenol was detected, but linalool oxide (*trans*, 6-membered), linalool oxide (*cis*, 6-membered) nerol, and nerolidol were not detected.

metabolize the incorporated $^{14}CO_2$. The volatile fractions were prepared from both the greened tea seedlings and the mechanically injured seedlings by the same method mentioned above. Radioactivities in the chloroform-soluble fraction and its volatile fraction were measured by a liquid scintillation spectrometer. As shown in Table 3, remarkably high radioactivity was observed in the volatile fraction of injured seedlings compared with that of intact seedlings. The volatile fractions were subsequently analyzed by gas chromatography (Table 4), and it was made clear that the most radioactivities were found in the peaks corresponding to both *cis*-3-hexenol and *trans*-2-hexenol in the fraction of injured seedlings. Little radioactivity was detected in the peak of aromatic and terpenoid alcohols.

Problems further to be solved

1) It is generally believed that *cis*-3-hexenol, so-called leaf alcohol, exists in intact leaves from many natural sourecs[8], but our

Table 4. Gas chromatographic separation of the concentrated volatile fractions obtained from intact and injured seedlings

Effluent fraction (retention time in min)	Compound	Intact seedlings			Injured seedlings		
		Radio-activity (dpm)	Recovery rate of unlabelled compounds (%)	Total radio-activity (dpm)	Radio-activity (dpm)	Recovery rate of unlabelled compounds (%)	Total radio-activity (dpm)
0–9.8		16	—	—	304	—	—
9.8–11.1	*cis*-3-Hexen-1-ol	6	6.10	98	928	5.43	17,090
11.1–13.4	(*trans*-2-Hexen-1-ol)	0	—	—	576	—	—
13.4–18.5		9	—	—	11	—	—
18.5–20.8	Linalool	0	9.90	0	17	7.21	236
20.8–38.7		2	—	—	43	—	—
38.7–44.0	Nerol	2	11.78	17	4	8.42	48
44.0–48.0	Geraniol	2	13.80	14	2	9.74	21
48.0–51.0	Phenylmethanol	0	3.38	0	10	2.18	459
51.0–55.0	2-Phenylethanol	0	4.87	0	1	2.83	35
55.0		3	—	—	29	—	—

observation indicates that in intact tea leaves only small quantity of *cis*-3-hexenol is contained in its form and that the alcohol is released or produced from an unknown form of the alcohol or an unknown precursor when the leaves are injured. What mechanisms are involved in the alcohol formation due to injuries?

2) Aromatic and terpenoid alcohols themselves or their precursors are not synthesized from radioactive $^{14}CO_2$ in tea seedlings during the 1 month growth under weak light intensity. What kinds of growth conditions and how long growth period are necessary for tea plant to form these alcohols themselves or their precursors? It is presumed that the mechanisms of producing volatile alcohols might be related to enzymatic reactions. What are the essential factors determing the quantities and qualities of the alcohols in black tea, the precursor or enzyme activities?

3) Terpenoid alcohols are very important factors for determining the quality of black tea. How can we regulate or increase these alcohols by the manufacturing process or the cultivation condition? How high-grown and low-grown tea are different in fresh tea leaves?

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