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Anaerobically Treated Tea and Its Hypotensive Effects

Katsuhiro HAKAMATA

Abstract

The present paper deals with anaerobically treated tea called "Gabaron tea". In the tea leaves stored under anaerobic conditions, γ-aminobutyric acid (GABA) and alanine increased considerably, while glutamic acid and aspartic acid decreased. Based on the result of 15N-glutamic acid feeding experiment for tea leaves, it is presumed that glutamic acid may be a source of nitrogen for the increased GABA and alanine. Gabaron tea is manufactured from green tea leaves which are stored under anaerobic conditions to increase GABA, a hypotensive compound. Although Gabaron tea has an undesirable odor, the major taste component of green tea shows small differences. When Gabaron tea is orally given to spontaneously hypertensive rats, their blood pressure decreased significantly as compared with the common green tea and the deionized water group. It is concluded that anaerobic conditions under appropriate time and temperature provide an improved tea containing a large amount of GABA with a relatively good quality. It is estimated that an adequate firing with 110-120□ is effective in reducing the undesirable odor of the Gabaron tea.

Discipline: Tea industry

Additional key words: firing, Gabaron tea, glutamate metabolism, γ-aminobutyric acid (GABA), spontaneously hypertensive rats (SHR)

Introduction

An increased interest in natural foods in recent years has stimulated consumers in Japan to take more natural healthy materials for diet. It is known that the tea, which has been used in medical purposes for a long time, contains pharmacological components. Under such background, attempts have been made in Japan to develop a method for enriching physiologically active components by taking advantage of inherent chemical ingredients and physiological functions of tea so that the healthy effects of tea could be further increased.

Tsushida et al.[8] found that a large amount of γ-aminobutyric acid (GABA) could be accumulated in the green tea leaves which had been plucked and kept under anaerobic conditions for some time. They manufactured green tea, oolong tea and black tea from those tea leaves to chemically analize the properties of each product[9]. Ohmori et al.[3] orally gave the green tea thus obtained to spontaneously hypertensive rats (SHR) everyday. The result indicated that there was an obvious effect of the green tea in suppressing the increase in blood pressure. The author attempted to improve the production process of this type of green tea, which had an undesirable odor specific to anaerobically treated teas, with the purpose of giving a better taste[1,2]. The present paper reviews the results of those studies which dealt with the anaerobically treated tea, called Gabaron tea.

Formation of γ-aminobutyric acid (GABA) in tea leaves

GABA is formed in a plant which is stored under anaerobic conditions[6,7]. GABA is a non-protein constituting amino acid, which widely exists both in animals and plants. It plays an important role in

Department of Tea Processing Technology, National Research Institute of Vegetables, Ornamental Plants and Tea (Kanaya, Shizuoka, 428 Japan)

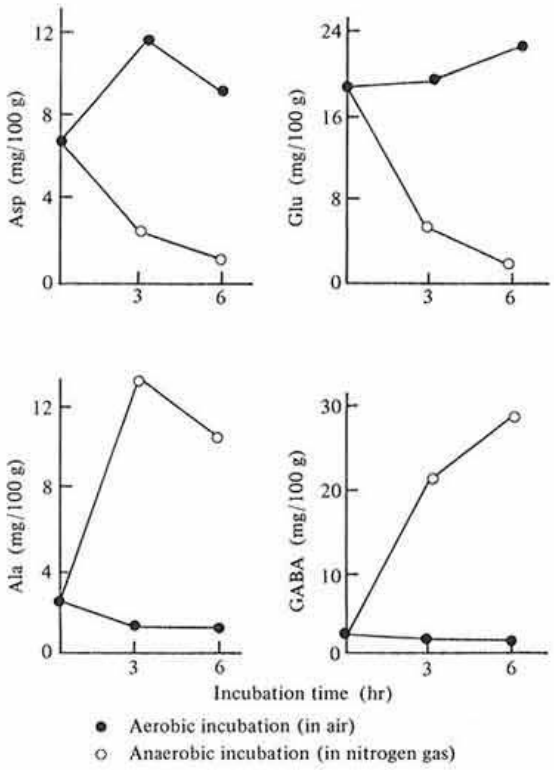


Fig. 1. Contents of amino acids in tea leaves during the aerobic and anaerobic incubation[8]

higher animals for neuro-transmission.

In their study on glutamate metabolism in tea leaves, Tsushida et al. found that in the tea leaves which had been stored under anaerobic conditions, GABA and alanine were considerably increased, while glutamic acid and aspartic acid were decreased (Fig. 1)[8]. In regard to the atom percentages and the amounts of ^{15}N in 8 major amino acids in the tea leaves, their changes were further subjected to analysis during the aerobic and anaerobic incubation period after treatment with ^{15}N-glutamic acid for 6 hr. The result showed that the atom percentages of ^{15}N in GABA and alanine increased considerably after 6 hr of incubation under the anaerobic condition. This indicates that the nitrogen source of these amino acids each showing an increase was glutamic acid (Table 1).

Under their analysis on six enzymes relating to the glutamate metabolism, Tsushida et al. identified that a glutamic acid decarboxylase had the lowest optimum pH value, i.e. 5.8[8]. Considering that the pH value of cells would be lowered under anaerobic conditions, it was presumed that the above enzyme would more advantageously affect the formation of GABA, compared with other enzymes. They purified the glutamic acid decarboxylase, demonstrating that this enzyme was activated by pyridoxal phosphate and reacted only L-glutamic acid as a substrate.

Manufacture of anaerobically treated tea and its characteristics

As described earlier, GABA increased in the green tea leaves which had been stored under anaerobic conditions. Stanton showed that GABA was effective in loweirng blood pressure[5]. Therefore, it would be expected that a new type of tea having a strengthened healthy effect could be obtained by producing a tea from green tea leaves containing a large amount of GABA, and that its consumption could be increased in the future.

Tsushida et al. kept plucked tea leaves in various gases at a room temperature for 5 to 10 hr and monitored changes in the amino acid contents in the tea leaves (Table 2)[9]. Nitrogen- and carbon dioxide-gas lots each showed a considerable decrease in aspartic acid and glutamic acid as well and a significant increase in alanine and GABA; while in air and oxygen lots, the results obtained were completely contrary to those changes. After 10 hr treatments under those gases, the GABA contents in the nitrogen- and carbon dioxide-gas lots were about 8 and 10 times as much as those in the air lot, respectively. This fact indicates that more GABA could be obtained under an anaerobic treatment. On the other hand, theanine which is the major taste component of green tea, as well as caffeine and tannin, which are both typical ingredients of green tea, showed little changes (Table 3)[9].

Based on these findings, Tsushida et al.[9] manufactured green tea, oolong tea and black tea from the tea leaves which had been anaerobically treated under nitrogen gas for 6 hr. Although each of these products had an undesirable odor specific to anaerobically treated teas, the contents of GABA remarkably increased, i.e. from 150 to 200 mg/100 g. On the other hand, the contents of glutamic- and aspartic-acid decreased, while no changes were seen with theanine contents. When the green tea leaves

Table 1. Atom percentages of ^{15}N and amounts of ^{15}N in amino acids in tea leaves during aerobic and anaerobic incubation after feeding ^{15}N-glutamic acid for 6 hr[8]

Incubation time (hr)	Asp	Asn	Glu	Gln	Ser	Ala	GABA	Theanine
				Aerobic incubation				
0 Atom%	17.71	7.74	47.07	12.17	11.54	13.57	—	0.48
0 ^{15}N (μmol/g)	1.80	0.21	9.48	0.67	0.65	0.46	—	0.14
3 Atom%	16.24	10.07	36.15	13.93	—	12.05	—	0.51
3 ^{15}N (μmol/g)	1.79	0.39	7.11	0.83	—	0.42	—	0.14
6 Atom%	9.64	7.35	31.32	12.68	5.75	5.74	—	0.44
6 ^{15}N (μmol/g)	1.02	0.59	6.20	0.95	0.58	0.24	—	0.12
12 Atom%	12.90	6.43	24.56	5.39	4.89	2.27	—	0.43
12 ^{15}N (μmol/g)	0.68	1.37	4.52	0.47	0.78	0.21	—	0.11
				Anaerobic incubation				
3 Atom%	6.57	8.53	28.06	10.24	9.08	11.46	9.91	—
3 ^{15}N (μmol/g)	0.03	0.22	0.97	0.26	0.55	2.42	1.50	—
6 Atom%	5.95	9.06	29.89	8.20	11.61	18.87	18.48	0.82
6 ^{15}N (μmol/g)	0.02	0.27	0.76	0.21	0.63	3.64	3.49	0.28
12 Atom%	1.67	2.77	6.15	2.94	2.87	5.11	13.22	0.33
12 ^{15}N (μmol/g)	0.04	0.10	0.60	0.13	0.14	0.86	4.53	0.09

—: Not determined.

Table 2. Contents of amino acids in tea leaves during the incubation in various gases[9]

Amino acid	Amino acid (mg%)							
	Air		Oxygen		Nitrogen		Carbon dioxide	
	5 hr	10 hr	5 hr	10 hr	5 hr	10 hr	5 hr	10 hr
Asp	149.6	176.7	113.6	237.6	11.7	2.0	3.7	8.6
Glu	133.6	236.3	140.4	148.0	4.8	8.0	4.3	5.7
Ala	14.6	40.1	13.7	44.2	165.1	123.1	67.8	58.6
Ser	42.1	115.7	52.8	77.6	39.9	36.8	29.9	31.1
Asn	10.0	62.4	13.0	65.8	13.8	12.6	10.8	12.8
Gln	24.5	37.4	43.2	77.1	13.3	15.2	9.4	11.7
Theanine	309.8	327.2	343.6	334.4	389.9	334.4	290.6	320.1
Arg	3.0	6.0	4.5	11.9	18.8	10.3	14.3	19.7
GABA	12.7	28.5	4.0	12.7	173.9	233.9	180.2	290.9

were anaerobically treated prior to withering in the production of oolong tea and black tea, GABA decreased considerably during the withering (Table 4). Tsushida et al. named this tea "Gabaron tea" after GABA.

The aromatic components of this tea were analyzed by a gas chromatography[9]. The result indicated that the contents of methyl and ethyl esters of fatty acids such as ethyl palmitate, methyl linoleate and methyl linolenate increased to a greater extent, compared with common green tea. However, it was reported that the aroma of these compounds differed from predominant aroma of the Gabaron tea. Although some stuides on the aromatic components of the Gabaron tea have been conducted thereafter, the mechanism of the undesirable odor of the Gabaron tea has not been identified yet.

The Gabaron tea is produced from green tea leaves which are stored for some time under anaerobic conditions to increase GABA content. It is recognized to be effective in lowering blood pressure and thus highly valued as a healthy drink. Any tea leaves, regardless of variety or season, are available for producing the Gabaron tea, as far as they are rich

Table 3. Contents of caffeine and tannin in tea leaves during the incubation in various gases[9]

Compounds	Incubation time (hr)	Caffeine and tannin content (%)			
		Air	Oxygen	Nitrogen	Carbon dioxide
Caffeine	5	2.7	2.7	2.5	2.7
	10	3.0	2.7	2.7	2.5
Tannin	5	14.3	13.9	14.5	15.0
	10	15.0	14.0	14.5	14.2

Table 4. Contents of amino acids in oolong tea and black tea made from the tea leaves incubated in nitrogen gas[9]

Tea	Amino acid (mg/100 g)								
	Asp	Glu	Ala	Ser	Asn	Gln	Theanine	Arg	GABA
Oolong 1	85.7	47.1	63.3	55.7	17.5	95.4	491.1	15.4	135.9
Oolong 2	39.1	46.2	58.5	44.3	24.0	42.6	451.1	16.5	245.9
Black 1	95.4	176.1	31.4	64.2	9.6	45.4	411.2	21.6	81.2
Black 2	29.5	22.4	88.7	44.3	22.6	25.0	377.0	12.9	176.5

Oolong 1 and black 1 were made from the tea leaves incubated in nitrogen gas before withering, and oolong 2 and black 2 were made from those tea leaves after withering.

in glutamic acid. Therefore, green leaves of the inexpensive second or third harvest can be processed to produce Gabaron tea, which gives an additional economic value.

Effect of Gabaron tea on blood pressure

Stanton reported that GABA was effective in lowering blood pressure[5].

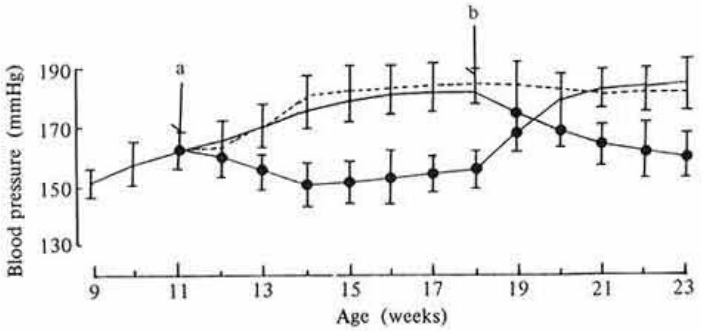
Ohmori et al. examined the Gabaron tea (green tea) produced by Tsushida et al.[9] regarding its effects on the blood pressure of the SHR, and found its positive effects (Fig. 2)[3]. More specifically, male rats of the SHR at the age of 9 weeks were divided into 3 groups, each having 8 animals and deionized water, common green tea and the Gabaron tea were administered to these groups, respectively. The Gabaron tea infusion was prepared by extracting 50 g of the Gabaron tea leaves in 1 *l* of deionized boiling water for 1 min.

During the testing period, the SHR in the Gabaron tea group showed a growth process almost comparable to that in the deionized water and common green tea groups. No significant difference in body weight gain was observed among these groups.

The Gabaron tea was given at the 10 week-age when the increase in blood pressure was not clearly identified, and after 20 week-age when the blood pressure had increased remarkably. In each case, the blood pressure decreased by 25 to 30 mmHg, compared with the control group (deionized water) and the common green tea group. However, when the administration of the Gabaron tea was stopped, the blood pressure rapidly increased and reached almost the same level of the deionized water and common green tea groups within 2 to 3 weeks. These results suggest that the Gabaron tea have an obvious effect for lowering blood pressure, though its mechanism is still to be identified yet. The GABA might presumably be the major factor of the above-stated effect.

Stanton[5] indicated the amount of GABA necessary for lowering the blood pressure of an animal by 20% when injected; namely, 11 μg/kg for dog, 19 μg/kg for rabbit, 174 μg/kg for pig and 96 mg/kg for cat. This variation indicates that the sensitivity for GABA varies among the animals. Therefore, the effective dose for man could not be specified unless clinical tests are conducted. Ohmori et al. examined this subject, reporting that the Gabaron tea was clinically effective[4]. However, further studies are required to disclose the mechanism of lowering blood pressure by the GABA as well as by the Gabaron tea.

The Gabaron tea is not effective in lowering blood



a: SHR are divided into three groups.
b: Feeding stuff was exchanged between the control group (fed on ordinary green tea) and the experimental group.
---- Control SHR fed by water
—— SHR fed by ordinary green tea
-●- SHR fed by Gabaron tea
Mean of 8 determinations ± S. D.

Fig. 2. The effect of Gabaron tea extract on systolic blood pressure of SHR[3)]

Table 5. Effects of temperature on the quality of anaerobically treated tea leaves[1)]

Temperature (°C)	Color of steamed leaf (score)			Offensive odor			Offensive taste		
	>15	10–15	<10	Few	Slight –Moderate	Strong	Few	Slight	Moderate –Strong
	hr	hr	hr	hr	hr	hr	hr	hr	hr
10	<25	30–40	–	<15	20–35	40	<15	20–35	40
20	5	10–20	25	< 2	5–10	>15	< 5	10	>15
30	2	5	10	< 2	5	>10	–	2	> 5

Table 6. Effects of heating on the quality of Gabaron tea[2)]

Heating (°C, 30 min)	Offensive odor	Offensive taste	Color value a*	GABA (mg%)
Control	Strong	Strong	−5.89	182.1
100	Slight	Slight	−5.40	154.9
110	Slight	Slight	−4.67	163.0
120	Slight	A little	−3.90	146.0
130	Few (strong roasted flavor)	Few (roasted taste)	−3.12	121.0
140	Few (strong roasted flavor)	Few (roasted taste)	−2.26	78.8

a*: CIE (1976) L*a*b*.

pressure rapidly and drastically, as the case in conventional hypotensors. Therefore, it is recommended to use the Gabaron tea not as a substitute for a drug but as a daily drink for health.

Improvement in aroma and taste of Gabaron tea

The original Gabaron tea had a disadvantage in

terms of its odor characteristic, which was not acceptable for drinking. This was a serious barrier to its commercialization.

A study was undertaken by the author and his team to identify suitable conditions for the anaerobic treatment in order to improve the aroma and taste of the Gabaron tea (Table 5)[1]. The materials were subjected to exposure under different temperatures (10, 20, 30°C) and periods (0–40 hr). The result indicated that under a longer treatment period and a higher temperature, the quality deteriorated considerably and the offensive odor were more nasty. It was also found that the GABA content showed a rapid increase during the early stage of the anaerobic treatment (1 to 5 hr) under each temperature, a greater increment took place under a higher temperature. From this result, it was concluded that the GABA content of the Gabaron tea could be increased by minimizing the deterioration of its quality under the anaerobic treatment of the tea leaves at either 10°C for 15 to 20 hr, 20°C for 5 to 10 hr or 30°C within 2 hr. Such treatments under an anaerobic condition enables to obtain an improved tea containing a large amount of GABA with a relatively good quality. Another experiment followed the above study to examine an adequate firing (*hiire* in Japanese) in order to relieve the offensive odor of the Gabaron tea (Table 6)[2]. In the production processing of green tea, a firing is employed in the refining stage to improve the aroma and taste of the product. Under the treatment where the Gabaron tea was heated at 110–120°C, the offensive odor became much lighter, though the GABA content decreased by 10–20%. The taste and aroma of the tea product thus obtained was acceptable for drinking. Therefore, it is concluded that a firing is effective in improving the quality of the Gabaron tea. When the Gabaron tea was heated at 130–140°C, the offensive odor was relieved completely, but the GABA content and qualities of the tea were considerably lowered.

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