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Comparison of bioactive components in GABA tea and green tea produced in Taiwan

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Abstract

The aim of this study is to investigate the bioactive components of GABA (γ-aminobutyric acid) tea as compared with green tea produced in Taiwan. Using in total 56 tea samples (28 green tea and 28 GABA tea), moisture content, Hunter *L*, *a* and *b* values, phenolic compounds, amino acids including GABA, fatty acids and ascorbic acid were determined. The results showed that moisture, total free amino acids, crude fat, Hunter *L* value, total nitrogen, free fatty acids and reducing sugar did not differ significantly between GABA tea and green tea. However, GABA tea had higher Hunter *a* and *b* values, while green tea had higher total catechin and ascorbic acid contents ($p < 0.05$). Of major catechins, epicatechin and epigallocatechin gallate were found to be lower in GABA tea than in green tea. For free amino acids, GABA, alanine, ammonia, lysine, leucine and isoleucine were found to be significantly higher in GABA tea, while the glutamic acid, aspartic acid, and phenylalanine were higher in green tea ($p < 0.05$). Theanine, tryptophan, valine, threonine and methionine were not found to be different between the two kinds of tea.

Keywords: GABA tea; Green tea; Physicochemical analyses; GABA; Glutamic acid

1. Introduction

γ-Aminobutyric acid (GABA) is an amino acid, $C_4H_9NO_2$, which is not present in proteins, but is known to be one of the major inhibitory neurotransmitters in the sympathetic nervous system and to play an important role in cardiovascular function (Takahashi, Tiba, & Iino, 1955). GABA is widely distributed and, together with alanine, has been reported to accumulate in plants (Millin & Rustidge, 1967; Streeter & Thompson, 1972a, 1972b; Tsushida & Murai, 1987), Chlorella (Lane & Stiller, 1970) and mammalian tissues (Wood & Watson, 1969) under anaerobic conditions. Using ^{14}C-glutamic acid to study the accumulation of GABA and alanine in radish leaves (*Raphanus sativus* L.), Streeter and Thompson (1972a, 1972b) clarified the mechanism responsible for the accumulation. Saikusa, Horino, and Mori (1994) also demonstrated the accumulation of GABA in rice.

In Japan, Tsushida, Murai, Omori, and Okamoto (1987) accidentally found that a large amount of GABA accumulated in green tea under anaerobic condition. They examined further the GABA content of green, oolong and black tea made under anaerobic condition and found that GABA accumulated in all teas. Due to GABAs being reported to reduce blood pressure in experimental animals (Omori et al., 1987; Stanton, 1963) and humans (Elliott & Hobbiger, 1959), GABA tea was produced on a commercial basis for people with hypertension. Further researches demonstrated that GABA was also able to reduce the blood pressure in experimental animals (Abe et al., 1995; Hakamata, 1990; Lin et al., 2000).

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GABA tea is now being produced in Taiwan. Green tea has been well known as bioactive drink due to its high content of catechins (Balentine, Wiseman, & Bouwen, 1997; Dufresne & Farnworth, 2000; Fujiki et al., 1992; Ohe, Marutani, & Nakase, 2001; Yeng & Chen, 1994; Yeng & Chen, 1996). The aim of this study is to compare the levels of bioactive compounds in GABA tea and green tea made with different varieties, in different seasons and in different production areas in Taiwan to compare the differences between the bioactive compounds in these two kinds of tea.

2. Materials and methods

2.1. Materials

Fifty-six tea samples, including 28 green teas and 28 GABA teas, were collected and analysed. They were made with cultivars of Chin-Shin Oolong (CSOolong), Shy-Jih-Chue (SJChue), Taiwan Tea Experiment Station No. 12 (TTES 12) and Taiwan Tea Experiment Station No. 13 (TTES 13), in the summer and spring seasons, and in the production area of Tao-Chu-Miao (TCM) and Min-Jian, LuKu and Jia-Yi. For GABA tea, fresh young leaves after picking were put into a nitrogen-filled chamber for 8 h and then shaken continuously under aerobic conditions for 3–4 h. These two steps were repeated twice (i.e., 22–24 h total), followed by a further anaerobic fermentation (8 h), prior to blanching, rolling and drying (Tsai, Juan, & Chang, 1995; Tsushida et al., 1987). For green tea, young leaves were simply submitted to blanching and drying. All samples were stored in a cold and dark place and pulverized by mill, screened through a 20-mesh sieve and mixed thoroughly before use. The entire experiment was done in triplicate.

2.2. Analysis of moisture content

Two grams of samples was weighed into a weighing bottle and dried in a moisture analyser (HR73 Moisture Analyzer, METTLER TOLEDO, Switzerland) at 105 °C to constant weight (Iwasa, 1975).

2.3. Analysis of total nitrogen

The Kjeldahl method was used for the analysis of total nitrogen (Iwasa, 1975).

2.4. Analysis of colour difference of tea extracts

Each sample (3 g) was infused with 150 ml boiled water for 5 min. After quick cooling by ice water to below 10 °C, the sample was filtered through a filter paper (Whatman No. 1). The colour parameters (Hunter *L*, *a*, *b* values) were measured with a Hunterlab SAV colorimeter (Σ80 Color measuring system, NIPPON DENSHOKU IND, CO., Japan). The white plate supplied with the Hunterlab SAV colorimeter was used as background. The sample was scanned six times to get the mean Hunter *L*, *a*, *b* values, using distilled water as reference. This method was according to Liang, Lu, Zhang, Wu, and Wu (2003).

2.5. Analysis of total catechins

Total catechin content of each extract was analysed by the ferrous tartrate method (Chen & Chen, 1995; Sakanaka & Yamamoto, 1997, chap. 9). A calibration curve was prepared and the results were presented as percentage of gallic acid equivalents (GAE per cent).

2.6. Analysis of total free amino acids

Total free amino acids were analysed using nihydrin. A calibration curve was prepared, and the results were presented as theanine equivalents per cent (Co & Sanderson, 1970; Iwasa, 1975).

2.7. Analysis of reducing sugar

Reducing sugar was analysed with the dinitrosalicylic acid (DNS) reagent, using D-glucose as a standard, both being obtained from Sigma Chemical Co., St. Louis, MO (Anan, Takayanagi, & Ikegaya, 1984). A calibration curve was prepared and the results were expressed as D-glucose equivalents per cent.

2.8. Analysis of ascorbic acid

Ascorbic acid analysis was carried out by HPLC (Hitachi Corp., Japan), using a reverse phase C-18 column (Phenomenex, Torrance, CA, 300 mm × 4.6 mm × 5 μm) and a mobile phase of 0.1% trifluoroacetic acid in water. The flow rate was 1 ml min^{-1}, the injected volume was 10 μl, and a UV detector (Model L-4000 UV Detector) was set at 254 nm. All data were compared to a standard curve of pure ascorbic acid (Iwasa, 1975).

2.9. Analysis of caffeine and catechins

Ground sample (1 g) was added to 90 ml boiled water, heated on a water-bath at 80 °C for 20 min. After cooling and filtering, the volume was made up to 100 ml with distilled water. The caffeine and catechin concentration were then measured by HPLC (Hitachi Corp., Japan) with a reverse phase C-18 column (Phenomenex, Torrance, CA, 300 mm × 4.6 mm × 5 μm) and the UV detector operating at 280 nm. Pure caffeine, catechin (C), epicatechin (EC), gallocatechin (GC), epigallocatechin (EGC), epicatechin gallate (ECG) and epigallocatechin gallate (EGCG), purchased from Sigma Co., were used as

standards (Goto, Yoshida, Kiso, & Nagashima, 1996; Liang et al., 2003; Sakanaka & Yamamoto, 1997, chap. 9).

2.10. Analysis of fat and free fatty acids

Fat was extracted by Soxhlet extraction (Soxtec System HT 1043, Extraction Unit, Tecator Co.) with petroleum ether and together with free fatty acids were analysed according to Chen and Chen's method (1995).

2.11. Analysis of amino acids

One gram of the tea sample was added to 90 ml boiled water in water-bath at 80 °C for 1 h. After filtering, the extract was freeze-dried. The amino acids were analysed on a Beckman amino acid analyser with a column (6-cm long, 4.6-mm i.d.) filled with P/N 855-3501 ion-exchange resin (Beckman Co., Ltd., USA). Norleucine was used as internal standard (Ericson, Wictorin, & Lundberg, 2002; Saikusa et al., 1994).

2.12. Statistical analyses

All data were analysed by the *t* test of Matched Pairs Procedure of SAS (SAS Institute Inc., Cary, NC, USA) statistical software (version 8.2, 2004) and compared at $\alpha = 0.05$ level.

3. Results and discussion

3.1. Physicochemical properties of GABA tea and green tea

The physicochemical properties of GABA and green tea samples are shown in Table 1. The contents of moisture, total free amino acids, total nitrogen, reducing sugar and crude fat did not differ significantly between GABA and green teas ($p > 0.05$). The total catechin and ascorbic acid contents of GABA tea were significantly lower than those of green tea ($p < 0.05$). In general, the moisture content of Taiwan-made tea was kept below 5%. Tsai, Chen, and Chang (1990) reported that the nitrogen content of tea leaf was not affected significantly by processing (harvesting and fermentation). Anan (1983) reported that fat and reducing sugar of green tea fell at the end of processing. In this study, the fat and reducing sugar of GABA tea with anaerobic fermentation did not differ significantly from that of green tea.

Table 1
Physicochemical properties of GABA tea and green tea

Properties	Green tea	GABA tea
Moisture	4.44 ± 0.92[a1]	4.35 ± 1.17[a]
Ascorbic acid	0.92 ± 0.21[a]	0.58 ± 0.34[b]
Total free amino acids	1.54 ± 0.66[a]	1.39 ± 0.61[a]
Total nitrogen	3.99 ± 0.53[a]	3.91 ± 0.52[a]
Reducing sugar	1.49 ± 0.29[a]	1.35 ± 0.31[a]
Crude fat	3.32 ± 0.20[a]	2.69 ± 0.11[a]
Total catechin	1.68 ± 0.40[a]	1.44 ± 0.42[b]
Hunter *L*	91.97 ± 6.20[a]	90.33 ± 6.22[a]
Hunter *a*	−0.70 ± 0.69[b]	0.98 ± 1.02[a]
Hunter *b*	8.95 ± 2.52[b]	20.98 ± 9.49[a]

[a,b] The means in each row followed by the same letter are not significantly different at the 5% level.
[1] Mean ± standard deviation: % on dry weight basis.

The only difference in processing steps between the two kinds of tea is that green tea was blanched and dried right after fresh-leaf picking, while the GABA tea underwent anaerobic fermentation during tea-making. Table 1 shows that the total catechins (determined by the ferrous tartrate method) and ascorbic acid of GABA tea was significantly lower than that of green tea ($p < 0.05$). It implies that catechins and ascorbic acid of GABA tea underwent oxidation during anaerobic fermentation (Millin & Rustidge, 1967).

Colour is one of important qualities for any kind of tea. The Hunter *L*, *a* and *b* values for green and GABA tea infusions are shown in Table 1. The Hunter *L* values were not significantly different ($p > 0.05$). It means the infusions of both teas were all quite clear and bright. However, the Hunter *a* and *b* values of GABA tea were significantly higher than those of green tea ($p < 0.05$). The Hunter *a* and *b* values are important for evaluating the colour change of sample. Some studies indicated that the different fermentation condition affected the colour of infusions (Liang et al., 2003). The colour of GABA tea infusion was darker and redder than that of green tea, due to three times of anaerobic fermentation plus two times of aerobic fermentations taken place in the processing of GABA tea.

3.2. Free fatty acids of GABA tea and green tea

Lipid compounds are not major constituents in a tea infusion, but nevertheless they play an important role in the development of the aroma (Dufresne & Farnworth, 2000). For this study, the free fatty acid contents in GABA and green teas are shown in Table 2. The results indicate that the contents of free fatty acids, myristic, palmitic, stearic, *cis*-9-oleic, linoleic, linolenic, arachidic eicosenoic, erucic, and lignoceric were not significantly different between GABA and green teas. However, Tsushida et al. (1987) showed that the ethyl palmitate, methyl linoleate and methyl linolenate increased to a greater extent in GABA tea as compared with common green tea.

3.3. GABA and major free amino acids in GABA tea and green tea

The content of total free amino acids of the two teas was found not to differ significantly (Table 1). The

Table 2
Contents of free fatty acids in GABA tea and green tea

Fatty acids	Green tea	GABA tea
Methyl myristate	1.15 ± 1.65[a1]	1.39 ± 1.85[a]
Methyl palmitate	42.28 ± 23.42[a]	57.63 ± 90.82[a]
Methyl stearate	24.26 ± 10.94[a]	27.51 ± 26.40[a]
Methyl *cis* -9-oleate	19.47 ± 12.30[a]	17.62 ± 10.22[a]
Methyl linoleate	22.68 ± 9.65[a]	20.91 ± 13.44[a]
Methyl linolenate	15.43 ± 6.51[a]	14.56 ± 10.39[a]
Methyl arachidate	44.57 ± 25.48[a]	44.08 ± 28.11[a]
Methyl eicosenoate	10.68 ± 6.52[a]	11.96 ± 8.82[a]
Methyl behenate	11.07 ± 11.35[a]	11.05 ± 12.60[a]
Methyl erucate	0.84 ± 2.42[a]	2.87 ± 11.81[a]
Methyl lignocerate	8.92 ± 5.71[a]	13.62 ± 14.62[a]

[a] Same as in Table 1.
[1] Mean ± standard deviation: ppm on dry weight basis.

Table 3
Contents of free amino acids in GABA tea and green tea

Amino acids	Green tea	GABA tea
Aspartic acid	121.46 ± 52.20[a1]	23.60 ± 5.72[b]
Glutamic acid	158.72 ± 55.21[a]	41.35 ± 15.95[b]
GABA	16.94 ± 8.46[b]	180.97 ± 51.43[a]
Alanine	24.18 ± 11.43[b]	51.86 ± 18.30[a]
Ammonia	6.95 ± 2.52[b]	10.95 ± 3.80[a]
Theanine	722.68 ± 503.76[a]	613.80 ± 310.94[a]
Threonine	17.34 ± 6.16[a]	22.10 ± 5.74[a]
Phenylalanine	19.65 ± 15.59[a]	12.28 ± 6.61[b]
Tryptophan	13.11 ± 8.25[a]	9.61 ± 5.29[a]
Lysine	9.65 ± 7.57[b]	13.54 ± 5.68[a]
Methionine	0.17 ± 0.17[a]	0.25 ± 0.48[a]
Isoleucine	8.29 ± 7.47[b]	12.49 ± 4.44[a]
Leucine	9.79 ± 8.19[b]	15.75 ± 5.60[a]
Valine	12.50 ± 10.59[a]	16.58 ± 7.41[a]

[a,b] Same as in Table 1.
[1] Mean ± standard deviation: mg/100 g on dry weight basis.

results of analysing the individual amino acids in these two tea samples are shown in Table 3. The contents of theanine, threonine, tryptophan, valine and methionine did not differ significantly between GABA tea and green tea. GABA, alanine, lysine, leucine, isoleucine and ammonia were found significantly higher in GABA tea than in green tea ($p < 0.05$). In contrast, glutamic acid, aspartic acid and phenylalanine were higher in green tea ($p < 0.05$). Tsushida et al. (1987) in their study of glutamic metabolism in tea leaves found that GABA and alanine were increased, while glutamic acid and aspartic acid were decreased in the tea leaves which had been stored under anaerobic conditions. The present study has shown the same result. The anaerobic process causing high GABA and aniline but low glutamic and aspartic acids in GABA tea is clearly seen. Likewise, the ammonia higher in GABA tea than in green tea was the result of the metabolic production as indicated by the report of Tsushida et al. (1987).

Tea contains many amino acids. Among them, theanine (γ-glutamic acid ethylamide), specific to the tea plant, is the most abundant, accounting for 50% of total amino acids. Theanine has a sweet and savoury taste and shows the highest correlation to the quality of green tea (Balentine et al., 1997). Furthermore, it has been found that theanine has considerable biological impact. For example, it has been reported that theanine decreases the level of norepinephrine and serotonin in brain, and intake of theanine by hypertensive rats results in decreased blood pressure (Juneja, Chu, Okubo, Nagato, & Yokogoshi, 1999). Recently, the cooperative effects of antitumour agents and theanine on cancer have been reported (Sugiyama & Sadzuka, 2003). The present study shows that GABA tea with anaerobic fermentation has a theanine content not significantly different from that of green tea (Table 2). It indicates that GABA tea not only has a high amount of GABA, but also a similar amount of theanine as green tea.

Amino acids essential for human consist of threonine, lysine, valine, isoleucine, leucine, tryptophan, methionine and phenylalanine (Robinson, Lawler, Chenoweth, & Garwick, 1990). In the present study, threonine, valine, tryptophan, and methionine did not differ significantly between GABA and green teas ($p > 0.05$). Lysine, isoleucine and leucine were even significantly higher in GABA than in green tea ($p < 0.05$). Only the phenylalanine content of GABA tea was found to be significantly lower than that of green tea ($p < 0.05$). Valine, isoleucine and leucine are referred to as the branched chain amino acids. They are needed nutrients for liver and cerebral neuron cell and proved good for alcoholism, hepatic encephalopathy and *liver coma* (Robinson et al., 1990). Rothuizen, Kok, Slob, and Mol (1996) reported that GABA tea modulated the signal of hypothalamic–pituitary–adrenocortical in rat liver. It is conspectus that GABA tea high in GABA, valine, isoliucine and leucine may improve the function of liver.

The above results show that overall GABA tea contains more essential amino acids than green tea, although their total amino acids contents show no difference.

3.4. Phenolic compounds of GABA tea and green tea

The polyphenols generally can be classified into the following four categories: flavanols with their gallic acid esters, flavonols with flavonol glycosides, leucoanthocyanins, phenolic acids and depsides, and oxidized and polymerized phenolic compounds. They constitute about 30% of the weight of made green tea and the flavanols with their gallic acid esters make up about 80% of total polyphenols. These phenolic compounds usually are the most abundant water-soluble components in the tea (Balentine et al., 1997). In the literature, it is found that oolong, pu-erh and black teas contain less catechins, such as EGCG, EGC, ECG and EC, than green tea because the processing by aerobic

Table 4
Contents of polyphenolic compounds in GABA tea and green tea

Compounds[1]	Green tea	GABA tea
GC	2.48 ± 1.24[a2]	3.17 ± 2.01[a]
C	0.76 ± 0.31[a]	0.73 ± 0.46[a]
EC	0.62 ± 0.23[a]	0.44 ± 0.67[b]
ECG	0.85 ± 0.64[a]	0.66 ± 0.58[a]
EGC	2.85 ± 1.67[a]	3.71 ± 2.06[a]
EGCG	4.69 ± 1.55[a]	3.26 ± 1.78[b]
Caffeine	3.22 ± 1.14[a]	3.34 ± 1.36[a]

[a,b] Same as in Table 1.
[1] GC, gallocatechin; C, catechin; EC, epicatechin; ECG, epicatechin gallate; EGC, epigallocatechin; EGCG, epigallocatechin gallate.
[2] Mean ± standard deviation: % on dry weight basis.

fermentation during tea making reduces the levels of catechins significantly (Balentine et al., 1997; Zuo, Chen, & Deng, 2002).

In the present study, analysis for caffeine, GC, C, EGC and ECG (Table 4) shows that anaerobically fermented GABA tea does not differ significantly from green tea ($p > 0.05$), except for EGCG and EC, which were significantly lower in GABA than in green tea ($p < 0.05$). The lower concentrations of the two catechins brought about by oxidation also resulted in the difference in total catechins between these two kinds of tea. It is noteworthy, that more of the non-ester type (free type) catechins (EGC, GC) is present in GABA than in green tea, but less of the ester type catechins (EGCG, ECG). Some studies have indicated that more free type catechins impart to tea infusions brothy and smooth characteristics, but that the ester type catechins exhibit astringency (Goto et al., 1996; Valentová, Skrovánková, Panovská, & Pokorný, 2002; Wang & Helliwell, 2000). Our sensory work did show the GABA tea with multiple anaerobic and aerobic fermentations to have its own characteristics, but no unpleasant aroma and taste (unpublished data). The causes of these effects in GABA tea from multi-anaerobic fermentation or other factors are of interest and further studies on this topic are necessary.

Several studies reported that the antioxidative activity of teas was due mainly to phenolic compounds (Balentine et al., 1997; Dufresne & Farnworth, 2000; Nakane, Hara, & Ono, 1994, cha 5; Sakanaka & Yamamoto, 1997, chap. 9; Yeng & Chen, 1996). Other effects of phenolic compounds, antibacterial, antitoxin, antimutagens and antiinflammation have also been reported (Fujiki et al., 1992; Sakanaka & Yamamoto, 1997, chap. 9; Yeng & Chen, 1994). Of the various types of teas, the antioxidative activity of unfermented green tea was the strongest. Green tea contains considerable amounts of catechins, such as EC, ECG, EGC and EGCG (Balentine et al., 1997; Dufresne & Farnworth, 2000; Nakane et al., 1994, chap. 5). Table 4 shows that most phenolic compounds did not differ significantly between GABA and green teas ($p > 0.05$), but total catechin, EC and EGCG were significantly lower in GABA than green tea ($p < 0.05$). Overall GABA tea has an antioxidant activity similar to that of green tea. In fact, our recent data comparing the antioxidant activity of these two kinds of tea shows the same result (to be published).

4. Conclusion

The present results show that the main difference between GABA and green tea are the contents of GABA, glutamic acid, alanine, aspartic acid, total catechins, EGCG and EC, especially the first two. Most of the other compounds examined, such as theanine, threonine, valine, methionine, tryptophan, crude fat, total free amino acids, total nitrogen, caffeine and all fatty acids did not differ significantly between the two kinds of tea.

It may be said that GABA tea is almost the same as green tea in terms of the above-mentioned bioactive compounds. In addition, GABA tea contains a high level of GABA which affects blood pressure, and the nervous system and cardiovascular system. Overall, GABA tea is close to green tea in bioactivity.

As parallel to the present study, the effect of different variety, production area and season on these bioactive compounds in GABA tea and green tea was investigated. The part result on free amino acids content of Taiwan GABA tea with different variety, production area and season has been published (Wang, Tsai, Lin, & Ou, 2005) and the manuscript for rest of results will be submitted shortly.

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