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Determination and Comparison of γ-Aminobutyric Acid (GABA) Content in Pu-erh and Other Types of Chinese Tea

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Supporting Information

ABSTRACT: Two previous studies have reported that pu-erh tea contains a high level of γ-aminobutyric acid (GABA), which is the major inhibitory neurotransmitter in the central nervous system and has several physiological functions. However, two other researchers have demonstrated that the GABA content of several pu-erh teas was low. Due to the high value and health benefits of GABA, analysis of mass-produced pu-erh tea is necessary to determine whether it is actually enriched with GABA. A high-performance liquid chromatography (HPLC) method was developed for the determination of GABA in tea, the results of which were verified by amino acid analysis using an Amino Acid Analyzer (AAA). A total of 114 samples of various types of Chinese tea, including 62 pu-erh teas, 13 green teas, 8 oolong teas, 8 black teas, 3 white teas, 4 GABA teas, and 16 process samples from two industrial fermentations of pu-erh tea (including the raw material and the first to seventh turnings), were analyzed using HPLC. Statistical analysis demonstrated that the GABA content in pu-erh tea was significantly lower than that in other types of tea ($p < 0.05$) and that the GABA content decreased during industrial fermentation of pu-erh tea ($p < 0.05$). This mass analysis and comparison suggested GABA was not a major bioactive constituent and resolved the disagreement GABA content in pu-erh tea. In addition, the GABA content in white tea was found to be significantly higher than that in the other types of tea ($p < 0.05$), leading to the possibility of producing GABA-enriched white tea.

KEYWORDS: γ-aminobutyric acid (GABA), pu-erh tea, bioactive constituents, determination, comparison

INTRODUCTION

γ-Aminobutyric acid (GABA) is a four-carbon nonprotein amino acid that acts as a major inhibitory neurotransmitter in the central nervous system and is a multifunctional molecule that has different situational functions in the central nervous system, the peripheral nervous system, and some nonneuronal tissues.[1,2] GABA has various physiological functions in animals and humans, such as neurotransmission and induction of hypotensive, diuretic, and tranquilizing effects.[3] In addition, the concentration of GABA in the brain may be related to various neurological disorders including epilepsy, seizures, convulsions, Huntington's disease, and Parkinsonism.[4]

Due to the physiological functions of GABA, the development of functional foods containing GABA in high concentrations has been actively pursued. Several GABA-enriched foods have been characterized: GABA tea,[5−7] rice germ,[8] brown rice,[9] tempeh-like fermented soybean,[10] dairy products,[11,12] sourdough bread,[13] black raspberry juice,[14] and distilled alcoholic beverages.[15] GABA-enriched food has been proved to be beneficial in cases of sleeplessness, depression, and autonomic disorders[16] and is effective in relieving chronic alcohol-related symptoms[17] in addition to possessing sedative[16] and antihypertension[18,19] properties. The intake of GABA-enriched food intake also stimulates the immune cells,[20] has inhibitory action on cancer cells,[21] and may prevent diabetic conditions.[22]

In 1987, Tsushida and Murai found that the GABA content of fresh tea leaves was increased by 8.9-fold under anaerobic conditions.[5] This then led to the development of GABA tea or Gabaron tea, which contains >150 mg of GABA/100 g of tea.[3,5−7,23] Further research has demonstrated that GABA tea was able to reduce blood pressure in experimental animals and humans.[19,23] Recently, GABA tea was also shown to aid sleep.[24] GABA tea can be consumed daily and has the same potential health benefits as GABA and tea. It is for these reasons that GABA tea has become popular in Asia, and GABA green tea, GABA oolong tea, and GABA black tea are now being mass produced and commercialized in Japan and China.[23]

Because of the high value and popularity of GABA tea, several methods have been developed to determine GABA, either separately or with other amino acids, in different types of tea, including fresh tea shoots,[5,6,26] green tea,[7,25,27−30] Gabaron tea,[7,25,27,29,30] black tea,[28,29] oolong tea,[29,30] pu-erh tea,[26,29,31] and microbe-fermented tea leaves [26,31,32] (Table 1). GABA was detected in 12 samples of pu-erh tea, which is a type of microbe-fermented tea originally produced in the Yunnan province of southwestern China, and in 7 samples of microbe-fermented tea leaves.[26,29,31,32] However, the results were inconsistent among different research groups. Chen and colleagues reported that the GABA content of pu-erh teas was higher and that short-term fermentation with *Streptomyces bacillaris* or *Streptomyces cinereus* enhanced the GABA content (>150 mg/100 g) (Table1).[26,31] However, Syu et al. found that GABA was not detectable in a ripe pu-erh tea and that its concentrations in two raw pu-erh teas were

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Table 1. Review of the Measurement of GABA in Tea

author, year[ref]	measured amino acid	tea samples (GABA content, *n* = number of samples)	method	validation of the method
Tsushida, 1987 [5]	glutamic acid (Glu), glutamine (Gln), alanine (Ala), GABA, serine (Ser), theanine (Thea), aspartic acid (Asp), and asparagine (Asn)	fresh tea shoots (0.89 mg/100 g); tea shoots treated with anoxic incubation (353.5 mg/100 g); tea shoots treated with oxic incubation (60 mg)	AAA, *o*-phthalaldehyde (OPA)-HPLC-fluorescence detector (FLD)	
Sawai, 2001 [6]	Asp, Glu, Asn, Ser, Gln, arginine (Arg), Ala, Thea, GABA	fresh tea leaf (3 ± 5 mg/100 g); fresh tea stem (9 ± 3 mg/100 g); leaf treated with cycling anaerobic and aerobic incubation (200−800 mg/100 g)	OPA-HPLC-FLD	
Huang, 2005[25]	GABA, Glu	pan-fired green tea (12 mg/100 g); Gabaron tea (272 mg/100 g)	HPLC-OPA-FLD	
Wang, 2006 [7]	Asp, Glu, GABA, Ala, ammonia, Thea, threonine (Thr), phenyl-alanine (Phe), tryptophan (Trp), lysine (Lys), methionine (Met), isoleucine (Ile), leucine (Leu), valine (Val)	green teas (16.94 ± 8.46 mg/100 g, *n* = 28); GABA teas (180.97 ± 51.43 mg/100 g, *n* = 28)	AAA	
Jeng, 2007 [26]	GABA	fresh leaves (127 ± 1 mg/100 g); pu-erh tea (125 ± 7 to 460 ± 25 mg/100 g, *n* = 7), microbe-fermented tea leaves (584.2, 739.2 mg/100 g)	HPLC	
Lin, 2007 [27]	GABA and Ala	GABA-rich tea (159 and 108 mg/100 g, *n* = 2); jasmine green tea (trace, *n* = 1)	OPA/2-ME, capillary electrophoretic (CE)-FLD	recovery = 94.22%; RSDs of migration time and peak area were 0.88, 3.51% (intraday) and 0.61, 3.76% (interday)
Hsieh, 2007 [28]	Arg, Trp, tyrosine (Tyr), Phe, Leu, Ile, histidine (His), Asn, Thr, Thea, Val, Met, glutamine (Gln), GABA, Ser, Ala, glycine (Gly), Asp, proline (Pro), cysteine (Cys), Lys	Taiwanese green tea (24.7 ± 2.6 mg/100 g); Taiwanese black tea (0.52 ± 2.7 mg/100 g); Japanese green tea (53.6 ± 2.6 mg/100 g)	CE-LED-IF	RSD < 2.7%
Syu, 2008 [29]	Asn, Arg, Gln, Ser, Glu, Asp, Thr, Thea, Gly, Ala, Met, Pro, Val, GABA, Trp, Phe, Ile, Leu, His, Lys, Tyr	green tea (ND−105.4 ± 9.9 mg/100 g, *n* = 16); oolong tea (ND−101.2 ± 11.6 mg/100 g, *n* = 14); black tea (ND−55.5 ± 10.1 mg/100 g,*n* = 3); pu-erh ripe tea (ND, *n* = 1); pu-erh raw tea (44.6 ± 11.9 and 46.1 ± 18.2 mg/100 g, *n* = 2); GABA tea (197.5 ± 8.4 mg/100 g, *n* = 1)	dabsylation, HPLC	
Su, 2010 [30]	GABA and Ala	oolong tea (6.0 mg/100 g, *n* = 1); jasmine green tea (2.5 mg/100 g, *n* = 1); GABA-rich tea (157.24 mg/100 g, *n* = 1)	OPA/2-ME-CE-FLD	recoveries = 92.33−97.87%; RSDs of slope were 2.48% (intraday) and 1.79% (interday)

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Table 1. Continued

author, year[ref]	measured amino acid	tea samples (GABA content, n = number of samples)	method	validation of the method
Chen, 2010 [31]	GABA	pu-erh teas (291 mg/100 g, 467 mg/100 g, n = 2); microbe-fermented tea leaves (712, 813, and 862 mg/100 g, n = 3)	HPLC	
Hou, 2010 [32]	GABA	microbe-fermented tea leaves (from 13.1 ± 0.4 to 19.6 ± 1.5 mg/100 g, n = 4)	HPLC	

only 44.6 and 46.1 mg/100 g, respectively (Table 1).[29] In a recent study by Chen, the GABA content of microbe-fermented tea samples was found to be only 13.1−19.6 mg/100 g.[32] Thus, the GABA content in pu-erh tea has yet to be definitively determined, with differing results obtained even by Chen and his colleagues.

Pu-erh tea has been shown to have antioxidant,[33,34] anticancer,[35] hypolipidemic,[36−42] antimutagenic,[43] and antimicrobial [43,44] properties. Indeed, several of the benefits of pu-erh tea, such as the lowering of blood pressure and blood sugar and the reversal of alcohol intoxication, were documented in various ancient Chinese medical texts. However, the bioactive constituents of pu-erh tea remain essentially unknown. Chen's results suggested that pu-erh tea contains high concentrations of GABA and therefore has the potential health benefits of GABA.[26,31] This led to the hypothesis that GABA is a major bioactive constituent in pu-erh tea. On the other hand, the results of Syu et al.[29] and Hou et al.[32] do not support this hypothesis. Due to its high value and health benefits, the GABA content of any food product is closely related to its price and health-promoting properties. Therefore, the GABA content of pu-erh tea must be confirmed in further investigations.

To our knowledge, GABA has not been determined in another type of Chinese tea, white tea. White tea is usually processed by withering and drying only, and in the absence of high-temperature fixation and rolling, it appears to retain more of its beneficial components.[45] It is therefore of interest to test whether white tea has a higher GABA content.

In this work, a HPLC approach for the detection of GABA in tea was developed and validated using an Amino Acid Analyzer analysis. Ninety-eight samples of various types of Chinese tea, including 62 pu-erh teas from the original production areas, were analyzed. This mass determination of the GABA content of pu-erh tea and comparison with other types of Chinese teas provided information relating to the bioactive constituent in pu-erh tea and resolved the disagreement regarding GABA content in pu-erh tea. In addition, during this work we also found it possible to produce GABA-enriched white tea.

MATERIALS AND METHODS

Chemicals and Reagents. GABA was purchased from Sigma-Aldrich (St. Louis, MO). Acetonitrile and ethanol for use in the mobile phases were of HPLC-grade reagent purchased from Tedia Co. Inc. (Fairfield, OH). Sodium acetate used in the mobile phases was of analytical reagent grade and was used without further purification. OPA regent (10 mg/mL, Agilent P/N 5061-3335) and borate buffer (0.4 M, pH 10.4; Agilent P/N 5061-3339) used for derivatization were purchased from Agilent (Agilent Technologies, Palo Alto, CA). Deionized water was prepared using the EPED-T purification system (Yi Pu Yi Da Co., Nanjing, Jiangshu, China) and was degassed under vacuum and filtered through a 0.45 μm nylon membrane prior to use in HPLC analysis.

Tea Samples and Preparation of Tea Infusion. Ninety-eight Chinese teas of various types, including 62 pu-erh teas, 13 green teas, 8 oolong teas, 8 black teas, 3 white teas, and 4 GABA teas, were purchased from local markets (Supporting Information Tables 1 and 2). Sixteen process samples from two industrial fermentations of pu-erh tea (including the raw material and the first to seventh turnings) were obtained from the Nanjian Phoenix Tuocha Tea Factory. Tea samples of 5.0 g were ground into powder using a grinder (Joyoung Co., Jinan, Shandong, China). Two grams of the tea powder was extracted with 50 mL of distilled water for 2 h in a water bath (85 °C). The tea water extract was centrifuged at 12000 rpm for 10 min at room temperature. The resulting supernatant was filtered through a 0.22 μm nylon membrane and subjected to HPLC or AAA to determine the GABA content as described below.

HPLC-FLD Analysis. GABA content was determined using an Agilent 1200 series HPLC system consisting of an LC-20AB solvent delivery unit, a SIL-20A autosampler, a CTO-20A column oven (40 °C), a G1321A fluorescence detector (FLD) (Ex, 230 nm; Em, 450 nm), and an LC Ver1.23 workstation (Agilent Technologies). The separation was completed using an Agela Venusil AA column (4.6 × 250 mm, 5 μm) fitted with a C18 guard column (Agela Technologies Inc., Tianjing, China). The mobile phases were solvents A (90 mM NaAc, 7% acetonitrile, pH 6.5) and B (80% acetonitrile). Elution conditions were as follows: 0−15 min, solvent A was reduced from 95 to 85% and solvent B from 5 to 15% (linear gradient); 15−16 min, solvent B was increased to 100% (linear gradient); 16−20 min, solvent B was kept at 100%; 20−21 min, solvent A was increased from 0 to 100% (linear gradient); 21−25 min, solvent A was kept at 100%; 25.5 min, solvent A was changed to 95%; the flow rate was 1.0 mL/min. The temperature of the column oven was set at 40 °C. The precolumn online derivatization was undertaken using OPA (10 mg/mL) according to the instructions provided in the Agilent Technical Note, with the following modifications:[46] step 1, draw off 5.0 μL of borate buffer (0.4 M, pH 10.4); step 2, draw off 1.0 μL of OPA reagent (10 mg/mL); step 3, draw off 0.0 μL of double-distilled water; step 4, draw off 1.0 μL of sample; step 5, draw off 0.0 μL of double-distilled water; step 6, mix 8 μL in air; step 7, inject. The FLD was set at an excitation of 230 nm, an emission of 450 nm, and a PTM gain of 12 to monitor the derivatized amino acids. Evaluation and quantification were carried out using the workstation. GABA was identified in the tea liquors by comparing the retention time of the unknown peaks with that of a GABA standard. Each tea was extracted twice, and each extraction was analyzed twice.

Validation of HPLC-FLD Analysis. HPLC-FLD validation tests were carried out using calibration curves and tests of precision, selectivity, accuracy, limit of detection (LOD), and limit of quantification (LOQ).

Calibration curves were constructed from the mean peak areas of triplicate HPLC-FLD analyses of five concentrations (0.1, 0.05, 0.01, 0.005, 0.001 mg/mL) of the GABA standard. Precision (reproducibility) of the method was determined by calculating the relative standard deviation (RSD) from repeated injections of the GABA standard

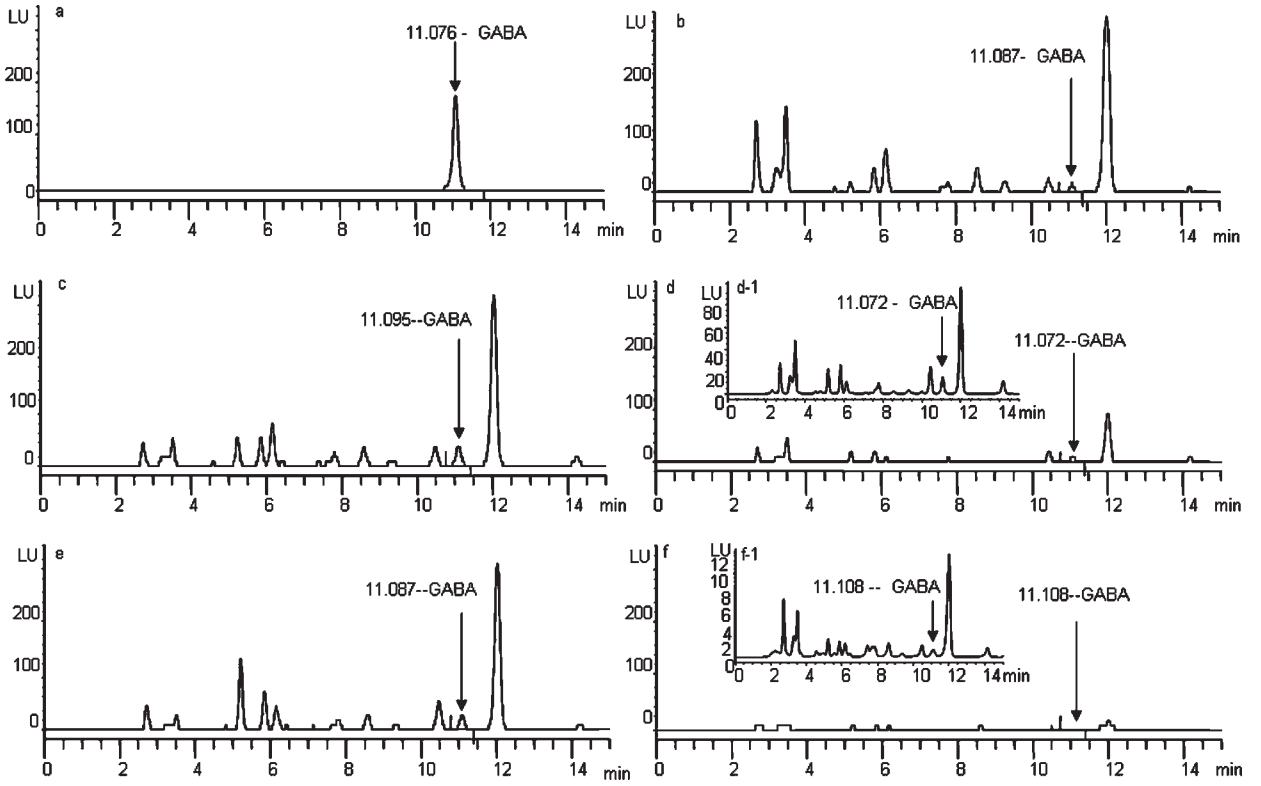

Figure 1. Representative HPLC patterns of GABA standard (a) and GABA in various teas, including green tea (b), black tea (c), oolong tea (d and d-1), white tea (e), and pu-erh tea (f and f-1). Panels a−f are shown at the same scale (0−300 LU); panels d-1 and f-1 are shown at scales of 0−100 LU and 0−14 LU, respectively.

solution. The intraday precision was determined using five replicate injections, whereas the interday precision was determined from five injections for 5 days, for both the retention times and peak areas. RSDs were calibrated using the formula RSD (%) = (SD/mean) × 100%. The selectivity criterion was that the GABA peak should have a chromatographic baseline with suitable resolution from all of the other sample components in all types of tea. Accuracy was evaluated by a recovery test. One milligram of the GABA standard solution was added to a 1.0 g sample of tea power and was then extracted and analyzed as described above. Average recoveries were calibrated using the formula recovery (%) = [(amount found − original amount)/amount spiked] × 100%. The LOD and LOQ were calculated at signal-to-noise ratios (S/N) of 3 and 10, respectively.

Amino Acid Analyzer (AAA) Analysis of GABA. Fourteen samples of various types of tea were sent to the Instrumental Analysis Center of Shanghai Jiaotong University for determination of the HPLC-measured GABA content using an automatic AAA (L-8900; Hitachi, Tokyo, Japan) attached to a Hitachi HPLC packed column with ion-exchanging resin 2622 PF (4.6 × 60 mm). Sample preparation was according to the manufacturer's protocols and consisted of three steps: step 1, the sample was deproteinized by thorough mixing with 40 mg of sulfosalicylic acid/mL (1:3) for 10 min and centrifuged at 19500 rpm for 90 min; step 2, the supernatant was poured off and adjusted to pH 2.2; step 3, the supernatant was filtered through a 0.45 mm filter, and 20 μL was loaded on a Hitachi L-8900 AAA. The analysis was performed according to the manufacturer's standard protocols.

Statistical Analysis. One-way ANOVA was used to identify statistical differences, followed by the least-significant difference (LSD) method for paired data. Results are expressed as the mean ± SD. All data were analyzed using SPSS 11.0 software packages (SPSS Inc., Chicago, IL), and $p < 0.05$ was considered to be significant.

RESULTS AND DISCUSSION

Development and Validation of HPLC-FLD Analytical Method. Precolumn online derivatization and determination of GABA in tea were achieved. A HPLC chromatogram of a GABA standard is shown in Figure 1a. The mean GABA retention time was 11.116 ± 0.042 min. GABA has a suitable resolution (>0.5 min) distance from all of the other amino acids in all types of tea (Figure 1). The RSDs of the retention times and peak areas of the intraday tests were 0.1 and 3.2%, respectively. The RSDs of the retention times and peak areas of the interday samples were 0.3 and 4.0%, respectively. Calibration curves were obtained based on five concentrations of the GABA standard. The coefficient of determination (r^2) was >0.99. The LOD and LOQ values were 0.559 and 1.863 ng, respectively.

A HPLC-FLD method to detect GABA in tea was developed. To further verify the HPLC results, partial samples were submitted to the Instrumental Analysis Center of Shanghai Jiaotong University and analyzed using an AAA. Due to the high costs of AAA analysis, only 14 samples of various types of tea were subjected to both HPLC and auto-AAA analysis. The GABA content is shown in Table 2. There was no significant difference ($p > 0.05$) between the GABA content determined by auto-AAA and HPLC; this demonstrates that our HPLC results are acceptable.

Determination of GABA in Chinese Teas. According to the processing methods and the characteristic quality of manufactured tea, there are six types of tea in China: green tea, yellow tea, dark tea (containing brick tea and pu-erh tea), white tea, oolong tea, and black tea.[47] In this work, 98 samples of various types of Chinese tea, including 62 pu-erh teas, 13 green teas, 8 oolong

Table 2. GABA Contents Determined by HPLC and AAA

		GABA contents (mg/100 g) determined by	
tea type	sample name	HPLC	AAA
pu-erh tea	Shengye pu-erh tuocha	0.9	0.9
	Nanjian Phoenix pu-erh Tuocha (Tulin)	0.9	1.2
	Tea Science kindfund yellow label pu-erh tea	1.4	1.3
	Longji-shunde-kindfund pu-erh tea	1.7	1.3
green tea	Huang shan maofeng	13.8	12.2
	Shifeng longjing	33.9	39.8
	Taiping kowkui	20.4	23.9
black tea	Yunnan black tea	31.1	34.0
	Keemun black tea	41.5	38.8
oolong tea	Jinjiang Yuan tieguanyin	15.79	16.6
	Wuyi Iron Arhat	16.4	17.3
	Wuliang Mountain organic tea	20.7	21.1
	Gui Fei oolong tea	14.8	18.3
white tea	white tea	50.5	43.8

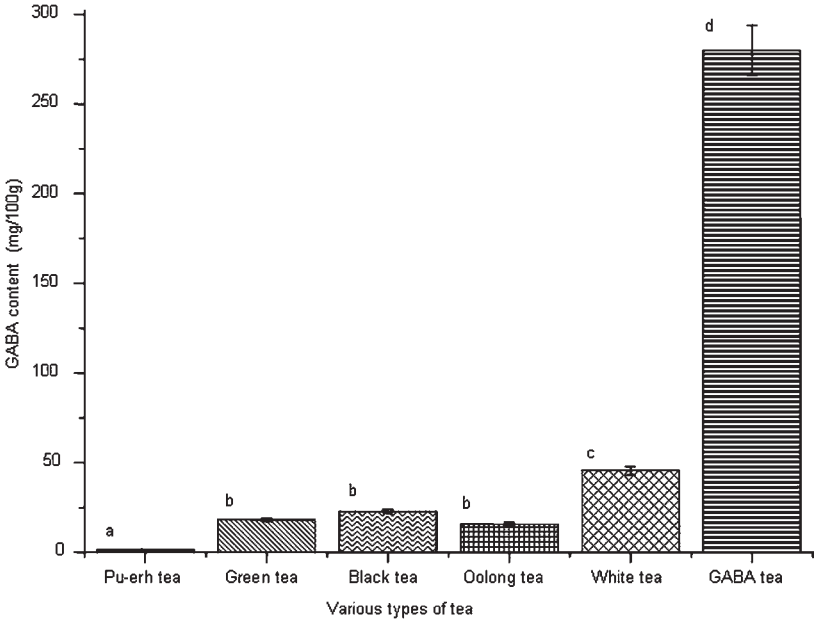

Figure 2. Comparison of means of the GABA contents of various types of Chinese tea: pu-erh tea ($n = 62$), green tea ($n = 13$), black tea ($n = 8$), oolong tea ($n = 8$), white tea ($n = 3$), and GABA tea ($n = 4$). The values represent the mean ± SD. Samples of pu-erh tea are further presented in Supporting Information Table 1; samples of green, black, oolong, white, and GABA teas are further presented in Supporting Information Table 2. The different letters above the bars indicate significant differences among the values ($p < 0.05$).

teas, 8 black teas, and 3 white teas, were analyzed by HPLC. The GABA content of these samples is shown in Supporting Information Tables 1 and 2, and a representative HPLC chromatogram of various tea amino acids is shown in Figure 1.

GABA Contents in Green Tea, Black Tea, and Oolong Tea. HPLC analysis showed that each sample of green tea, black tea, and oolong tea in this study contained GABA (Supporting Information Tables 1 and 2). The mean concentrations were 18.2 ± 11.0, 22.9 ± 14.7, and 15.9 ± 7.9 mg/100 g, respectively (Figure 2). Our results are consistent with those of previous studies (Table 1), both of which demonstrated that most green, black, and oolong teas contain GABA.

GABA Contents in White Tea. The GABA content of white tea was investigated for the first time in this study, and the concentration was found to be 45.7 ± 7.7 mg/100 g (Figure 2). A previous study has also shown that the amino acid content gradually

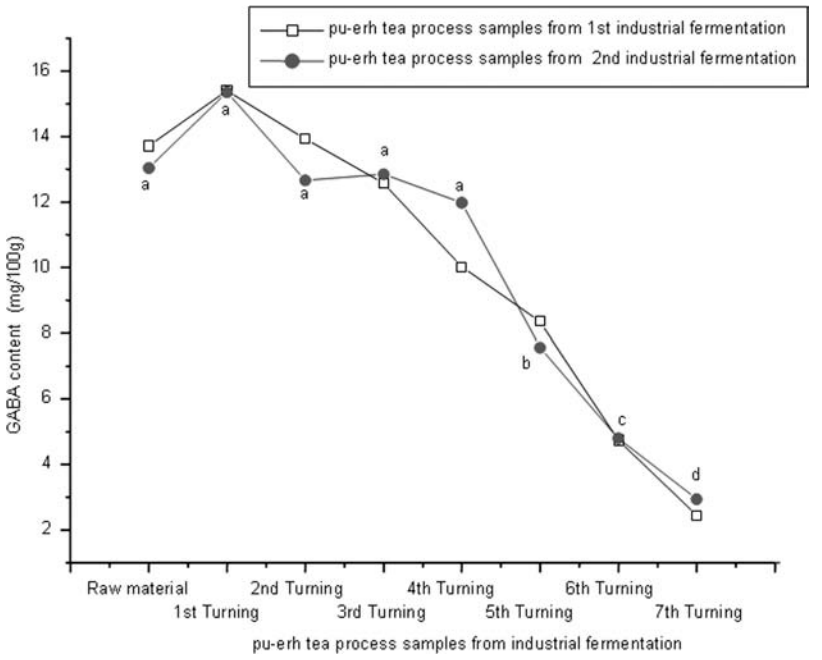

Figure 3. GABA content of process samples from two industrial fermentations of pu-erh tea. Values represent the mean ± SD. Sample data are further presented in Supporting Information Table 1. The different letters indicate significant differences among the values ($p < 0.05$).

increases during the withering process.[45] Our analysis showed that the GABA content in white tea was significantly higher than that in green tea, black tea, and oolong tea ($p < 0.05$). This shows that there is potential for producing GABA-enriched white tea.

GABA Content in GABA Tea. GABA tea is now being commercialized in China. Analysis of four samples of GABA tea from the Chinese market revealed a GABA content of >150 mg/100 g (Supporting Information Table 2). This confirms that GABA tea produced in China conforms to GABA tea standards.

GABA Contents in Pu-erh Tea. HPLC analysis showed that the GABA content in pu-erh tea was 1.6 ± 1.0 mg/100 g (Figure 2). This is consistent with the paper by Syu et al., who used a spectrophotometric detector and found that they were unable to detect GABA in a ripe pu-erh tea.[29] We used a FLD, which is one of the most sensitive detection methods in liquid chromatography, and were able to detect low levels of GABA present in pu-erh tea. Recently, Hou et al. found that the GABA content is 13.1−19.6 mg/100 g in fermented tea samples.[32] We all showed that pu-erh tea contains low concentrations of GABA.

Comparison of GABA Content in Various Types of Tea. The comparison of GABA content in various types of tea is shown in Figure 2. This comparison revealed that pu-erh tea has a lower GABA content than green, black, oolong, or white tea ($p < 0.05$). However, the study by Chen et al. suggested that pu-erh tea has a higher GABA content than other types of tea (green tea, black tea, oolong tea).[26,31] We considered that the analytical method used by Chen et al. for determining the GABA content needed further evaluation because, according to these authors, the GABA content in fresh leaves was 127 ± 1 mg/100 g,[26] which is 14.3-fold greater than that reported by Tsushida,[5] 42.3-fold greater than that reported by Sawai,[6] and 11.6-fold greater than that reported by Hung.[25] Taken together, we are drawn to the conclusion that the GABA content of pu-erh tea is lower than that of other types of tea. We presume that this is a result of the microbial fermentation used during the processing of pu-erh tea. Because there are several studies which demonstrate that the free amino acid and theanine contents decrease during fermentation,[48,49] we therefore assume that the GABA content also decreases. To confirm this assumption, the GABA content was measured for 16 process samples from two industrial fermentations of pu-erh tea (Figure 3). The results demonstrate that the GABA content among the raw material and the first, second, third, and fourth turnings did not significantly differ ($p > 0.05$), but decreased sharply after the fourth turning. This suggests that GABA-rich pu-erh tea (microbially fermented pu-erh tea) could be produced using a raw material with a high GABA content if the fermentation is stopped after the fourth turning.

According the report by Chen et al., tea merchants and tea consumers put a high value on GABA in pu-erh tea. They claim that GABA plays an important role in the health benefits of pu-erh tea, for example, leading to the lowering of blood pressure and blood sugar and reducing the effects of alcohol intoxication.[50] Our results, however, indicate that GABA is not the main bioactive constituent in pu-erh tea.

In conclusion, this mass determination of GABA concentration in pu-erh tea and the comparison with other types of Chinese tea resolved the disagreement of GABA content in pu-erh tea and demonstrated that the GABA content in pu-erh tea was significantly lower than that in other types of tea ($p < 0.05$). It is interesting to note that in this work we also found that the GABA content of white tea was significantly higher than that in other types of tea ($p < 0.05$), thus making it possible to produce GABA-enriched white tea.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. GABA contents in pu-erh tea and other types of tea. This material is available free of charge via the Internet at http://pubs.acs.org.

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ABBREVIATIONS USED

Ala, alanine; AAA, Amino Acid Analyzer; Arg, arginine; Asn, asparagine; Asp, aspartic acid; CE, capillary electrophoretic; Cys, cysteine; FLDr, fluorescence detector; GABA, γ-aminobutyric acid; Glu, glutamic acid; Gln, glutamine; Gly, glycine; HPLC, high-performance liquid chromatography system; His, histidine; Ile, isoleucine; Leu, leucine; LOD, limit of detection; LOQ, limit of quantification; Lys, lysine; Met, methionine; OPA, *o*-phthalaldehyde; Phe, phenylalanine; Pro, proline; RSD, relative standard deviation; Ser, serine; Thea, theanine; Thr, threonine; Trp, tryptophan; Tyr, tyrosine; Val, valine.

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