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Determination of Theanine, GABA, and Other Amino Acids in Green, Oolong, Black, and Pu-erh Teas with Dabsylation and High-Performance Liquid Chromatography

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Dabsyl chloride (dimethylaminoazobenzene sulfonyl chloride), a useful chromophoric labeling reagent for amino acids and amines, was developed in this laboratory in 1975. Although several methods have been developed to determine various types of amino acids, a quick and easy method of determining theanine, GABA, and other amino acids has not been developed in one HPLC system. In this paper are analyzed the free amino acid contents of theanine and GABA in different teas (green tea, black tea, oolong tea, Pu-erh tea, and GABA tea) with a dabsylation and reverse phase high-performance liquid chromatography (HPLC) system coupled with a detector at 425 nm absorbance. Two reverse phase columns, Hypersil GOLD and Zorbax ODS, were used and gave different resolutions of dabsyl amino acids in the gradient elution program. The data suggest that the tea source or the steps of tea-making may contribute to the theanine contents variations. High theanine contents of high-mountain tea were observed in both green tea and oolong tea. Furthermore, the raw (natural fermented) Pu-erh tea contained more theanine than ripe (wet fermented) Pu-erh tea, and the GABA contents in normal teas were generally lower than that in GABA tea.

KEYWORDS: Theanine; GABA; amino acids; tea; dabsylation

INTRODUCTION

Tea is the most popular and widely consumed beverage in the world because of its refreshing taste, attractive aroma, and potential health benefits (*1, 2*). It has many physiological and pharmacological activities due to the presence of components such as amino acids, polyphenols, carbohydrates, caffeine, purine alkaloids, and vitamins (*3*). Tea is made from the leaves of the plant *Camellia sinensis* L., which is now widely cultivated in Southeast Asia as well as in several central African countries. Generally, tea can be broadly classified according to the production method as unfermented tea (green tea), half-fermented tea (oolong tea), full-fermented tea (black tea), or postfermented tea (Pu-erh tea). Green tea and oolong tea are favored in Oriental countries, whereas black tea is favored in Western countries.

Pu-erh tea, mainly produced in the Yunnan province of China, is consumed widely in Southeast Asia. Pu-erh tea can be categorized, depending on the way it is manufactured, as either natural fermented (raw) or post wet fermented (ripe). Both forms of Pu-erh undergo secondary fermentation and oxidation, resulting in a unique type of tea (*4*). In Japan, GABA tea, also called Gyabaron tea because it is rich in γ-aminobutyric acid (GABA), is popular. GABA is an important neurotransmitter with the chief inhibitory activity in the mammalian central nervous system. GABA is known to exhibit antihypertensive effects (*5*), and teas rich in GABA have been demonstrated to induce a fall in blood pressure in rats (*6*).

Theanine (glutamic acid γ-ethyl amide; 5-*N*-ethyl glutamine), as the only free form (nonprotein) amino acid in teas, is very important because of its biological effects and flavor characteristics. For example, theanine has been demonstrated to increase serotonin, dopamine, and GABA levels in the brain, which impart neuroprotective effects (*7, 8*). Moreover, theanine decreases blood pressure in spontaneously hypertensive rats (*9*). Theanine is also, incidentally, the main component responsible for the exotic taste of tea. The brothy sweet umami taste of green tea is due to amino acids, especially theanine (*10*).

Several methods have been developed to determine the presence of various amino acids. For example, amino acids can be determined in tea according to the ninhydrin assay method. High-performance liquid chromatography (HPLC) methods that involve precolumn derivatization with *o*-phthaladehyde (OPA) (*11*) and phenylisothiocyanate (PITC) (*12*) with fluorescence and diode array UV detection are used for amino acid determination. An improved capillary electrophoretic (CE) separation system and an indirect UV detection system were proposed for amino acid analysis (*13*). Estimates of dabsyl-amino compounds

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can be carried out by HPLC with a UV−visible detector at 425 nm absorbance, a method previously developed by our laboratory (*14*). Dabsyl chloride (4-dimethylaminoazobenzene-4′-sulfonyl chloride) is a useful chromophoric labeling reagent for amino acids (*15*). It reacts readily with all kinds of amino acids and amino compounds to form chromorphoric dabsyl derivatives, which can be detected on a thin layer of chromatographic plate and liquid chromatography. The dabsyl derivatives could be used for the qualitative and quantitative assays of naturally occurring amines and amino acids because of their stable properties and strong absorbance visibly at 425 nm. In previous studies, the determination of theanine in teas by HPLC with fluorescence and the differentiation of green, white, black, oolong, and Pu-erh teas according to free amino acid content have been reported by Alcazar et al. (*4*). However, the determination of theanine and GABA with other amino acids by dabsylation has not been developed in one HPLC system. In the present study, we used dabsylation coupled with HPLC systems to determine each free amino acid and focused on analyzing the theanine and GABA contents of green, black, oolong, and Pu-erh teas.

MATERIALS AND METHODS

Chemicals and Reagents. Standard amino acids, including theanine (Thea) and GABA, were purchased from Sigma-Aldrich (St. Louis, MO). Amino acid stock solutions of 1 mg/mL were prepared in 0.1 M HCl and stored at −20 °C. Dimethylaminoazobenzene sulfonyl chloride (dabsyl chloride) was also purchased from Sigma-Aldrich. Acetonitrile and acetone (HPLC grade) were purchased from Romil (Cambridge, U.K.). Hydrochloric acid, glacial acetic acid, sodium hydrogencarbonate, and sodium acetate were purchased from Merck (Darmstadt, Germany). Ultrapure water was obtained from a Milli-Q system of Millipore (Bedford, MA). HCl (1 M), sodium hydrogencarbonate (1 M, pH 9), and dabsyl chloride (1 mg/mL) were freshly prepared and dissolved in acetone before dabsylation of samples.

Tea Samples. Yunnan high-mountain tea 1, Yunnan high-mountain tea 2, Meng-pa-zhai 2007 (spring), Nanru mountain 2005, Xi-hu lake Longjing, and Lion mountain Longjing teas were purchased from China, whereas Ah-Li mountain oolong and Li-shan mountain oolong teas were purchased from Taiwan. Three Pu-erh tea samples were purchased from Yunnan, China. Other commercial tea leaves and tea bags were purchased from a local tea market in Taipei, Taiwan.

Preparation of Tea Samples. One gram of the tea sample was extracted with 50 mL of hot distilled water (80−90 °C) for 30 min. The tea water extract was cooled to room temperature and then filtered through a 0.45 μm nylon filter membrane (Phenomenex). After filtration, the sample's pH was adjusted to about 3 by adding 100 μL of 1 M HCl and then partitioning with an equal volume of ethyl acetate twice. Water layer pH was adjusted to about 9 by adding 2 mL of 1 M $NaHCO_3$ (pH 9) and then partitioning with an equal volume of ethyl acetate once. The water layer containing amino acids was subjected to dabsylation as described below.

Amino Acids Dabsylation. The amino acid solution (1 mL, pH 9.0) was mixed with 1 mL of dabsyl chloride (1 mg/mL, in acetone) and reacted at 67 °C for 10 min. The pH of the reactive mixture was kept at 9 by adding 1 M $NaHCO_3$ solution. After that, the reaction was stopped by an ice bath, and then the dabsyl sample was filtered through a 0.45 μm nylon filter membrane. When the dabsylation was complete, the free amino acids became dabsyl-amino acids, namely, dabsyl-theanine, dabsyl-GABA, etc. In addition to reacting with amino acids, dabsyl chloride could also react with water to produce sodium dimethylaminoazobenzene 4-sulfonate (DABS-ONa) as demonstrated by a large and early peak in the HPLC pattern (**Figure 1**, peak 1).

HPLC Gradient System for Determination of Amino Acids in Tea Samples. The determination of dabsyl-amino acids derived from tea samples was carried out by HPLC with a spectrophotometric detector at 425 nm as previously described. The HPLC system consisted of a Waters 600E solvent delivery system, a model U6K universal injector, and a Jasco UV−visible 975 detector operating at 425 nm. A reversed phase column (Hypersil GOLD Thermo, 250 mm × 4 mm i.d., 5 μm particle size) coupled with a C18 cartridge was used. The column temperature was maintained at 30 °C. The composition of the optimized mobile phase A was acetonitrile/0.045 M CH_3COONa (pH 4) = 30:70. Mobile phase B was acetonitrile/0.045 M CH_3COONa (pH 4) = 75:25. The gradient elution profile between 0 and 15 min was kept at 100% of solvent A; 15−20 min, linear gradient change to 85% of solvent A; 20−35 min kept at 85% of solvent A; 35−50 min, linear gradient change to 75% of solvent A; 50−65 min, linear gradient change to 0% of solvent A; 65−75 min kept at 0% of solvent A; 75−85 min, linear gradient change to 100% of solvent A. The mobile phase was filtered through a 0.22 μm membrane filter and degassed prior to use. Ten microliters of each dabsyl sample was injected into the chromatograph column, and the column was ready for the next sample run after column conditioning of >30 min.

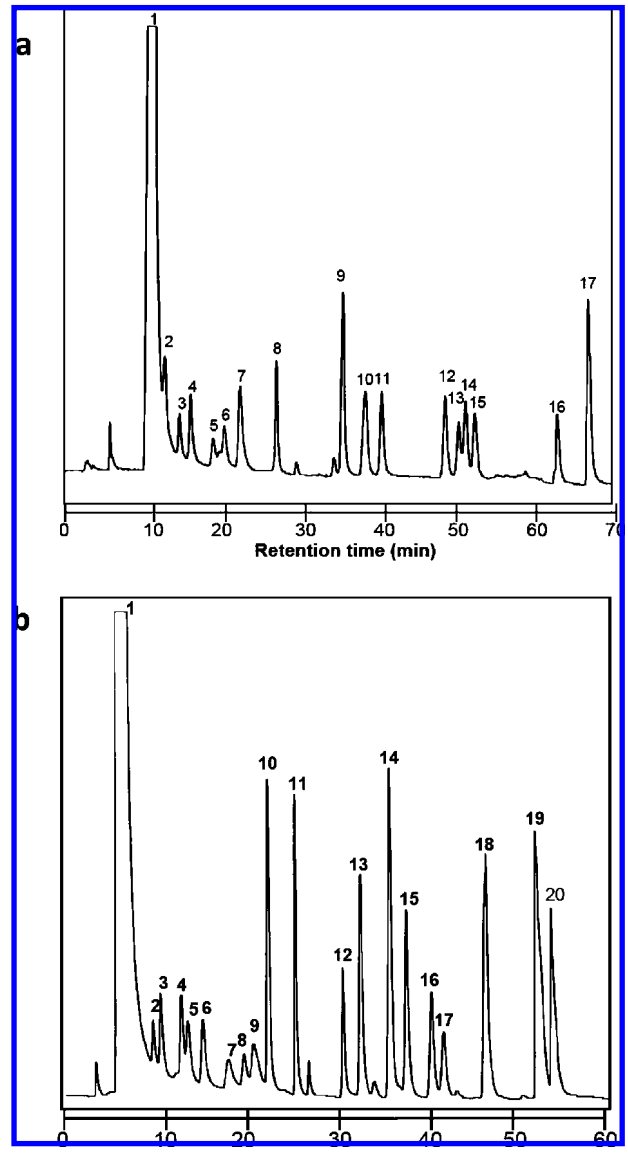

Figure 1. Representative HPLC patterns of standard dabsyl-amino acids by gradient elution and detected at 425 nm. The panels illustrate typical RP-HPLC chromatograms of authentic dabsyl free amino acids and the order of elution by retention time. (**a**) Hypersil GOLD Thermo column: 1, DABS-ONa + Asn; 2, Arg + Gln; 3, Ser; 4, Glu + Asp; 5, Thr; 6, Thea; 7, Gly; 8, Ala; 9, Met + Pro; 10, Val; 11, GABA; 12, Trp; 13, Phe; 14, Ile; 15, Leu; 16, His + Lys; 17, Tyr. (**b**) Zorbax ODS column: 1, DABS-ONa + Cys; 2, Asn; 3, Gln; 4, Ser; 5, Asp; 6, Glu; 7, Arg; 8, Thr; 9, Thea; 10, Gly; 11, Ala; 12, Met; 13, Pro; 14, Val; 15, GABA; 16, Trp; 17, Phe; 18, Ile + Leu; 19, His + Lys; 20, Tyr.

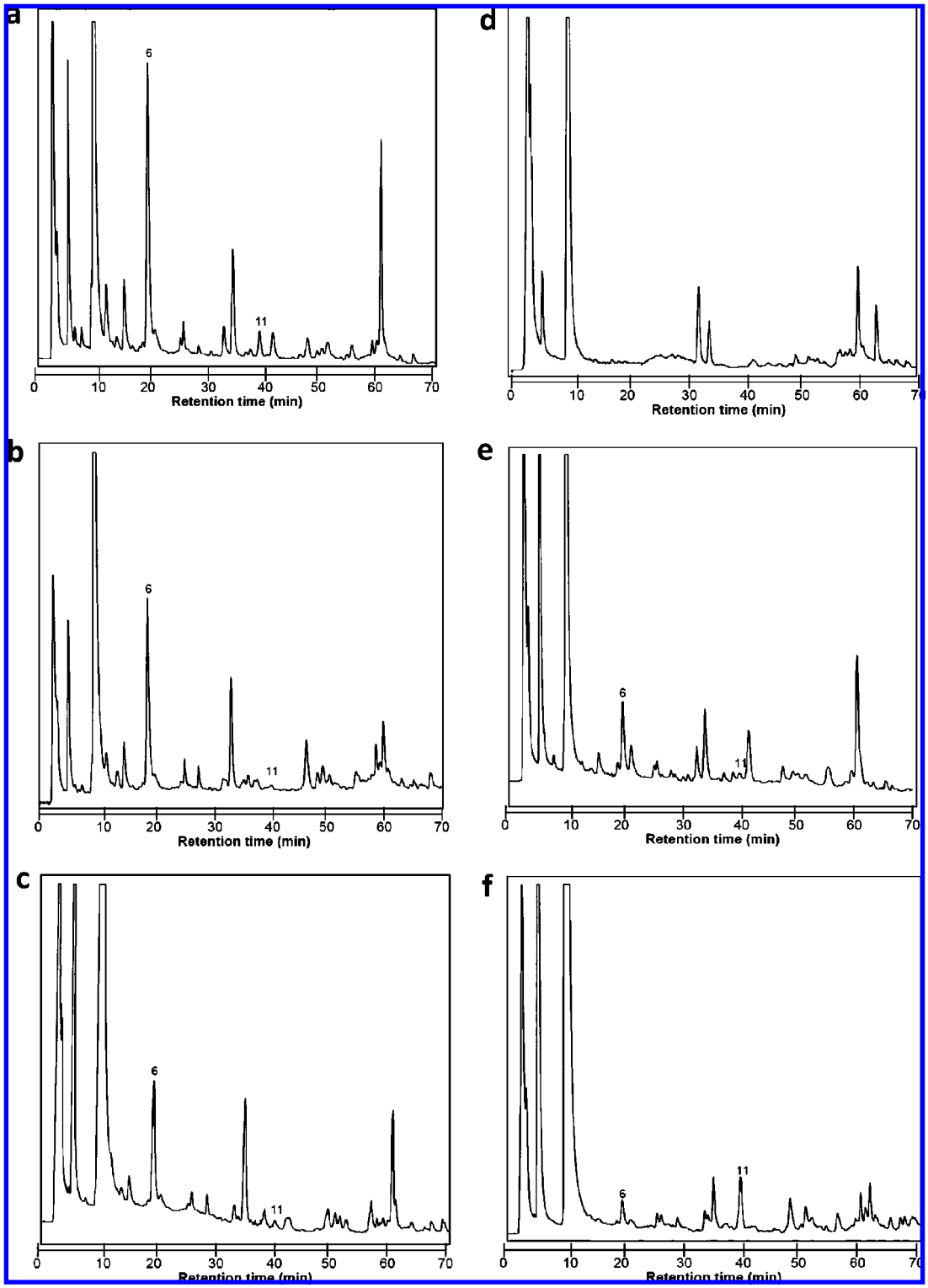

Figure 2. Representative HPLC patterns of dabsyl-amino acids including theanine and GABA contents in various teas, including green tea (**a**), oolong tea (**b**), black tea (**c**), Pu-erh ripe tea (**d**), Pu-erh raw tea (**e**), and GABA tea (**f**). The numbers 6 and 11 indicate theanine and GABA, respectively. Samples were derivatized with dabsyl chloride, and detection was at 425 nm. A Hypersil GOLD Thermo column was used.

Another reversed phase column (Zorbax ODS, 250 mm × 4.6 mm i.d., 5 μm particle size) coupled with a C18 cartridge was used. The gradient elution profile between 0 and 15 min was kept at 100% of solvent A; 15−20 min, linear gradient change to 75% of solvent A; 20−40 min kept at 75% of solvent A; 40−50 min, linear gradient change to 0% of solvent A; 50−60 min kept at 0% of solvent A; 60−65 min, linear gradient change to 100% of solvent A. The column was ready for the next sample run after column conditioning of >30 min. Other parameters of this system were the same as for the Hypersil GOLD Thermo column described above.

HPLC Method Validation. The reversed phase column (Hypersil GOLD Thermo) was used for sample analysis, and the precision, selectivity, accuracy, detection quantification limits, and linearity ranges were estimated. To evaluate the precision of the method, five replicate analyses of a standard solution on different days were performed for both the retention time and the peak area of the dabsyl-amino acid standards. The resolutions were >0.5 min for all of the determined peaks besides peaks 1 and 2. The accuracy was assessed by recovery experiments. Known amounts of dabsyl-amino acid standards were added to the tea dabsylated sample. The recovery rate was calculated

Table 1. Quantification of Theanine, GABA, and Other Amino Acid Contents in Green Teas

tea	theanine[c]	GABA	Ser	Thr	Gly	Ala[d]	Val[e]	Trp	Phe	Ilu	Leu	Tyr[f]
General Green Tea Leaves												
Xi-hu lake Longjing, China	1307.86 ± 99.57[a]	53.71 ± 7.81	86.85	ND[b]	ND	42.48	ND	26.73	12.67	16.81	66.20	5.34
Tenren, green tea (Longjing), Taiwan	1400.90± 81.58	26.35 ± 7.35	135.03	ND	ND	47.43	ND	36.11	26.37	33.06	34.50	6.93
Qingxin-biluo (Min-lao tea factory)	1492.96 ± 64.87	90.78 ± 17.39	199.90	186.35	49.78	93.153	254.23	95.52	147.07	137.74	103.98	5.23
Lion mountain Longjing, China	1562.85 ± 70.96	68.43 ± 9.80	ND	ND	ND	ND	45.22	8.46	18.91	74.65	ND	4.38
Zhen-pin (high grade) Longjing, China	3029.98± 171.58	105.40 ± 9.94	176.50	105.33	35.32	85.40	51.56	51.60	12.78	15.04	74.20	13.3
High-Mountain Green Tea												
Yunnan high-mountain tea 1, China	1336.75 ± 79.70	58.28 ± 13.04	112.50	ND	ND	58.49	74.78	25.47	30.02	22.20	39.24	4.32
Yunnan high-mountain tea 2, China	1668.44 ± 72.58	52.07 ± 10.32	87.59	ND	ND	42.52	71.19	ND	ND	ND	ND	5.97
Meng-pa-zhai (2007 spring), Yunnan, China	2111.38 ± 67.57	ND	91.62	ND	ND	58.07	70.82	71.00	76.04	17.60	28.02	8.61
Nanru mountain (2005 spring), China	2116.52 ± 85.77	ND	88.13	ND	ND	38.12	ND	ND	ND	ND	ND	9.77
Powder of Commercial Green Tea												
Tenren, green tea powder, high grade, Taiwan	686.53 ± 75.85	20.56 ± 7.82	163.16	ND	ND	36.84	127.55	15.02	30.51	31.05	35.62	3.38
Tenren, green tea powder, Taiwan	786.85 ± 75.37	19.83 ± 8.28	142.28	ND	ND	29.73	71.43	47.71	11.24	51.49	37.37	2.42
Anxin, green tea powder	791.47 ± 74.24	31.70± 7.62	137.26	ND	ND	ND	95.86	74.81	27.47	54.16	38.12	9.97
Tenren, cold steep green tea powder	936.15 ± 80.13	19.61 ± 8.09	136.40	ND	ND	37.13	45.88	18.01	24.94	63.14	49.01	6.68

[a] Value (μg/g) = mean ± SD ($n \geq 3$). [b] ND, nondetectable. [c] Peak 6 = Thea (major) + Lys (minor). [d] Peak 8 = Ala (major) + His (minor). [e] Peak 10 = Val (major) + Tyr (minor). [f] Peak 17 = Tyr (major).

Table 2. Quantification of Theanine, GABA, and Other Amino Acid Contents in Oolong Tea

name	theanine[c]	GABA	Ser	Thr	Gly	Ala[d]	Val[e]	Trp	Phe	Ilu	Leu	Tyr[f]
Tenren Oolong Tea												
Tenren oolong tea bag	170.06 ± 63.03[a]	19.17 ± 7.58	140.68	ND[b]	ND	ND	80.86	26.39	34.56	57.42	50.47	5.32
Tenren oolong (low quality)	699.86 ± 67.67	19.97 ± 7.51	102.12	ND	ND	ND	51.62	30.33	18.97	25.28	35.18	4.68
Tenren oolong (middle quality)	1130.43 ± 81.08	23.95 ± 8.16	187.47	ND	ND	48.96	72.30	63.08	51.38	39.88	31.46	9.39
Tenren oolong (high quality)	1677.74 ± 69.92	34.61 ± 8.21	189.59	ND	ND	60.43	80.19	45.08	52.76	54.96	46.50	7.99
Dong Ding Oolong Tea												
Nantou Deer valley, Dong Ding	406.24 ± 73.05	25.79 ± 7.67	133.52	ND	ND	28.68	ND	43.92	27.37	58.02	34.54	5.32
green hill tender leaf tea (super)	441.90 ± 75.78	31.16 ± 7.68	144.45	ND	ND	37.57	45.37	18.85	72.72	46.60	30.23	10.36
Hua-tai tender leaf tea	466.88 ± 75.20	31.32 ± 7.38	102.50	ND	ND	ND	59.90	33.06	36.17	45.73	35.33	8.27
Tenren, Dong Ding oolong Tea	553.63 ± 72.87	39.78 ± 7.97	140.68	ND	ND	33.81	45.86	26.39	34.56	57.42	50.47	5.77
Dong Ding spring tea	744.30 ± 69.25	ND	110.59	ND	ND	ND	ND	34.25	35.26	34.60	59.42	7.91
High-Mountain Oolong Tea												
Ah-Li mountain oolong tea	2430.34 ± 147.41	46.29 ± 9.82	104.10	125.54	44.02	79.59	97.83	52.51	27.77	57.80	34.74	14.33
Lisan high-mountain oolong tea	2831.40 ± 265.89	101.16 ± 11.57	104.28	ND	44.94	31.81	47.30	28.11	13.28	37.83	27.90	5.52

[a] Value (μg/g) = mean ± SD ($n \geq 3$). [b] ND, nondetectable. [c] Peak 6 = Thea (major) + Lys (minor). [d] Peak 8 = Ala (major) + His (minor). [e] Peak 10 = Val (major) + Tyr (minor). [f] Peak 17 = Tyr (major).

by comparing the obtained amounts with those added, and their values ranged between 90 and 100%. Calibration curves were constructed over six different concentrations. Each standard was analyzed in triplicate, and the peak area was plotted against the corresponding concentration.

Data Analysis. For all of the measurements, more than three replicate samples were taken for analysis. All of the values were averaged, and the standard deviation (SD) was obtained for statistical data analysis.

RESULTS

The separation of standard dabsyl-amino acids is shown in **Figure 1**. By the reversed phase column (Hypersil GOLD Thermo), the dabsyl free amino acids derived from Ser, Thr, Thea, Gly, Ala, Val, GABA, Trp, Phe, Ile, Leu, and Tyr could be detected in baseline-separated peaks on the chromatogram (**Figure 1a**). A better separation of standard dabsyl amino acid was achieved by the Zorbax ODS column (**Figure 1b**); the dabsyl-Asn, -Gln, -Ser, -Asp, -Glu, -Arg, -Thr, -Thea, -Gly, -Ala, -Met, -Pro, -Val, -GABA, -Trp, -Phe, and -Tyr could be detected separately on the chromatogram (**Figure 1b**). Chromatograms of the tea samples analyzed by the Hypersil GOLD Thermo column are shown in **Figure 2**, including those of green, oolong, black, Pu-erh, and GABA teas. The quantitative pattern of amino acids, particularly of theanine and GABA, is shown in **Tables 1–3**. The presence of theanine and GABA was confirmed by cochromatography with an authentic standard. Under these conditions, the calibration curves for measuring both dabsyl-theanine and dabsyl-GABA showed a coefficient of linearity near unity ($R^2 = 0.99$). The lower limit of quantification (LLOQ) was >1000 ng, whereas the limits of detection (LOD) were 11.62 ng (for dabsyl-theanine) and 3.43 ng (for dabsyl-GABA), respectively.

By the manufacturing method, tea samples were divided into three groups, green, oolong, and other teas. **Table 1**, first group, shows the theanine content of green tea, which varied from 686.53 to 3029.98 μg/g. After subclassification of the green teas into three groups—general tea leaves, high-mountain tea leaves, and the powder of tea bags—the variance of theanine content became 686−936 μg/g in the powder of tea bags, but 1307−3029 μg/g in general and high-mountain tea (**Figure 3a**). The GABA contents were low on average; only Ah-Li mountain oolong tea and Zhen-pin Longjing tea reached 90 μg/g. In the second group, the theanine content of oolong tea varied from 170.06 to 2831.40 μg/g (**Table 2**). After subclassification of oolong tea into three groups, the theanine content varied from 406.24 to 744.30 μg/g in Dong Ding oolong tea, from 2430.34 to 2831.40 μg/g in high-mountain oolong tea, and from 170.06 to 1677.74 μg/g in Tenren oolong tea. Interestingly, the theanine content in the Tenren oolong tea group correlated to its sale price and quality. **Figure 3a** illustrates that oolong tea samples show significant amounts of theanine present by their source, and these results indicate high-mountain tea contains a higher level of theanine. The GABA contents were low, aside from that of Lisan high-mountain oolong tea. Like Zhen-pin, Lisan high-mountain oolong tea contains high theanine and GABA contents. Taken together, the theanine content in high-mountain green and oolong teas averaged >2000 μg/g (**Figure 3b**).

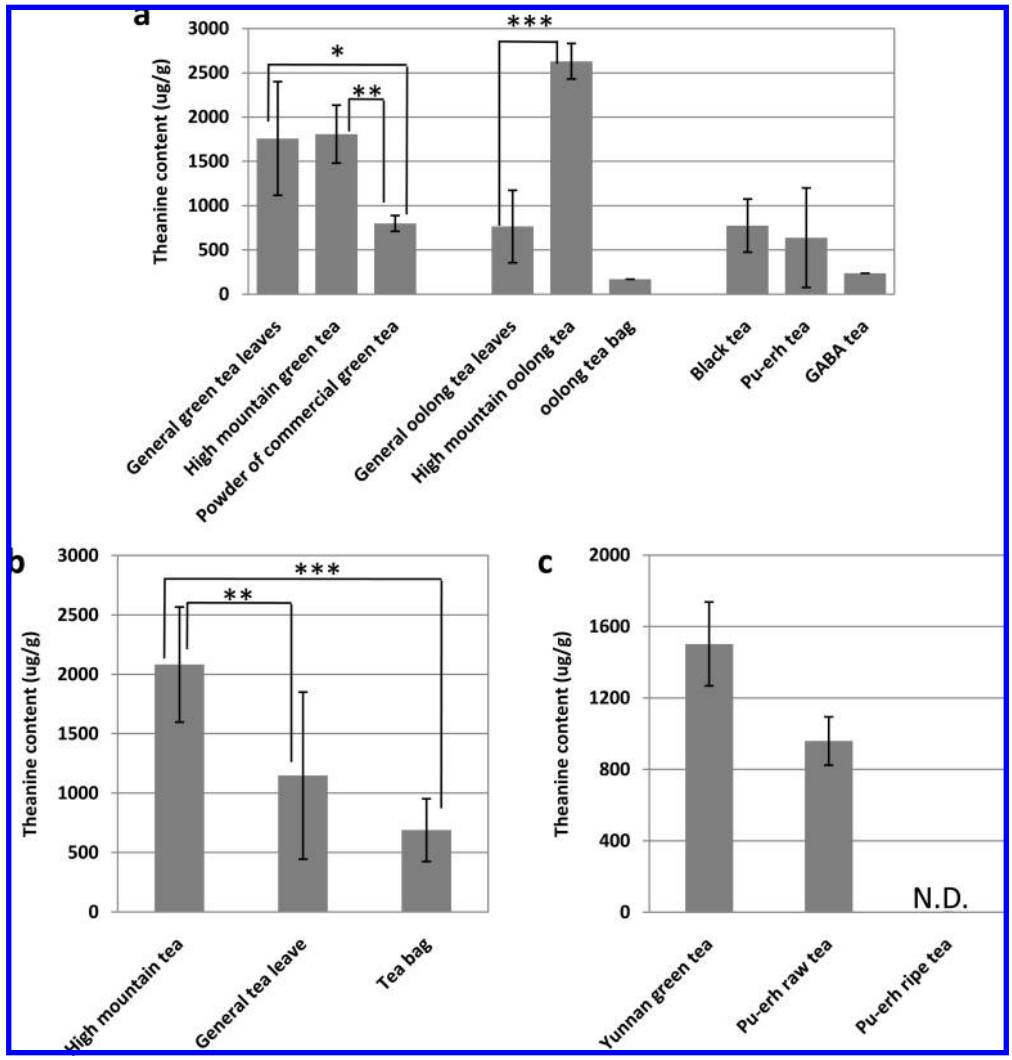

Figure 3. Comparison of theanine and GABA contents in various teas: (**a**) average theanine contents from teas categorized by piling steps as nonfermented teas (green tea), semifermented teas (oolong tea), and other teas (black tea, Pu-erh tea, and GABA tea); (**b**, **c**) comparison of theanine contents by tea manufacturing source and procedures. Bars depict mean ± SE of each group. ∗, ∗∗, and ∗∗∗ represent a statistically significant difference betweem groups, $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

Table 3. Quantification of Theanine, GABA, and Other Amino Acid Contents in Black Tea, Pu-erh Tea, and GABA Tea

name	theanine[c]	GABA	Ser	Thr	Gly	Ala[d]	Val[e]	Trp	Phe	Ilu	Leu	Tyr[f]
Black Tea												
Stassen pure Ceylon black tea	470.77 ± 72.74[a]	55.45 ± 10.11	113.50	ND[b]	ND	ND	ND	81.29	27.37	58.02	36.37	6.32
London black tea	784.87 ± 64.26	ND	87.55	ND	ND	16.52	64.17	23.18	30.12	22.96	31.81	10.47
Lipton yellow-label black tea	1070.19 ± 95.36	34.50 ± 8.10	119.46	ND	ND	33.90	74.84	83.45	71.78	48.81	54.69	7.20
Pu-erh tea												
Pu-erh ripe tea 2004, wet fermentation, Yunnan, China	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	4.59
Pu-erh raw tea, Yi-Wu main mountain, China	862.95 ± 69.53	44.56 ± 11.90	115.73	ND	ND	46.35	54.40	8.44	16.39	12.90	31.37	6.33
Pu-erh raw tea 2004, natural fermentation, Yunan, China	1054.49 ± 71.56	46.07 ± 18.16	86.01	ND	ND	12.87	63.02	6.43	16.42	15.04	31.37	4.12
Specially Made Tea												
GABA tea	237.94 ± 73.69	197.51 ± 8.40	ND	ND	ND	ND	56.17	75.18	25.24	55.06	35.16	5.44

[a] Value (μg/g) = mean ± SD ($n \geq 3$). [b] ND, nondetectable.; [c] Peak 6 = Thea (major) + Lys (minor). [d] Peak 8 = Ala (major) + His (minor). [e] Peak 10 = Val (major) + Tyr (minor). [f] Peak 17 = Tyr (major).

Black tea, Pu-erh tea, and GABA tea were involved in the third group. In black teas, the theanine content varied from 470.77 to 1070.19 μg/g, and their GABA contents were all below 55.45 μg/g (**Table 3**). The subclassified Pu-erh teas were Pu-erh ripe tea and Pu-erh raw tea. For Pu-erh ripe tea, neither theanine nor GABA content could be detected. In contrast, the theanine content was from 862.95 to 1054.49 μg/g and the GABA content was about 45 μg/g in Pu-erh raw tea. Compared to the green tea, Yunnan high-mountain teas 1 and 2 are also sources of leaves for making Pu-erh tea. The theanine content decreases in the following order: Yunnan high-mountain green tea, Pu-erh raw tea, and Pu-erh ripe tea (**Figure 3c**). GABA teas have a high GABA content, manufactured as they are by a special procedure under anaerobic conditions. The commercial GABA tea sample contained less theanine (237.94 μg/g) and more GABA (197.51 μg/g) compared to green teas.

DISCUSSION

Dabsyl chloride can react with the amine group to form a stable product such as dabsyl amino acid. Dabsyl products with

a strong absorbance at 425 nm can be applied to couple with TLC or HPLC separate system (*15*). Compared to OPA or PITC, dabsyl-amino acid is relatively stable and can be stored for >1 month at 4 °C. Moreover, the detection limit of dabsyl-theanine (11.62 ng) is significantly better than that of OPA (120 ng) (*16*). Furthermore, the lower limit of quantification of dabsyl-theanine can be >1000 ng. Incidentally, Pro and Pro-OH are secondary amino acids that cannot directly react with PITC or OPA reagents (*17*). However, they can react with dabsyl chloride directly by the dabsyl method. In this study, we demonstrated a HPLC system with a reversed phase column (Hypersil GOLD Thermo or Zorbax ODS) is suitable for the analysis of dabsyl-amino acids, separately. It is worthy of note that the running time for the Hypersil GOLD system in **Figure 1a** is longer (70 min) as compared with Zorbax ODS system (55 min) in **Figure 1b**. Furthermore, the resolution capacity of the Zorbax ODS column for dabsyl-amino acids (**Figure 1b**) is better than that of the Hypersil GOLD column (**Figure 1a**). In the initial phase of this study, we have focused on determining the contents of theanine and GABA by using the Hypersil GOLD system (**Tables 1–3**).

Theanine is a unique free amino acid found almost exclusively in tea plants. It is the main component responsible for giving tea its taste and possesses some pharmacological activities. For these reasons, theanine is usually used as an index for determining the characteristics and quality of teas (*10*). Alcazar et al. have shown amino acid patterns in teas; however, the theanine contents of various teas were diverse, even in the same kind of tea (*4*). In the presented data in this paper, we tried to explain the theanine diversity in some teas. When extracted from green and oolong tea leaves (**Figure 3b**), the highest theanine is found predominately in high-mountain teas, in general teas, and then in tea bags. A high level of theanine in the high-mountain tea and a low level in the tea bag partially explained how theanine content diversity may due to sample source. In Pu-erh teas, as shown in **Figure 3c**, the theanine of Yunnan high-mountain green tea (1500 μg/g) exceeded that of Pu-erh raw tea (900 μg/g), which in turn exceeded that of Pu-erh ripe tea (ND). Yunnan high-mountain green tea is made with steps similar to those used to obtain Pu-erh raw tea, but without the secondary fermentation and oxidization. The difference between the Pu-erh raw and ripe teas was principally in the piling steps. The disparity of tea-making steps may explain the theanine content diversity in Pu-erh teas. In our samples, besides GABA tea, other teas seemed to have a lower GABA content (<100 μg/g).

Because dabsyl chloride reacts with primary amines, secondary amines, and some hydroxyl groups, it is a powerful reagent for the microdetermination of amino acids, aliphatic amines, and polyamines (*14*). Free amino acids, such as His, Lys, and Tyr, have more than one functional group, which means they could form multiple derivatized products. Separating these products from other dabsyl-amino acids could prove to be challenging. This problem, however, could be solved because in tea sample dabsylation, dabsyl chloride is a major reactant and amino acid content is relatively low, so that the dabsyl-amino acid would be the major "dabsyl-saturated" product, as demonstrated by the fact that the major peak of Tyr is peak 17 and peaks 6, 8, and 10 could be taken as Thea, Ala, and Val (Hypersil GOLD Thermo column).

In summary, we focus on determining the theanine and GABA contents in teas by a dabsylation HPLC system. We found that the theanine variability in green and oolong tea samples may be partially due to the sample source. High-mountain teas usually contain high levels of theanine. Second, different teamaking steps, such as those employed with Pu-erh teas, may contribute to the theanine diversity.

ABBREVIATIONS USED

HPLC, high-performance liquid chromatography; LOD, limit of detection; LOQ, limit of quantification; GABA, γ-aminobutyric acid; DABS-Cl, dabsyl chloride, dimethylaminoazobenzene sulfonyl chloride; OPA, *o*-phthaladehyde; PITC, phenylisothiocyanate; Lys, lysine; Asn, asparagine; Arg, arginine; Gln, glutamine; Ser, serine; Glu, glutamic acid; Asp, aspartic acid; Thr, threonine; Thea, theanine; Gly, glycine; Ala, alanine; His, histidine; Tyr, tyrosine; Met, methionine; Pro, praline; Val, valine; Trp, tryptophan; Phe, phenylalanine; Ile, lsoleucine; Leu, leucine; ND, nondetected; EA, ethyl acetate; aa, amino acid.

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