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Tentative identification, quantitation, and principal component analysis of green pu-erh, green, and white teas using UPLC/DAD/MS

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Abstract

Tea (*Camellia sinensis* L.), an important drink and a natural medicine for thousands of years, contains many health beneficial compounds. Growing season, geographical region, and fermentation methods create many variations in tea compositions, which contribute to each tea's uniqueness. In this study, a simple, rapid, and efficient ultra-performance liquid chromatography (UPLC) method combined with diode array detector (DAD) and mass spectroscopic (MS) detection and chemometrics analysis was used to analyse three different types of teas (green pu-erh, green tea, white tea). Using the developed method, 68 compounds were identified and 54 were quantified based on retention times, UV spectra, and MS spectra by referencing to available standards and data in the literatures. The results showed the chemical differences between the tested teas. Principal component analysis (PCA) was applied to classify and distinguish between tea samples.

Keywords

Camellia sinensis; Tentative identification; Quantification; UPLC/DAD/MS; PCA

1. Introduction

Tea (*Camellia sinensis* L.) has been used as an important drink for over 1000 years because of its health benefits for the human body. It is one of the most widely consumed beverages in the world, next to water (Alan Crozier, Jaganath, & Clifford, 2009; Dufresne & Farnworth, 2001; Editorial committee of encyclopedia of Chinese medicinal plant-sEds, 1999). Tea is cultivated in more than 30 countries. The growing seasons, geographical regions, processing, and fermentation methods create many varieties that contribute to each tea's uniqueness. Teas are commercially distinguished into three major categories according

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to the degree of fermentation: non-fermented (green, white, and green pu-erh teas), partially fermented (oolong and paochong teas), and fully fermented (black and pu-erh teas), although other classifications have been proposed (Alcazar et al., 2007; Fernandez, Martin, Gonzalez, & Pablos, 2000; Lin, Lin, Liang, Lin-Shiau, & Juan, 1998). In the fermentation process, the enzymatic oxidation of polyphenols takes place, leading to the formation of theaflavins and thearubigins, which are responsible for their characteristic aroma and colour (Palmer, 1984). In general, green and oolong teas are consumed mainly in Asia and Northern Africa, white tea in Asia and Europe, pu-erh tea in Asia, and black tea worldwide (Wu et al., 2007).

Taxonomically, *C. sinensis* (L.) O. Kuntze has two subspecies, *C. sinensis* var. *sinensis* (China tea) and *C. sinensis* var. *assamica* (Masters) Kitamura (Assam tea or pu-erh tea in China). There are two definitions for white teas. The traditional Chinese definition of white tea belongs only to “khenghe bai hao” and “fudin bai hao”, found only in Fujian province of China. The definition in other countries of white tea is less sophisticated, it could be any tea made from newly grown buds and young leaves of the tea plants. The buds may be shielded from sunlight during growth to reduce the formation of chlorophyll, giving the young leaves a white appearance (Hilal & Engelhardt, 2007).

Many studies have been reported on the determination of chemical components of teas, especially on tea phenolics. It has been shown that teas contain purine (xanthine) alkaloids, phenolic compounds (catechins, *O*-glycosylated flavonols, *C*-glycosylated flavones, proanthocyanidins, and phenolic acids and their derivatives), terpenoids, fatty acids, essential oils, and amino acids. Oolong and black teas also contain the fermented oxidation products of catechins, theaflavins, and polymeric thearubigins (Alan et al., 2009; Chiu & Lin, 2005; Clifford, Stoupi, & Kuhnert, 2007; Del Rio et al., 2004; Dufresne et al., 2001; Editorial committee of encyclopedia of Chinese medicinal plant-sEds., 1999; Ito, Sugimoto, Kakuda, & Kubota, 2002; Kato & Shibamoto, 2001; Lakenbrink, Engelhardt, & Wray, 1999; Miketova et al., 2000; Naef, Jaquier, Velluz, & Maurer, 2006; Zeeb, Nelson, Albert, & Dalluge, 2000; Zhou, Zhang, Xu, & Yang, 2005; Zhu et al. 2004). Biological studies have demonstrated the health benefits from tea phenolics, especially epigallocatechin gallate (EGCG), the major catechin constituting over 10% of the dry weight of green teas (Alan et al., 2009; Dufresne et al., 2001; Editorial committee of encyclopedia of Chinese medicinal plant-sEds., 1999; Friedman, 2007; Friedman et al., 2007; Jie, Lin, Zhang, Lv., He, & Zhao, 2006; Lambert & Yang, 2003).

Modern analytical methods are also used for tea classification and quality control based on the chemical components of teas. Reported methods include gas chromatography mass spectrometry (GC/MS, Pongsuwan, Fukusaki, Bamba, Yonetani, Yamahara, & Kobayashi, 2007) and liquid chromatography mass spectrometry (LC/MS) for characterisation of teas (Pongsuwan, Bamba, Harada, Yonetani, Kobayashi, & Fukusaki, 2008; Xie et al., 2009), capillary electrophoresis (CE) analysis of major amino acids in tea leaves and beverages (Hsieh & Chen, 2007), near infrared spectroscopy (NIR) identification of green, black, and oolong teas (Zhao, Chen, Huang, & Fang, 2006), NMR measurements (Fujiwara, Ando, & Arifuku, 2006), and HPLC with UV detection (Fernandez et al., 2000; Zheng, Watson,

Johnston, Clark, Edrada-Ebel, & Elseheri, 2009). Some of these methods were coupled with chemometrics for studies of the chemical compounds and chromatograms of tea extracts.

In a previous study, we used a standardised profiling method based on HPLC/DAD/MS to examine 41 green teas and 25 fermented teas (Lin, Chen, & Harnly, 2008). However, the differences between green pu-erh teas, green teas, and white teas were not investigated. As part of our continuous effort in botanical research, a newly developed UPLC/DAD/MS method was used for tentative identification and quantification of the major constituents in green pu-erh teas, green teas, and white teas. The method proved to be an effective alternative for the standardised profiling method used previously. Principal component analysis (PCA) was performed to categorise the tested teas.

2. Material and methods

2.1. Standard compounds and other chemicals

(+)-catechin (C), (–)-epicatechin (EC), (–)-epigallocatechin (EGC), (–)-epigallocatechin 3-O-gallate (EGCG), gallic acid (GA), caffeine (CAF), theobromine (TB), theophylline (TP), apigenin, *p*-coumaric acid, caffeic acid, chlorogenic acid, kaempferol, quercetin, and kaempferol 3-*O*-*p*-coumaroylglucoside were obtained from Sigma Chemicals Co. (St. Louis, MO). Quercetin 3-*O*-glucoside, kaempferol 3-*O*-glucoside, and myricetin were purchased from Extrasynthese (Genay, Cedex, France). HPLC grade methanol, acetonitrile and formic acid were purchased from VWR International, Inc. (Clarksburg, MD). HPLC grade water was prepared from distilled water using a Milli-Q system (Millipore Laboratory, Bedford, MA).

2.2. Tea samples

A total of 15 tea samples were obtained from various sources. The 5 green pu-erh teas and 5 white teas were purchased through internet. The 5 green teas were purchased at local stores in China. The detailed information for the tea samples are shown in Table 1. All the samples were finely powdered and passed through a 20-mesh sieve and then kept in desiccators at room temperature.

2.3. Standard solutions and sample preparation

Approximately 3.0–5.0 mg of each standard was accurately weighed using a Mettler M3 Micro Balance and was then brought to a concentration of 1.0 mg/mL with methanol–water (60:40, v/v) to provide stock solutions. The stock solutions were mixed and diluted to provide a series of standard solutions at appropriate concentrations for the calibration curves. All the solutions were stored at 4 °C in refrigerator.

100 mg of each tea sample was sonicated (FS30 Ultrasonic sonicator, Fisher Scientific, Pittsburgh, PA) with 5.00 mL of methanol–water (60:40, v/v) for 60 min at room temperature. The slurry mixture was centrifuged at 5000 g for 15 min (IEC Clinical Centrifuge, Damon/IEC Division, Needham, MA). The supernatant was filtered through a 0.45 µm PVDF syringe filter (VWR Scientific, Seattle, WA).

2.4. UPLC/MS conditions

A Waters ACQUITY UPLC system (Waters, Milford, MA) equipped with a binary solvent delivery manager, a column manager, a sample manager, and a diode array detector (DAD) was used. The system was coupled with a Waters Micromass Quattro Micro™ mass spectrometer (Waters, Milford, MA). The instrument was fitted with an Acquity UPLC® HSS C18 column, 1.8 μ m, 2.1 $\times$ 100 nm (Waters, Milford, MA). Chromatographic separation was carried out with mobile phase A (H₂O containing 0.1% formic acid) and mobile phase B (acetonitrile containing 0.1% formic acid). The linear gradient program was as follows: 0–12 min, 5–26% B; 12–14 min, 26–65% B. The flow rate was 0.5 mL/min, the column temperature was 40 °C, the autosampler temperature was 10 °C, and the injection volume was 5 μ L. The DAD was set at 270 and 348 nm for quantitation and UV spectra were recorded from 190–400 nm for tentative identification. Both positive and negative ion modes were used with the ESI-MS. Positive ion mode parameters were as follows: capillary voltage, 3 kV; cone voltage, 20 V; extractor voltage, 3 V; RF lens voltage, 0.3 V; the desolvation gas flow, 650 L/h; the desolvation temperature, 275 °C; and source temperature, 100 °C. Negative ion mode parameters were as follows: capillary voltage, 3.5 kV; cone voltage, 30 V; extractor voltage, 2 V; RF lens voltage, 0.2 V; desolvation gas flow, 700 L/h; desolvation temperature, 300 °C; and source temperature, 100 °C. Data were collected in centroid mode over a mass range of *m/z* 100–1100 with acquisition time of 400 ms and inter-scan delay of 100 ms.

2.5. Quantification of the chemical compounds

Since only 18 standards were available, GA was used as the molar equivalent to quantify its derivatives; EC for epicatechin 3-*O*-benzoate; C for all the tea catechins; EGCG for catechin gallates; caffeic acid for its derivatives; quercetin 3-*O*-glucoside for all the quercetin 3-*O*-glycosides; kaempferol 3-*O*-glucoside and kaempferol 3-*O*-*p*-coumaroylglucoside for all of its kaempferol 3-*O*-glycosides without and with *p*-coumaroyl, respectively. All samples were run in triplicate for both quantitation and multivariate statistical analysis.

2.6. Multivariate statistical analysis

In this study, 3 approaches were used for PCA analysis. The 1st one was to use all the aligned data points of a LC/DAD chromatogram, the 2nd one was to use the quantitative results, and the 3rd one was to use characteristic fingerprints (the relative peak areas of significant chromatographic peaks).

1. All the data points from the LC/DAD chromatogram of each sample was exported from Masslynx and saved in *.csv format. Then all the data were combined into 1 matrix in Microsoft Excel. The matrix was imported to SpecAlign software (Version 2.4 Cartwright Group, PTCL, University of Oxford) for chromatogram alignment, after which, several thousands of data points were converted into decades of peak intensities.
2. The contents of the 54 determined chemical compounds were used as input data, where the first column consist of different tea samples and the remaining columns to the right consist of the contents of the compounds.

3. This approach selected the chromatogram of an authentic sample of a certain botanical as a reference fingerprint (RF, in this study, WT1). The most obvious peak of the RF was selected as the reference peak (RP, peak at retention time 3.71 min in this case). The areas of all other peaks in the chromatograms were normalised against the area of the RP and the ratios of the peaks were entered into a peak table and used for PCA analysis. The detailed process has been discussed previously (Chen, Ozcan, & Harnly, 2007; Chen, Song, & Lin, 2009).

The different kinds of data were introduced into SIMCA-P 11.5 software package (Umetrics) AB, Umea°, Sweden) for the PCA analysis. Unit variance scaling (autoscaling) was performed before PCA scatter plots were obtained based on two first components.

3. Results and discussion

3.1. Optimisation of the UPLC method

Good separation was achieved after screening a series of mobile phases, UPLC columns, and gradient profiles (Fig. 1). Among the standards used, GA, TB, TP, EGC, C, CAF, EC, EGCG and *p*-coumaric acid were quantified at 270 nm, while chlorogenic acid, caffeic acid, quercetin 3-*O*-glucoside, kaempferol 3-*O*-glucoside, myricetin, quercetin, kaempferol 3-*O*-*p*-coumaroylglucoside, apigenin, and kaempferol were quantified at 348 nm. In the previous study (Lin et al., 2008), the HPLC method needed a long analysis time of 70 min, whereas the UPLC/DAD/MS method required only 14min. The combination of the shortened running time with a lower flow rate (0.5 mL/min vs 1.0 mL/min) reduced solvent consumption from 70 to 7 mL per analysis.

3.2. Tentative identification of the chemical compounds

Representative UPLC chromatograms detected at 270 and 348 nm are shown in Fig. 2. The retention times (t_R), wavelengths of maximum absorbance (λ_{max}), deprotonated/protonated molecules ($[M-H]^-/[M+H]^+$), and major fragment ions (NI/PI) are listed in Table 2. Peak identification was based on analysis of the data in the table, comparison with standards, and comparison with data in previously published literatures. The identification process has been described in details previously (Lin et al., 2008). All the results are listed in Table 2.

3.3. Quantification of the chemical compounds

The described UPLC method was subsequently applied to simultaneously determine 54 compounds in the tea samples. The total contents (mg/g tea samples, mean $\pm$ SD) of phenolic acid relatives, catechins, purine alkaloids, proanthocyanidins, *O*-glycosylated flavonols, flavonols and flavones, and acylated glycosylated flavonols for the 3 different groups of teas are listed in Table 3. The results showed that catechins existed at the highest concentrations in all the tested samples. The total contents of catechins in the white teas and green teas were similar and greater than those in green pu-erh teas. Between the tea groups, the contents of phenolic acid derivatives were the highest in white teas, followed by that in green pu-erh teas, and much lower in green teas. Total flavonoid contents were highest in white teas, much lower in pu-erh teas and green teas. Other notables: TP was not detected in green teas, quercetin 3-*O*-dirhamnosylglucoside and kaempferol 3-*O*-*p*-cou-

maroyldirhamnosylhexoside were not detected in white teas, apigenin was not detected in green pu-erh teas.

3.4. Principal component analysis (PCA)

PCA is an unsupervised mathematical procedure that transforms a number of possibly correlated variables into a smaller number of uncorrelated variables called principal components. Its operation can be thought of as revealing the internal structure of the data in a way which best explains the variance in the data.

When chromatographic fingerprints are used for PCA, there are basically two sources of variation contained in chromatographic fingerprints. One comes from the experimental process, and the other from the samples themselves. The main variation arising from the experimental process are retention time shifts that make the comparison of fingerprints acquired from different systems or at different times difficult for PCA analysis. A retention time align step is usually needed for the PCA to be successful. Despite numerous studies, computerised peak alignment programs have had only limited success, as discussed in our previous studies (Chen et al., 2007; Lin et al., 2008).

Fig. 3a shows the PCA score plot from the 1st approach. Since all the chromatograms was collected from one system within a week, the alignment procedure finished flawlessly. However, as shown in the PCA score plot, this approach did not work very well even under those optimal conditions. The primary reasons are (1) tea is a very complex botanical and (2) the data used in PCA included all the information acquired and a lot of them were either redundant or no-critical.

Fig. 3b shows the PCA score plot from the 2nd approach. All the quantitative data were summarised into 1 matrix in Excel. The matrix was then used for PCA. The PCA score plot produced clear separation of the three tea groups (Green pu-erh tea, Green tea and White tea). Although the sample WT1 was separated from the white tea cluster, it is in the direction of white tea group. From the results, we found that the contents of gallic acid, 1,2,6-trigalloylglucose and caffeine in WT1 were much higher compared to other white teas. The method, although effective, is not practical since quantitation of all the major components of a botanical is a very expensive and time-consuming process.

Fig. 3c shows the PCA score plot from the 3rd approach. The biggest advantage of the approach is that it is nearly fool-proof, as the retention time shifts introduced by the instruments and other experimental conditions (fluctuation of the temperatures, the reagents used, and the columns, etc.) could be minimised. The tea samples could be classified into three groups, which were marked as “Green pu-erh”, “Green tea”, and “White tea”. The data showed that the teas of the same category were clustered into the same group. “Green tea” group and “White tea” group were separated from “Green pu-erh” group in PC1, also, the three groups were separated well in PC2. Although GP2 was a little farther from other GP samples, it was separated distinctly from green teas and white teas. It could be asserted that the samples classified into one group were associated with similar chemical properties/ components.

Among the 3 approaches, the 1st approach is the easiest, but unfortunately not very effective when used in very complex samples like teas even under the best possible scenarios. The second approach works, but is too labour intensive for PCA since it used the quantitation results of 58 compound. The 3rd approach gives the best separation among the 3 approaches and can be used to compare fingerprints obtained from different instruments, operated by different people, and under different conditions.

4. Conclusion

The results of this study show that the optimised UPLC fingerprint techniques combined with the chemometrics method are able to classify tea samples objectively and successfully in accordance with the different categories. The results also indicate that the UPLC method has the practical advantages of shorter analysis time and reduced solvent consumption, which makes it an attractive alternative to conventional HPLC technique in routine fingerprint analysis, especially in situations where high sample throughput and fast analytical speed are needed. The method developed in the present study can provide an important reference to the quality control of teas.

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Fig. 1.
UPLC/DAD chromatograms of mixed standards (total scan 200–400 nm).

Fig. 2. Representative UPLC chromatograms of tea samples: (a) green tea registered at 348 nm; (b) green pu-erh tea registered at 348 nm; (c) white tea registered at 348 nm; and (d) white tea registered at 270 nm.

Fig. 3.

(a) PCA plot based on the original profile matrix exported from Masslynx after spectra alignment by SpecAlign software; (b) PCA plot based on the contents of the 54 determined chemical compounds; (c) PCA plot based on the relative peak areas of main peaks.

Table 1

The detailed information of tested teas.

Label	Category	Information
GP1	Green pu-erh tea	Holy mountain trading company, green Tibetan mushroom pu-erh
GP2	Green pu-erh tea	Anjing, Sheng Tai Qing Raw Pu'er
GP3	Green pu-erh tea	Ba Jiao Ting
GP4	Green pu-erh tea	Bulang Shan
GP5	Green pu-erh tea	Guang Yao Hao
GT1	Green tea	Jiming Gong Tea, Sixth Grade, Chengkou, Chongqing City
GT2	Green tea	Mingqian green tea, Sichuan Province
GT3	Green tea	China Zhu Tea
GT4	Green tea	Japanese green tea
GT5	Green tea	Japanese Sencha
WT1	White tea	Adagio tea, white leaf tea, silver needle
WT2	White tea	Adagio tea, white leaf tea, snowbud
WT3	White tea	Adagio tea, white leaf tea, white symphony
WT4	White tea	Adagio teas, white leaf tea, white peony
WT5	White tea	Ji'an white tea

Table 2

Identified components in tested tea samples.

Peak No.	t_R (min)	UV λ_{max} (nm)	$[M-H]^-/[M-H]^-$ (m/z)	NI/PI aglycone, other ions (m/z)	Identification
1	0.49	ND	173/175	—/—	Theanine
2	0.71	254	173/175	—/—	Unknown
3	0.84	273	331/333	169/—	Galloylglucose
4A	1.04	273	343/345	169,191/151,173,187	3-Galloylquinic acid
4B	1.07	274	343/345	169,191/151,173,187	5-Galloylquinic acid
4C	1.08	274	169/171	—/—	Gallie acid
5	1.24	273	343/345	169,191/173,187	4-Galloylquinic acid
6	1.37	264	164/166	113,147/120,149	Unknown
7A	1.69	273	305/307	—/—	Galocatechin
7B	1.74	273	179/181	—/—	Theobromine
8	2.02	275	609/611	—/—	Galocatechin dimer
9	2.23	240,326	353/355	191,291/275	3-Caffeoylquinic acid
10	2.31	240,326	353/355	179,191/275	5-Caffeoylquinic acid
11	2.40	271	179/181	—/—	Theophylline
12A	2.44	278	456/458	—/—	Unknown
12B	2.49	272	456/458	—/—	Unknown
13	2.66	273	287/289	—/195	Unknown
14	3.02	210,270	305/307	—/—	(-)-epigallocatechin
15A	3.12	—	337/339	163,305/163,231	3- <i>p</i> -Coumaroylquinic acid
15B	3.16	310	337/339	163,305/163,209,231	5- <i>p</i> -Coumaroylquinic acid
16	3.26	274	183/185	169/—	Gallie acid methyl ester
17	3.32	276	483/485	—/—	Digalloylglucose
18	3.43	276	289/291	—/—	(+)-Catechin
19A	3.49	235sh,300sh,326	353/—	191/291	Chlorogenic acid
19B	3.50	282	295/297	113,191/—	Caffeoylmalic acid
20A	3.65	271	633/—	—	Strictinin
20B	3.71	270	—/195	—/157	Caffeine
21	3.80	235sh,300sh,326	353/355	179,191/195	4-Caffeoylquinic acid

Peak No.	t_R (min)	UV λ_{max} (nm)	[M-H] ⁻ /[M-H] ⁺ (m/z)	NI/PI aglycone, other ions (m/z)	Identification
22	3.97	240sh,292sh,323	179/181	135/157	Caffeic acid
23	3.97	278	577/579	—	Procyanidin dimer
24A	4.31	279	745/747	—	Gallocatechin catechingallate
24B	4.37	279	745/747	—	Gallocatechin catechingallate
25	4.78	210,279	289/291	—	(-)-epicatechin
26	4.91	213,274	457/459	—	(-)-Epigallocatechin 3-O-gallate
27	5.15	278	457/459	—	(-)-Gallocatechin 3-O-gallate
28	5.32	277	383/385	—	Epicatechin 3-O-benzoate
29	5.52	278	457/459	169/195	Gallocatechin gallate
30	5.58	226,310	163/165	119/120,142,157	<i>p</i> -Coumaric acid
31	5.70	276	897/899	730/195,437,731	Digallocatechin-catechin
32	5.74	276	897/899	730/195,437,731	Digallocatechin-catechin
33	5.82	277	729/731	635/195,446,451	Catechin-4 α -epicatechin 3-O-gallate
34	5.91	278	635/—	—	Trigalloylglucose
35	6.16	262,356	479/481	—/319	Myricetin 3-O-galactoside
36	6.31	262,355	479/481	—/319	Myricetin 3-O-glucoside
37	6.42	276	471/473	287/195,289	(-)-Epigallocatechin 3-O-(4''-O-methyl) gallate
38	6.62	276	471/473	287/195,289	(-)-Epigallocatechin 3-O-(3''-O-methyl) gallate
39	6.67	256,354	771/773	—/303,465,611	Quercetin 3-O-galactosylrutinoside
40	6.98	256,354	771/773	—/303,465,611	Quercetin 3-O-glucosylrutinoside
41	7.16	277	441/443	—	(-)-Epicatechin gallate
42	7.22	278	441/443	—	(+)-Catechin gallate
43	7.42	256,354	609/611	—/303,465	Quercetin 3-O-rhamnosylgalactoside
44	7.59	348	609/611	—/303,465	Rutin
45	7.68	210,256,354	463/465	—/120,157,303	Quercetin 3-O-glucoside
46	7.97	256,354	755/757	—/303,465	Quercetin 3-O-dirhamnosylglucoside
47	8.02	266,348	755/757	—/287,449,595	Kaempferol 3-O-glucosylrutinoside
48A	8.39	265,347	447/449	—/195,287	Kaempferol 3-O-galactoside
48B	8.43	265,347	739/741	—	Kaempferol 3-O-rhamnosylrutinoside
49	8.56	265,347	593/595	—/287,449	Kaempferol-3-O-rutinoside
50	8.74	283,330sh	603/605	—	Unknown

Peak No.	t_R (min)	UV λ_{max} (nm)	[M-H] ⁻ /[M-H] ⁺ (m/z)	NI/PI aglycone, other ions (m/z)	Identification
51	8.86	210,265,347	447/449	-/303	Kaempferol 3-O-glucoside
52	9.37	266,368	317/319	-/-	Myricetin
53	9.75	266,348	781/783	-/287	Kaempferol 3-O-acetyl-dirhamnosylhexoside
54	9.88	220,275	705/707	-/-	Quercetin dihexoside sulphate
55	10.03	221,282	499/501	-/-	Unknown
56	11.52	266,310	1063/1065	-/303	Quercetin 3-O-acylglycoside
57	11.92	266,368	301/303	-/-	Quercetin
58	12.73	266,310	593/595	-/287	Kaempferol 3-O-p-coumaroylglycoside
59	12.85	266,310	901/903	-/287	Kaempferol 3-O-p-coumaroylglycosyl-rhamnosylglactoside
60	12.87	266,310	901/903	-/287	kaempferol 3-O-p-coumaroyl-dirhamnosylhexoside
61	12.96	268	447/449	-/287	Quercetin 3-O-rhamnoside
62	13.01	260,310	885/887	-/287	Kaempferol 3-O-p-coumaroyl-dirhamnosylhexoside
63	13.08	260,310	593/595	-/287	Kaempferol 3-O-6''-p-coumaroylglycoside
64	13.25	218,267,338	269/271	-/-	Unknown
65	13.33	266,368	285/287	-/-	Kaempferol
66	13.56	226,285	467/469	-/-	Unknown
67	13.65	266,316	739/741	285/287	Kaempferol 3-O-di-p-coumaroylhexoside
68	13.72	266,316	739/741	285/287	Kaempferol 3-O-2'',6''-di-p-coumaroylglycoside

Table 3

Quantitation results (mg/g) of some important chemical compounds in tested tea samples.

Peak No	Compound	Contents (mg/g, mean ± SD)		
		Green pu-erh	Green tea	White tea
Phenolic acid relatives				
3	Galloylglucose	0.4116 ± 0.2175	0.2853 ± 0.1813	0.3547 ± 0.1158
4A + 4B + 4C	3-Galloylquinic acid + 5-galloylquinic acid + gallic acid	30.6355 ± 12.2719	16.6109 ± 5.8619	40.6541 ± 12.3919
9	3-Caffeoylquinic acid	0.1387 ± 0.0266	0.0425 ± 0.0282	0.0443 ± 0.0098
10	5-Caffeoylquinic acid	0.4065 ± 0.1020	0.0903 ± 0.0582	0.0356 ± 0.0112
16	Gallic acid methyl ester	0.1950 ± 0.1625	0.1106 ± 0.0342	6.7001 ± 6.3004
17	1,6-Digalloylglucose	0.2357 ± 0.1861	0.2114 ± 0.1476	0.7519 ± 0.4278
19A + 19B	Chlorogenic acid + caffeoylmalic acid	1.1004 ± 0.8073	0.3330 ± 0.1757	0.6435 ± 0.3064
21	4-Caffeoylquinic acid	5.1895 ± 1.7253	0.8125 ± 0.7868	0.0924 ± 0.0835
22	Caffeic acid	0.0191 ± 0.0427	2.5669 ± 1.3071	3.0477 ± 0.8248
30	p-Coumaric acid	3.8126 ± 1.2359	3.9910 ± 1.2861	2.9695 ± 1.6811
34	1,2,6-Trigalloylglucose	4.4141 ± 2.6049	2.9223 ± 1.6583	19.4409 ± 16.0670
	Total	46.5586 ± 14.5440	27.9767 ± 9.0805	74.7346 ± 31.8080
Catechins				
7A	Gallocatechin	0.4287 ± 0.0985	0.3800 ± 0.1826	0.4415 ± 0.3101
14	(-)-Epigallocatechin	15.5085 ± 18.2513	47.0838 ± 29.4833	17.2873 ± 14.3654
18	(+)-Catechin	3.8890 ± 1.6334	1.2264 ± 0.5175	0.6592 ± 0.4646
20A	(-)-Methylepigallocatechin gallate	0.6670 ± 0.1264	0.8923 ± 0.1131	0.8671 ± 0.2337
25	(&minus)-Epicatechin	18.4774 ± 4.4958	15.4012 ± 3.6785	9.3480 ± 3.7320
26	(-)-Epigallocatechin 3-O-gallate	88.3355 ± 37.5361	144.2102 ± 29.1159	169.7685 ± 4.7624
27	(-)-Gallocatechin 3-O-gallate	0.2405 ± 0.0681	0.3326 ± 0.2538	0.3849 ± 0.1964
28	Epicatechin 3-O-benzoate	1.0737 ± 0.4470	1.9573 ± 1.0008	1.2857 ± 0.9623
33	Catechin-4α-epicatechin 3-O-gallate	0.7991 ± 0.4140	0.3315 ± 0.2937	0.6431 ± 0.1915
38	(-)-Epigallocatechin 3-O-(3''-O-methyl) gallate	1.6149 ± 2.2873	1.6435 ± 1.1365	0.2546 ± 0.1547
	Total	131.0343 ± 58.9121	213.7414 ± 52.9722	200.9399 ± 15.6054
Purine alkaloids				
7B	Theobromine	2.1380 ± 0.7187	1.6380 ± 0.7871	1.9818 ± 1.2951
11	Theophylline	0.2277 ± 0.2538	ND	0.3489 ± 0.6978
20B	Caffeine	24.1144 ± 6.2303	29.8456 ± 4.3371	43.0819 ± 4.9826
	Total	26.4800 ± 6.5912	31.4836 ± 5.0835	45.4126 ± 4.0074
Proanthocyanidins				
24A + 24B	Gallocatechin catechingallate	0.4174 ± 0.1259	0.9587 ± 0.2905	0.7988 ± 0.4608
31	Digallocatechin-catechin	0.1082 ± 0.1162	0.6727 ± 0.7336	0.7224 ± 1.1990
32	Digallocatechin-catechin	0.3403 ± 0.2164	0.1761 ± 0.1385	3.1458 ± 1.6156
	Total	0.8660 ± 0.3216	1.8075 ± 0.9498	4.6670 ± 2.7229
O-Glycosylated flavonols				
40	Quercetin 3-O-glucosylrutinoside	0.5014 ± 0.3690	4.4178 ± 1.7759	6.4777 ± 3.2404
43	Quercetin 3-O-rhamnosylgalactoside	3.5999 ± 1.3702	1.2165 ± 1.3354	0.8465 ± 0.4835

Peak No	Compound	Contents (mg/g, mean $\pm$ SD)		
		Green pu-erh	Green tea	White tea
44	Rutin	0.8781 $\pm$ 0.8544	0.0933 $\pm$ 0.0260	0.0063 $\pm$ 0.0142
45	Quercetin 3- <i>O</i> -glucoside	2.5978 $\pm$ 0.7897	0.7904 $\pm$ 0.4351	0.7641 $\pm$ 0.5335
46	Quercetin 3- <i>O</i> -dirhamnosylglucoside	0.0054 $\pm$ 0.0121	0.0733 $\pm$ 0.1000	ND
47	Kaempferol 3- <i>O</i> -glucosylrutinoside	0.5145 $\pm$ 0.2190	5.3761 $\pm$ 2.0145	5.2526 $\pm$ 1.5471
48A + 48B	Kaempferol 3- <i>O</i> -galactoside + kaempferol 3- <i>O</i> -rhamnosylrutinoside	0.3127 $\pm$ 0.2498	1.1999 $\pm$ 1.3701	0.5445 $\pm$ 0.2332
49	Kaempferol-3- <i>O</i> -rutinoside	1.9691 $\pm$ 0.5651	1.0491 $\pm$ 0.6987	0.7587 $\pm$ 0.3128
51	Kaempferol 3- <i>O</i> -glucoside	1.3033 $\pm$ 0.3286	0.9552 $\pm$ 0.6125	0.9692 $\pm$ 0.3647
61	Quercetin 3- <i>O</i> -rhamnoside	0.0725 $\pm$ 0.0325	0.0840 $\pm$ 0.0607	0.1867 $\pm$ 0.0955
	Total	11.7548 $\pm$ 3.0255	15.2556 $\pm$ 3.9826	15.8064 $\pm$ 3.6666
<i>Flavonols and flavone</i>				
52	Myricetin	0.0372 $\pm$ 0.0365	0.0723 $\pm$ 0.0130	0.1788 $\pm$ 0.1449
57	Quercetin	0.1964 $\pm$ 0.1985	0.0176 $\pm$ 0.0363	0.0283 $\pm$ 0.0387
64	Apigenin	ND	0.0642 $\pm$ 0.0254	0.0533 $\pm$ 0.0143
65	Kaempferol	0.0529 $\pm$ 0.0219	0.0216 $\pm$ 0.0182	0.1042 $\pm$ 0.0998
	Total	0.2865 $\pm$ 0.2451	0.1756 $\pm$ 0.0336	0.3646 $\pm$ 0.1034
<i>Acylated glycosylated flavonols</i>				
53	Kaempferol 3- <i>O</i> -acetyl-dirhamnosylhexoside	0.0917 $\pm$ 0.0668	0.0442 $\pm$ 0.0645	0.0386 $\pm$ 0.0272
58	Kaempferol 3- <i>O</i> - <i>p</i> -coumaroylglucoside	0.0807 $\pm$ 0.0348	0.0692 $\pm$ 0.0269	0.2217 $\pm$ 0.1213
59 + 60	kaempferol 3- <i>O</i> - <i>p</i> -coumaroylglucosylrhamnosylglactoside + kaempferol 3- <i>O</i> - <i>p</i> -coumaroyldirhamnosylhexoside	0.0690 $\pm$ 0.0294	0.0253 $\pm$ 0.0290	0.0138 $\pm$ 0.0192
62	Kaempferol 3- <i>O</i> - <i>p</i> -coumaroyldirhamnosylhexoside	0.0176 $\pm$ 0.0110	0.0477 $\pm$ 0.0362	ND
63	Kaempferol 3- <i>O</i> -6''- <i>p</i> -coumaroylglucoside	0.0145 $\pm$ 0.0153	0.0141 $\pm$ 0.0055	0.0322 $\pm$ 0.0314
67	Kaempferol 3- <i>O</i> -di- <i>p</i> -coumaroylhexoside	0.0695 $\pm$ 0.0218	0.0433 $\pm$ 0.0234	0.4431 $\pm$ 0.5119
68	Kaempferol 3- <i>O</i> -2'',6''-di- <i>p</i> -coumaroylhexoside	0.0203 $\pm$ 0.0118	0.0190 $\pm$ 0.0126	0.0903 $\pm$ 0.0775
	Total	0.3633 $\pm$ 0.1392	0.2628 $\pm$ 0.1050	0.8398 $\pm$ 0.7088

ND: not detected.