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Comparison of the Chemical Constituents of Aged Pu-erh Tea, Ripened Pu-erh Tea, and Other Teas Using HPLC-DAD-ESI-MS^n

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Supporting Information

ABSTRACT: Pu-erh tea is a popular beverage in southwestern China and South Asian countries. To explain the differences of aged pu-erh tea and ripened pu-erh tea, the chemical constituents of these teas were identified by HPLC-DAD-ESI-MS^n. In addition, HPLC was used to determine the contents of the major polyphenols, gallic acid, caffeine, and theobromine, in various types of teas. These results showed that the majority of chemical constituents in ripened pu-erh tea and aged pu-erh tea were similar, but the contents of catechins and gallic acid presented significant differences between these two teas. After fermentation by microorganism, the levels of catechins in ripened pu-erh tea were decreased, but the contents of gallic acid and caffeine were conversely elevated compared with aged pu-erh tea.

KEYWORDS: pu-erh tea, HPLC-MS^n analysis, fermentation, catechins

INTRODUCTION

Pu-erh tea is traditionally made with leaves of old wild tea trees of *Camellia sinensis* var. *assamica*, which are found in southwestern China as well as the bordering tropical regions in Burma, Vietnam, Laos, and the very eastern parts of India. Pu-erh is well-known for the fact that it is a compressed tea. The production process of pu-erh tea is significantly different compared with those of other teas, such as green tea, black tea, or oolong tea. After picking, pu-erh tea leaves are turned into raw pu-erh tea by a short process referred to as "kill green". Then leaves are artificially fermented for 6 months to a year with microorganisms to produce ripened pu-erh tea or pressed into cake and stored in natural conditions to make aged pu-erh tea. These two kinds of pu-erh teas present different tastes and colors of tea infusion. For a long time, pu-erh teas were confused by consumers and researchers because few characteristic of these two teas were described scientifically.

Although there are plentiful studies of the chemical constituents of black tea and green tea,[1,2] the effects of the special fermentation process on the chemical constituents of pu-erh tea have not been explained simultaneously and in depth. Recently, some new compounds derived from epicatechin and organic acid in pu-erh tea after fermentation were found.[3,4] Nevertheless, the contents of the major compounds in aged pu-erh tea and ripened pu-erh tea lack a detailed description.

Fermentation is the most important procedure for forming special flavor and tea pigments, such as theaflavins and thearubigins derived from catechins by oxidation. It also influences the contents of major polyphenols in teas.[5] As a result of postfermentation with microorganisms, ripened pu-erh tea develops a taste that is wonderfully complex, silky smooth, and mellow, whereas the aged pu-erh tea stored for a few years shows a mouthfeel similar to that of green tea, even a bitterer flavor. This difference should be caused by different contents of polyphenols.

The special flavor of ripened pu-erh tea formed during the postfermentation process is attractive to many consumers, and its healthcare functions also have been trusted for a long time. It was reported that both ripened and aged pu-erh tea can lower the atherosclerotic risk in animal experiments.[6] Pu-erh tea was also found to possess protective effects against hydrogen peroxide-induced damage in human fibroblast HPF-1 cells.[7]

The major flavanols present in tea are catechin and its gallate derivatives.[8] The main flavonoids contained in tea are conjugates of quercetin, kaempferol, myricetin, and their glycoside conjugating moieties varying from mono- to di- and triglycosides.[9] Other compounds existing in tea were gallic acid and quinic esters of gallic and caffeic acids together with the purine alkaloids, theobromine and caffeine, and proanthocyanidins. In this study, quantitative and qualitative analyses of these compounds in ripened pu-erh tea, aged pu-erh tea, and other teas were performed by HPLC-DAD-ESI-MS^n.

MATERIALS AND METHODS

Samples. Fourteen ripened pu-erh teas and 14 aged pu-erh teas were picked from different production places of Yunnan province in 2005 and then manufactured according to individual fermentation process. Seventeen main commercial green teas from different production places were purchased from Maliandao tea shops. Eleven oolong teas were cultivated and processed in Fujian province, which is the well-known origin production place of Tieguanyin and Dahongpao teas. Seven kinds of black teas were from Anhui, Fujian, and Yunnan provinces. Six white teas were from Fuding city, Fujian province, which is the main origin of white tea. Two yellow teas were collected from Anhui province. All of the details of these teas are listed in the Supporting Information.

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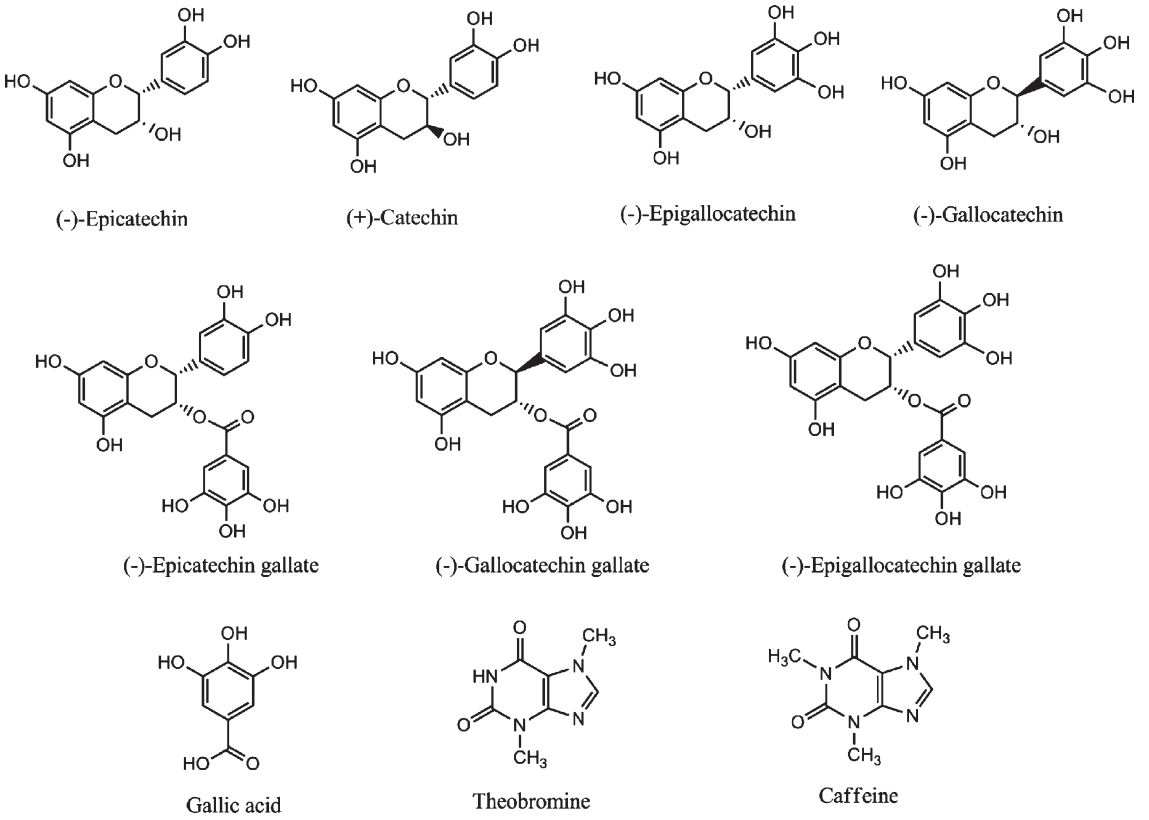

Figure 1. Structures of the major phenolics and purine alkaloids found in teas.

Chemicals. Gallic acid (GA, >98%), caffeine (>98%), theobromine (>98%), (+)-catechin (C, >98%), (−)-epicatechin (EC, >98%), (−)-gallocatechin (GC, >98%), (−)-epigallocatechin (EGC, >98%), (−)-gallocatechin gallate (GCG, >98%), (−)-epigallocatechin gallate (EGCG, >98%), and (−)-epicatechin gallate (ECG, >98%) standards were purchased from Shanghai Tongtian Biotechnology Co. and identified in our laboratory for quantitative analysis (Figure 1). The purity of other chemical constituents isolated from pu-erh tea for peak identification was no less than 95%. HPLC grade acetonitrile (CH_3CN) purchased from Bosio Co. Inc. and distilled water were used as mobile phase of HPLC. Other reagents were of analytical grade.

Sample Preparation. Each tea sample was milled and weighed for 2 g accurately and subsequently transferred into a 50 mL conical flask with cover. Fifty milliliters of hot water (90 °C) was added into the conical flask and maintained at the temperature of 90 °C for 30 min with intermittent shaking (every 10 min) according to the preparation method reported.[10] Then, each extract was filtered through a 0.5 μm Millipore filter before injection to HPLC-MS^n for analysis.

HPLC-DAD-ESI-MS^n Analysis. The Agilent G6300 series HPLC-MS system (Santa Clara, CA) consisted of a Surveyor MS pump, an autosampler, a diode array detector, and an LC/MSD ion trap mass spectrometer with Xcalibur software for data acquisition and analysis. Separations were carried out using an Agilent SB-Aq C_{18} reverse phase column (250 × 4.6 mm i.d., 5 μm) protected with a security guard cartridge (Gemini C_{18}, 4 × 2.0 mm i.d., Phenomenex). The elution used a linear gradient program from 5 to 30% acetonitrile in 0.6% formic acid aqueous solution over 60 min and then changed to 100% acetonitrile using 10 min. The flow rate was 0.8 mL/min. Ten microliter samples were injected. A 15 min re-equilibration time was used between HPLC runs. The DAD acquisition wavelength was set in the range of 200−400 nm. After passing through the flow cell of the DAD, the column eluate was split to 0.2 mL/min, which was directed to a trap mass spectrometer with an electrospray interface (ESI) operating in full scan MS mode from m/z 100 to 2000. Mass spectra were acquired in both negative and positive modes with an ion spray voltage of 3.5 kV, a capillary temperature of 350 °C, a capillary voltage of 35 V, a sheath gas pressure of 25 psi, and an auxiliary gas pressure of 12 psi.

For quantitative analysis, different concentrations of standards were analyzed by an Agilent 1100 series. Chromatographic conditions were the same as described above.

Method Validation. Calibration curves were established for each reference compound. Standard solutions of GA, GC, EGC, C, EC, EGCG, GCG, ECG, theobromine, and caffeine were prepared by dissolving them in a volume of acetonitrile/water (1:1, v/v) individually to generate stock concentrations of 1.7, 0.8, 1.4, 0.5, 0.9, 1.3, 0.6, 0.9, 1.7, and 6.5 mg/mL, respectively. Precision was evaluated by intraday ($n = 5$) assays. Recoveries of the quantified constituents were determined using a certain tea sample, for which the respective chemical contents had been predetermined. Each standard solute was spiked at a close concentration with the sample. Then, recoveries were calculated on the basis of the difference between the total amount determined in the spiked samples and the amount observed in the nonspiked samples.

Statistical Analysis. The mean value and standard deviation of analytes was calculated from the data obtained from the experiment. ANOVA was carried out to determine significant difference (**, $P < 0.01$) by using the software package SPSS Statistics 17.0 for Windows (release 17.0.1; SPSS Inc., Chicago, IL, 2008).

RESULTS AND DISCUSSION

Comparison of Chemical Constituents in Various Teas by HPLC-DAD-ESI-MS^n. To investigate the chemical constituents of teas, HPLC-DAD-ESI-MS^n was used to study the mass spectral fragmentation pattern of different chemical constituents. In

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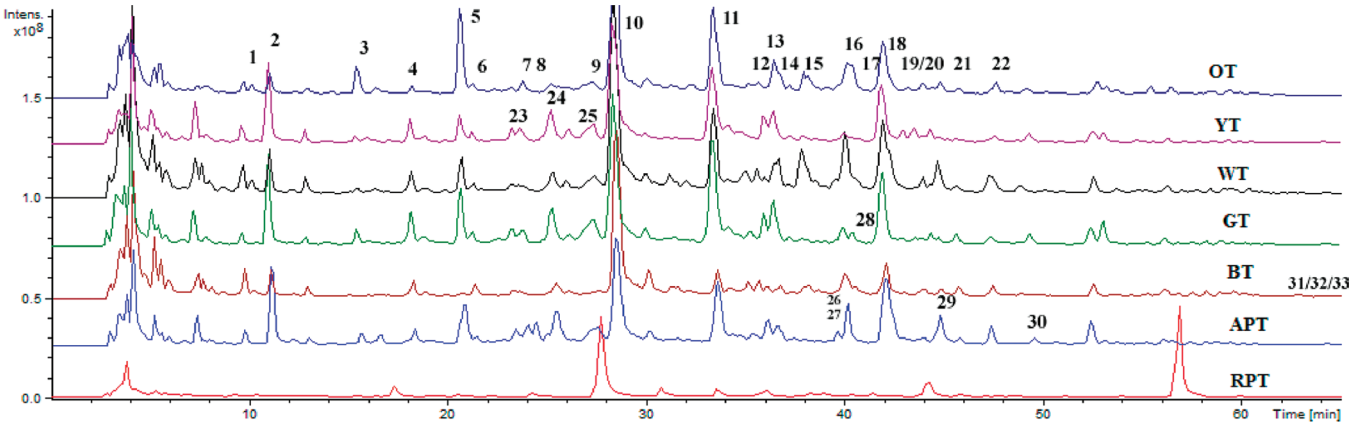

Figure 2. TICs (+MS) of different types of teas. APT, aged pu-erh tea; RPT, ripened pu-erh tea; BT, black tea; GT, green tea; WT, white tea; YT, yellow tea; OT, oolong tea.

Table 1. MS Data of Peaks of TICs (+MS/−MS) of Different Teas and Identification Results of Peaks by Comparison with Retention Time and Quasi-molecular Ion Peaks of Standards

peak	retention time (min)	APT	RPT	BT	GT	YT	OT	WT	+MS/−MS	identification
1	9.8	+[a]	+	+	+	+	+	+	171/169	gallic acid
2	11.2	+	+	+	+	+	+	+	345, 367/343	5-galloylquinic acid
3	15.7	+	+	+	+	+	+	+	307, 329/305	(−)-gallocatechin
4	18.3	+	+	+	+	+	+	+	181,203	theobromine
5	21.0	+	+	+	+	+	+	+	307, 635/305, 611	(−)-epigallocatechin
6	21.8		+	+					181, 203	caffeic acid
7	24.0	+	+	+	+	+	+	+	291, 313	(+)-catechin
8	24.4	+	+	+	+	+		+	355, 377/353	chlorogenic acid
9	27.6	+	+	+	+	+	+	+	291/289	(−)-epicatechin
10	28.6	+	+	+	+	+	+	+	195	caffeine
11	33.6	+	+	+	+	+	+	+	459, 481/457	(−)-epigallocatechin-3-gallate
12	35.6	+	+	+	+	+	+	+	565, 587/563	apegenin-6-*C*-α-L-arabinopyranosyl-8-*C*-*β*-D-glucopyranoside
13	36.5	+	+	+	+	+	+	+	459, 481/457	(−)-gallocatechin-3-gallate
14	36.7		+	+		+	+	+	481, 503	myricetin-3-*O*-*β*-D-galactopyranoside
15	38.2	+	+	+	+	+	+	+	595, 617	apigenin-8-*C*-glucose-rhamnose
16	40.2	+	+	+	+	+	+	+	611, 633/609	quercetin-3-*O*-[α-L-rhamnopyranosyl (1→6)-*O*-*β*-D-glucopyraniside]
17	41.5	+	+	+	+	+	+	+	741, 763	kaempferol-3-*O*-[α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→6)]-*β*-D-glucopyranoside
18	42.0	+	+	+	+	+	+	+	443/441	(−)-epicatechin-3-gallate
19	42.4	+	+	+	+	+	+	+	465, 487/463	quercetin-3-*O*-*β*-D-glucopyranoside
20	42.9	+	+	+	+		+	+	595, 617/593	kaempferol-3-*O*-α-L-rhamnopyranosyl (1→6)-*β*-D-galactopyranoside
21	45.8	+	+	+	+	+	+	+	449, 471/447	quercetin-3-*O*-α-L-rhamnoside
22	47.3	+	+	+	+	+	+	+	449, 471/447	kaempferol-3-*O*-*β*-D-glucopyranoside

[a] "+" indicates that the data of peaks are consistent with the mass data of chemical constituent standards.

addition, catechins and other standards isolated from pu-erh tea were analyzed to identify the peaks on mass total ion chromatogram (TIC). TICs of various teas are shown in Figure 2.

Each sample was analyzed using both negative and positive ionization modes. The quasi-molecular ion peaks of $[M + H]^+$ and $[M + Na]^+$ at positive model provided more dependable information of compounds than data from only the negative model, so multiple-stage mass spectrometry was mainly operated in positive mode. Twenty-two compounds on HPLC-MS were identified postively on the basis of their retention time, absorbance spectrum, and MS fragmentation pattern by cochromatography with standards isolated from pu-erh tea (Table 1).

Although the commercial teas were processed to different extents of fermentation technology, most of the main chemical

Table 2. MS^n Data of Other Compounds Present in Tea

peak	retention time (min)	APT	RPT	BT	GT	YT	OT	WT	+MS/−MS	MS^n
23	23.5	+[a]	+	+	+	+	+	+	507/**483**[b]	331, 313, 169,
24	25.6	+	+	+	+	+	+	+	657/**633**	463, 301
25	27.5	+	+	+	+	+	+	+	**579**, 601/577	409, 427, 291, 151, 139
26	39.6	+	+		+	+	+	+	**611**, 633/609	465, 303
27	39.7		+	+	+	+	+	+	**757**, 779/755	611, 449, 287
28	40.5				+		+		**473**, 495/471	347, 305, 289, 139
29	44.8	+	+	+	+	+	+	+	**595**, 617/593	449, 287
30	49.5	+		+	+	+	+	+	**427**, 459/425	275, 257, 139
31	62.7			+				+	717/**715**	563, 407
32	64.5			+				+	717/**715**	563, 407
33	65.1			+				+	869/**867**	715, 697

[a] + indicates that the data of peaks existed. [b] Boldface indicates the parent ion for MS^n analysis.

constituents from fresh leaves of tea (*Camellia sinensis* or *Camellia assamica*) could still be reserved after the production process. As shown in Table 1, some main catechins existed in all of the teas and also other flavones and purine alkaloids.

On the basis of mass fragmentation data coupled with previously published characterization studies, other compounds in teas were identified by multiple-stage mass spectrometry and absorbance spectrum (Table 2).

Peak 23 (t_R, 23.5 min; λ_{max}, 215, 280 nm) showed a molecular weight of 484 deduced from the $[M + Na]^+$ and $[M - H]^-$ at m/z 507 and 483. Analysis revealed that an $[M - H]^-$ at m/z 483 produced a major secondary fragment (MS^2) at m/z 313. This 170 amu loss equates with the cleavage of a gallic acid unit. Further MS^3 analysis of the MS^2 m/z at 331 produced an ion at m/z 169 due to loss of 162 amu of glucose. UV λ_{max} at 215 and 280 nm also supported the existence of gallic acid rather than flavones. According to published information,[11] peak 23 could be identified as gallic acid-3-*O*-(6′-*O*-galloyl)-*β*-D-glucoside.

Peak 24 (t_R, 25.6 min; λ_{max}, 218, 275 nm) gave a couple of ions of $[M + Na]^+$ and $[M - H]^-$ at m/z 657 and 633, respectively. MS-MS fragmentation of $[M - H]^-$ at m/z 633 yielded a major MS^2 ion m/z at 463 in negative mode. This loss of 170 amu corresponds to the cleavage of gallic acid. MS^2 m/z at 463 continued to lose 162 amu and yielded a major MS^3 ion m/z 301. The UV spectrum showed a λ_{max} at 218 and 275 nm, which is consistent with absorption spectrum of gallic acid. This showed that the aglycone quercetin did not exist in this compound. Peak 24 was therefore tentatively assigned as 3-*O*-galloyl-4,6(*S*)-hexahydroxydiphenoyl-D-glucose on the basis of previous results.[12]

Peak 25 (t_R, 27.5 min), chromatographed with the same absorbance spectrum as catechin, gave a couple of ions $[M + H]^+$ and $[M - H]^-$ at m/z 579 and 577, respectively. MS-MS fragmentation of $[M + H]^+$ at m/z 579 yielded a major MS^2 ion m/z at 291 in positive mode, which was similar to the $[M + H]^+$ ion of catechin. Another major MS^2 ion m/z at 427 yielded MS^3 fragment ions m/z at 139 and 151, which were the typical fragment ions of catechin. Considering the positive MS^n information and previous results,[13] the loss of m/z 288 confirmed the identification of peak 25 as a procyanidin.

Peak 26 (t_R, 39.6 min) showed a couple of ions $[M + H]^+$ and $[M - H]^-$ at m/z 611 and 609, respectively. $[M + H]^+$ m/z 611 gave a major MS^2 ion m/z 465 to lose a 146 amu rhamnose. The fragment at m/z 465 continued to lose 162 amu of glucose and yielded the $[M + H]^+$ ion of aglycone quercetin m/z at 303. Because quercetin-3-*O*-L-rhamnopyranosyl-(1→6)-D-glucopyraniside has already been identified as the peak of retention time 40.2 min on the TIC by comparison with authentic standard, these two glycosyls may be respectively connected to the C-3 and C-7 of flavonol from the information provided by fragment patterns. Peak 26 can be identified as quercetin-3-*O*-rhamnose-7-*O*-glucose.

Peak 27 (t_R, 39.7 min; λ_{max}, 280, 340 nm) had an $[M + H]^+$ at m/z 757, which fragmented to produce a major MS^2 ion at m/z 611. This 146 amu loss equates with the cleavage of a rhamnosyl unit. MS^2 ion m/z at 611 produced an MS^3 ion at m/z 449, which continued to yield a fragment ion m/z 287 corresponding to kaempferol supported by the UV spectrum of λ_{max} at 280 and 340 nm. The position of the conjugating sugar can be deduced from the sequence of glucose loss. According to the compound isolated from tea,[14] peak 27 could be tentatively assigned as kaempferol-3-*O*-[2-*O*-*β*-D-galactopyranosyl-6-*O*-α-L-rhamnopyranosyl]-*β*-D-glucopyranoside.

Peak 28 (t_R, 40.5 min) showed $[M + H]^+$ at m/z 473 yielding a MS^2 fragment m/z 289 corresponding to the special fragment ion of gallocatechin. Also, the characteristic fragment ions m/z at 139 and 151 from gallocatechin were found in the MS^3 fragmentation of m/z 289. Apart from the gallocatechin of 306, the other part of the compound of peak 30 was 172, corresponding to the residue of methyl-gallate. Compared with the compound isolated from tea,[15] this peak can be preliminary assigned as a gallocatechin-3-*O*-(3-methyl)-gallate.

Peak 29 (t_R, 44.7 min; λ_{max}, 280, 340 nm) produced a $[M + H]^+$ ion m/z at 595, which yielded a MS^2 m/z at 449 by the loss of 146 amu related to rhamnose. The MS^2 m/z 449 continued to lose 162 amu of glucose and yielded a fragment m/z 287 corresponding to the kaempferol. Supported by the UV spectrum with λ_{max} at 280 and 340 nm, peak 29 was identified as kaempferol 3-*O*-rutinoside according to the compound isolated from tea.[16]

Peak 30 (t_R, 49.5 min; λ_{max}, 280 nm) yielded an $[M + H]^+$ at m/z 427, which yielded a major MS^2 ion at m/z 257 with the loss of 170 amu related to gallic acid. In addition, the UV spectrum showed the λ_{max} at 280 nm, the same as flavan-3-ol. The MS^2 m/z 275 was supposed to be the $[M + H]^+$ of the epiafzelechin. According to the compound isolated from tea, peak 30 was supposed to be the epiafzelechin-gallate.[16]

Peaks 31, 32, and 33 were identified as theaflavin-3-gallate, theaflavin-3′-gallate, and theaflavin-3, 3′-digallate on the basis of

Table 3. Linearity, Correlation Coefficient (r),[a] and Limits of Detection (LOD) and Quantification (LOQ) of the Compounds Studied

compound	linearity (μg/mL)	r	LOD (ng)	LOQ (ng)
GA	0.09−174.1	0.9998	1.1	3.4
GC	0.42−83.52	0.9999	25.8	85.9
theobromine	0.08−20.81	0.9999	1.4	4.6
EGC	0.89−142.72	0.9999	21.8	72.7
C	0.025−0.624	0.9999	5.5	18.2
EC	0.09−23.84	0.9999	5.0	16.8
caffeine	4.90−245.40	0.9999	1.3	4.4
EGCG	0.16−128.00	0.9999	2.6	8.7
GCG	0.06−14.84	0.9999	2.6	8.8
ECG	0. 93−233.60	0.9999	1.6	5.3

[a] r is the correlation coefficient of each calibration curve, which was determined by six calibration points.

Table 4. Validation Results of the Analytical Method Using an Aged Pu-erh Tea Extract Solution

compound	recovery (%)	precision RSD (%)	repeatability RSD (%)	stability RSD (%)
GA	94.8	1.15	1.16	0.5
GC	83.3	3.59	3.37	1.2
theobromine	105.4	0.40	3.42	1.0
EGC	101.5	1.77	3.28	1.9
C	102.5	0.69	2.73	0.6
EC	93.7	0.37	1.89	0.5
caffeine	115.4	0.37	1.57	0.5
EGCG	83.4	0.54	2.82	1.0
GCG	108.8	0.77	2.19	2.6
ECG	81.0	0.44	2.55	0.7

MS-MS and elution order, because the HPLC elution of this study was similar to that of a previous paper.[17] Kuhnert already did many works on the mass spectrometric studies of theaflavins and thearubigins.[18,19] In the present study, the $[M - H]^-$ ion at m/z 715, the $[M - H]^-$ ion at m/z 867, and their MS^2 ions were the same as theaflavin-gallate and -digallate. These theaflavins were not detected in the ripened pu-erh tea, but were evidently observed in black and white teas. This main characteristic of pu-erh tea should be the difference between black tea oxidative fermentation and microbial pu-erh tea fermentation.

Validation of the Method. *Selectivity.* The selectivity of detection of each compound with good resolution was ensured by the determination of the retention time. The chromatographic conditions allowed the determination of 10 major compounds in 7 types of tea. A high concentration of formic acid was used in the mobile phase to achieve the best resolutions for phenolics and catechins.

Linearity and Limits of Detection (LOD) and Quantitation (LOQ). Calibration graphs for the catechins, gallic acid, caffeine, and theobromine were constructed using six levels of concentration, which covered the concentration ranges expected in each tea sample. The characteristics of the calibration curves, including the range of linearity, the correlation coefficient (r), LOD, and LOQ of each compound, are listed in Table 3.

Recovery, Precision, Repeatability, and Stability. The recovery was determined by spiking a tea extract sample with the addition of standard compounds. To test the precision of the assay method, the aged pu-erh tea sample to be analyzed was injected five times successively under the chromatographic conditions described above. The repeatability of the sample preparation method was tested by determining five equivalent samples from one tea. The stability of the aged pu-erh tea extract sample was tested by repeated injection over 24 h. Table 4 summarizes the results obtained.

Comparison of the Contents of Major Active Compounds of Various Teas by HPLC. Before HPLC analysis, every phenolic and purine alkaloid in all kinds of teas were identified by comparison with the retention time of standards, whereas peak identity and purity were also determined by MS^n analysis. Under the chromatographic conditions, all main compounds analyzed with good resolutions complied with the requirements of determination. The chromatograms of various teas are shown in Figure3.

The contents of these compounds in various teas were calculated as shown in Table 5.

It has been reported that catechin derivatives, particularly EGCG, not only possessed strong antioxidant activity but also can inhibit nitration reactions, modulate carcinogen-metabolizing enzymes, trap ultimate carcinogens, and inhibit cell proliferation.[20,21] This study showed that aged pu-erh tea contained a higher content of EGCG than postfermentation ripened pu-erh tea (**, $p < 0.01$). The levels of other catechins such as C, EC, EGC, GCG, and ECG in aged pu-erh tea were also significantly higher than those of ripened pu-erh tea (**, $p < 0.01$). On the contrary, the postfermentation process increased gallic acids by remarkably high levels in ripened pu-erh (**, $p < 0.01$) compared with aged pu-erh tea.

Analysis on the aged and ripened pu-erh tea was used to explore the effects of microbial fermentation on the contents of major polyphenols in the fresh leaves of pu-erh tea. After fermentation, the level of total catechins (TC) in ripened pu-erh tea decreased significantly compared with aged pu-erh tea (**, $p < 0.01$), whereas the postfermentation process of ripened pu-erh tea elevated the content of gallic acid, which may be accumulated from the decomposition of catechin gallate conjugates by microorganisms. The level of caffeine of pu-erh tea was also affected by the microbial fermentation as found in the the previous paper.[22] There was a significant difference in the content of caffeine between ripened pu-erh tea and aged pu-erh tea (**, $p < 0.01$).

It is known to all that major tea catechins of black tea are oxidized or condensed to other polyphenolic pigments such as theaflavins and thearubigins. Green tea is processed directly by drying and steaming the fresh tea leaves and, thus, no fermentation is involved. Because white and yellow teas are also unfermented, the contents of total catechins were 70.73 ± 22.06 and 72.29 mg/g, respectively, compared with the total content of catechins of 66.38 ± 35.87 mg/g in green tea.

Oolong tea is made from the fresh leaves of *Camellia sinensis*, which are subjected to a partial fermentation stage before drying. Its content of total catechins was 87.82 ± 35.13 mg/g, which was the highest value in all teas. This result revealed that partial fermentation may be a more suitable process for elevating the levels of catechins. Both black and ripened pu-erh teas, which undergo a full fermentation stage, showed similar levels of major catechins. The contents of catechins in black tea were

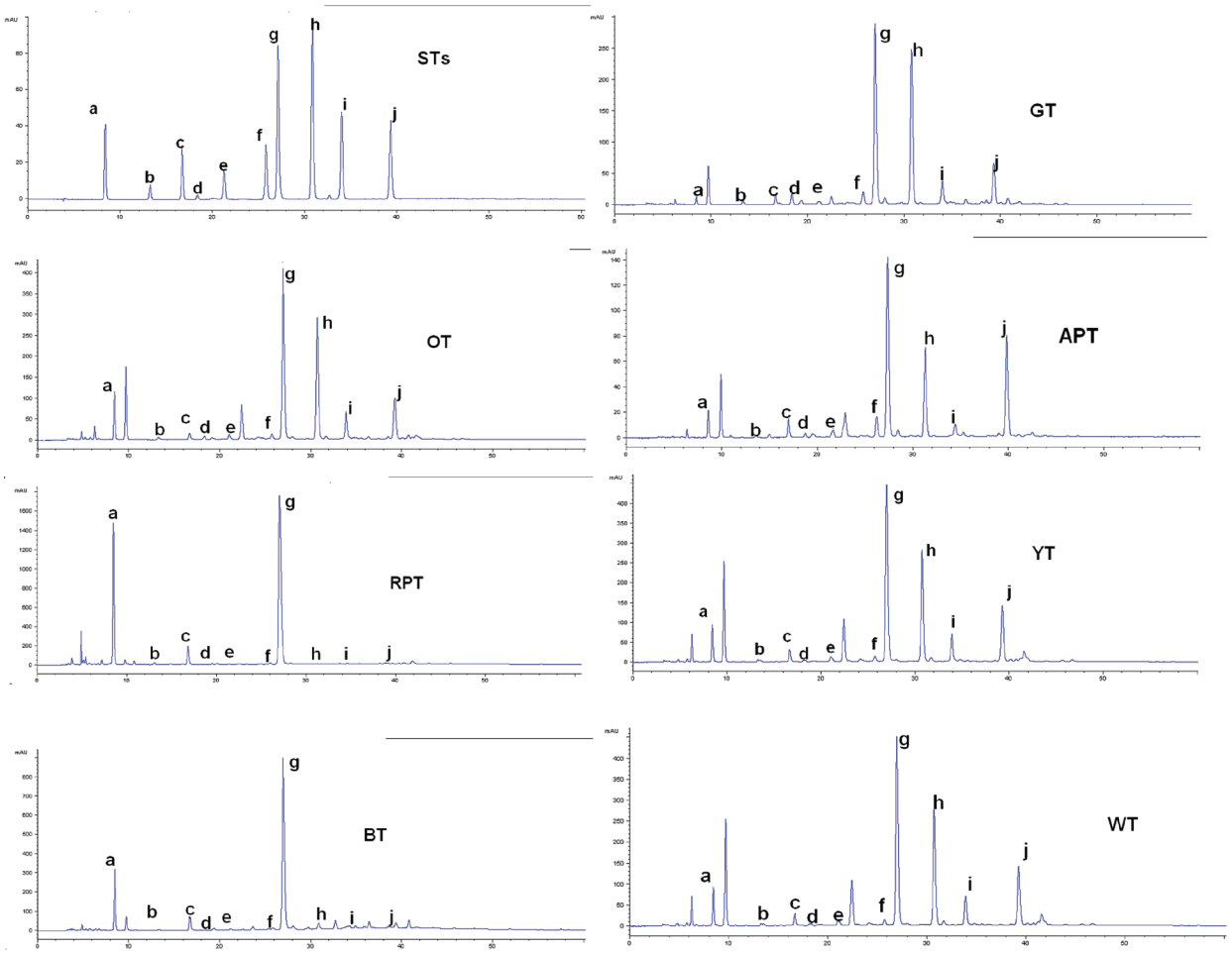

Figure 3. Chromatograms of samples made from seven kinds of teas and standards. Detection was carried out with UV at 280 nm. STs, standards; GT, green tea; OT, oolong tea; APT, aged pu-erh tea; RPT, ripened pu-erh tea; YT, yellow tea; BT, black tea; WT, white tea.

Table 5. Contents of the 10 Studied Compounds in Tea Samples

	contents (mg/g)						
compound	GT (17)[a]	BT (7)	WT (6)	YT (2)	OT (11)	RPT (14)	APT (14)
GA	1.21 ± 0.98[b]	3.91 ± 0.60	4.62 ± 1.10	2.63[c]	0.79 ± 0.69	6.51 ± 2.98**[d]	1.47 ± 1.27
GC	4.79 ± 2.79	4.03 ± 1.28	7.32 ± 2.42	3.05	14.03 ± 5.20	2.90 ± 0.80	3.64 ± 2.37
theobromine	1.26 ± 0.82	0.87 ± 0.21	0.83 ± 0.35	1.35	0.33 ± 0.09	0.92 ± 0.30**	0.75 ± 0.38
EGC	9.83 ± 6.64	0.60 ± 0.12	6.70 ± 2.03	6.74	29.32 ± 12.70	0.41 ± 0.26**	5.00 ± 2.49
C	2.11 ± 1.48	0.54 ± 0.17	2.57 ± 1.52	3.50	0.98 ± 0.37	0.22 ± 0.14**	3.16 ± 1.31
EC	2.98 ± 1.54	0.44 ± 0.12	1.91 ± 0.47	2.36	5.85 ± 2.17	0.57 ± 0.39**	6.01 ± 2.91
caffeine	20.04 ± 11.31	26.80 ± 3.94	33.78 ± 5.28	25.75	18.77 ± 4.23	15.52 ± 2.49**	11.38 ± 3.57
EGCG	33.80 ± 19.20	1.34 ± 0.43	35.79 ± 12.38	35.58	30.81 ± 10.91	0.08 ± 0.07**	9.43 ± 4.27
GCG	4.38 ± 2.20	0.21 ± 0.09	5.68 ± 3.47	6.40	1.51 ± 0.55	0.04 ± 0.01**	1.37 ± 0.65
ECG	8.20 ± 4.31	0.66 ± 0.35	10.40 ± 3.39	12.22	5.10 ± 2.53	0.05 ± 0.05**	9.86 ± 3.97
TC	66.38 ± 35.87	7.79 ± 1.78	70.73 ± 22.06	72.29	87.82 ± 35.13	4.28 ± 1.01**	38.69 ± 16.51

[a] The number of samples for each kind of tea. [b] Data are expressed as the mean ± standard deviation of samples from the same type of tea. [c] For yellow tea, only the mean value is listed because only two samples were collected. [d] **, $p < 0.01$ with respect to APT group.

significantly decreased, whereas the contents of gallic acid were elevated compared with green tea (**, $p < 0.01$).

In conclusion, the types of teas in this study covered almost all of the dominant commercial teas on the market and all of the types of teas classified as undergoing a fermentation process. This study also provided a clear description of the chemical constituents of ripened pu-erh tea, aged pu-erh tea, and yellow tea, which are not familiar to most consumers.

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■ ASSOCIATED CONTENT

Supporting Information. Detailed information on various teas. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

GA, gallic acid; C, (+)-catechin; EC, (−)-epicatechin; GC, (−)-gallocatechin; EGC, (−)-epigallocatechin; GCG, (−)-gallocatechin gallate; EGCG, (−)-epigallocatechin gallate; ECG, (−)-epicatechin gallate; TC, total catechins; APT, aged pu-erh tea; RPT, ripened pu-erh tea; BT, black tea; GT, green tea; WT, white tea; YT, yellow tea; OT, oolong tea; HPLC, high-performance liquid chromatography.

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