Comparison of ten major constituents in seven types of processed tea using HPLC-DAD-MS followed by principal component and hierarchical cluster analysis

Yi, Tao; Zhu, Lin; Peng, Wan-Ling; He, Xi-Cheng; Chen, Hong-Li; Li, Jie; Yu, Tao; Liang, Zhi-Tao; Zhao, Zhong-Zhen; Chen, Hu-Biao

Published in:
LWT - Food Science and Technology

DOI:
10.1016/j.lwt.2015.01.003

Published: 01/06/2015

Document Version:
Peer reviewed version

Comparison of ten major constituents in seven types of processed tea using HPLC-DAD-MS followed by principal component and hierarchical cluster analysis

Tao Yi [a,*,1], Lin Zhu [a,1], Wan-Ling Peng [a], Xi-Cheng He [a], Hong-Li Chen [b], Jie Li [c], Tao Yu [a], Zhi-Tao Liang [a], Zhong-Zhen Zhao [a], Hu-Biao Chen [a,*]

Affiliation

[a] School of Chinese Medicine, Hong Kong Baptist University, Hong Kong Special Administrative Region, China.

[b] Department of Chemistry, Lanzhou University, Lanzhou 730000, China.

[c] Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, 500 West 12th Avenue, Columbus, OH 43210, USA.

[*]**Corresponding Author**

School of Chinese Medicine, Hong Kong Baptist University, Hong Kong Special Administrative Region, P. R. China. Tel.: +852 3411 2081, +852 3411 2060; fax: +852 3411 5571, +852 3411 2461. E-mail address: yitao@hkbu.edu.hk (T. Yi), hbchen@hkbu.edu.hk (H. B. Chen).

[1] These authors contributed equally to this work.

Abbreviations: GT, green tea; **YT,** yellow tea; **WT,** white tea; **OT,** oolong tea; **BT,** black tea; **APT,** aged pu-erh tea; **RPT,** ripened pu-erh tea; **PCA,** principal component analysis; **HCA,** hierarchical cluster analysis.

ABSTRACT

A new HPLC-DAD-MS method was developed to compare the major constituents in 7 types of processed tea, namely green tea, yellow tea, white tea, oolong tea, black tea, aged pu-erh tea and ripened pu-erh tea. MS was used for identification in positive ion mode, and DAD was used for quantification at wavelength of 210 nm. Ten components were simultaneously determined in 74 tea samples representing 7 processing types, and then principal component analysis (PCA) and hierarchical cluster analysis (HCA) were used to distinguish and classify between the samples. The results demonstrate that the contents of the major constituents significantly varied among the 7 types of tea. Unique aspects of each type of processing were correlated with unique aspects of the chemistry of the tea. The 7 types of processed tea were successfully divided into four categories based on our determination and chemometrics analysis. Our present method was adaptable for the comparative study of processed tea, which significantly contributes to discrimination and quality evaluation of teas.

KEYWORDS: Processed tea, Major constituents, HPLC-DAD-MS, Principal component analysis, Hierarchical cluster analysis

Chemical compounds studied in this article

Gallic acid (PubChem CID: 370); Theobromine (PubChem CID: 5429); (-)-Gallocatechin (PubChem CID: 9882981); (-)-Epigallocatechin (PubChem CID: 72277); (+)-Catechin (PubChem CID: 9064); Caffeine (PubChem CID: 2519); (-)-Epicatechin (PubChem CID: 72276); (-)-Epigallocatechin gallate (PubChem CID: 65064); (-)-Gallocatechin gallate (PubChem CID: 199472); (-)-Epicatechin gallate (PubChem CID: 107905)

1. Introduction

Tea, next to water, is the second most popular beverage in the world (Shahidi, 1997). Tea contains multiple constituents, and catechins as well as alkaloids are considered to be the major bioactive components among these chemicals (Da Silva Pinto, 2013; Wang et al., 2011). It is well known that tea has extensive health benefits for humans, including anti-oxidation (He & Shahidi, 1997), anti-obesity (Sergent, Vanderstraeten, Winand, Beguin, & Schneider, 2012), anti-viral (Zhong, Ma, & Shahidi, 2012), cholesterol-lowering effect (Chan et al., 1999), and reducing the risks of cancer (Wu et al., 2007; Zhong, Chiou, Pan, Ho, & Shahidi 2012). Due to its powerful anti-oxidant and anti-microbial properties, tea extract has also been used as a natural preservative to increase shelf life of foods (Dong, Zhu, Li, & Li, 2013; Oh, Jo, Cho, Kim, & Han, 2013).

In general, teas are processed before use. There are basically 7 processing methods in China, producing the 7 main varieties of tea to meet the needs of different consumers (Lu & Shen, 2012; Zhou, Duan, Wu, & Si, 2013). They are green tea (GT), yellow tea (YT), white tea (WT), oolong tea (OT), black tea (BT), aged pu-erh tea (APT) and ripened pu-erh tea (RPT). Their processing protocols are illustrated in Diagram 1. However, the 7 processed teas are always confused, because few chemical characteristics of these teas have been described systematically.

*Insert **Diagram 1** here*

On the other hand, consumers have the opportunity of exposure to various teas, and they are eager to know their difference and how to distinguish between them. For tea producers and tea regulatory agencies, they also want to establish a specific quality standard based on

the characteristics of individual tea for quality assurance and quality control (QA & QC). Therefore, a comparative study of the major chemical constituents in various processed teas is urgently needed now.

Recently, chemical analysis of tea has been carried out using TLC (Cimpoiu, Hosu, Seserman, Sandru, & Miclaus, 2010), HPLC (Song, Li, Guan, Wang, & Bi, 2013; Rahim, Nofrizal, & Saad, 2014; Wang, Helliwell, & You, 2000; El-Shahawi, Hamza, Bahaffi, Al-Sibaai, & Abduljabbar, 2012) and UPLC-MS (Fraser et al., 2013; Pongsuwan et al., 2008). However, the existing studies mainly focus on the determination of a single or a few types of tea based on a few makers. Up to date, compositional data generated using the same extraction and the same validated methodology are still scarce, and comparative analysis of a comprehensive range of teas simultaneously by a single method has not been reported (Stodt & Engelhardt, 2013).

Inspired by the above-mentioned problem, in the present work, we aimed: (i) to develop a new HPLC-DAD-MS method for simultaneous determination of 10 major components (7 catechins, 2 alkaloids and 1 gallic acid) in 74 tea samples produced by 7 processing methods; (ii) to classify the tea samples by principal component analysis (HCA) and hierarchical cluster analysis (HCA) and, finally, (iii) to correlate the chemical composition of different teas with their processing methods. By obtaining a clearer view on chemical composition of 7 types of processed tea, this study has a considerable significance for consumer, producer and quality control authorities concerned to teas.

2. Materials and methods

2.1. Materials and reagents

Seventy-four tea samples, namely: 17 green tea (GT) samples, 7 yellow tea (YT) samples, 8 white tea (WT) samples, 13 oolong tea (OT) samples, 8 black tea (BT) samples, 10 aged pu-erh tea (APT) samples and 11 ripened pu-erh tea (RPT) samples were collected from China. Detailed information of these teas is listed in the Table S1 of the Supporting data.

Gallic acid (GA), caffeine (CAF), theobromine (TBM), (+)-catechin (C), (-)-epicatechin (EC), (-)-gallocatechin (GC), (-)-epigallocatechin (EGC), (-)-gallocatechin gallate (GCG), (-)-epigallocatechin gallate (EGCG), and (-)-epicatechin gallate (ECG) standards were purchased from Phytomarker Ltd. (Tianjin, China). The purity of these chemical standards was no less than 98%. The chemical structures of standards are shown in Figure 1. Formic acid of analytical grade was purchased from Merck (Darmstadt, Germany). Acetonitrile of HPLC grade was purchased from Lab-scan (Bangkok, Thailand). Water was purified using a Milli-Q water system (Millipore; Bedford, MA, USA).

*Insert **Figure 1** here*

2.2. HPLC-DAD-MS analysis

An Agilent 1100 high-performance liquid chromatography (HPLC) system with diode array detector (DAD), was used for quantitative analysis. Detection wavelength was set at 210 nm. An Alltima C_{18} column (5 μm, 4.6 × 250 mm) was used for chromatographic separation at 30 ^{o}C. The mobile phase consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) using a gradient program of 2% (B) in 0-1 min, and 2-30% in 1-30 min. The solvent flow rate was 1 mL/min. For mass spectrometric determination, the HPLC-DAD system was hyphenated to an Agilent 6540 quadrupole time-of-flight mass

spectrometry (MS) system by an electrospray ionization (ESI) interface (Santa Clara, CA, USA). The effluent from DAD was drained to the MS system with a split ratio of 3:1. The conditions of MS analysis in positive ion mode were as follows: scan range, 50-1700 m/z; drying gas (nitrogen), flow rate, 8 L/min; gas temperature, 300 ^{o}C; capillary voltage, 3 kV; fragmentor voltage, 150 V; nebulizer pressure, 310 kPa. All operations and data analysis were controlled by Agilent MassHunter Workstation software version B.04.00.

2.3. Preparation of standard and sample solutions

For the calibration, EGC, CAF, EGCG, GCG and ECG working solutions of 2-100 mg/L, C, EC and GC solutions of 1-50 mg/L, GA solutions of 0.5-25 mg/L and TBM of 0.2-10 mg/L were prepared in 50% methanol and stored at 4 ^{o}C before use.

Each tea sample was milled (ca. 0.84 mm); of the milled tea, 0.5 g was accurately weighted and transferred into a 50 mL conical centrifuge tube. The tea sample was extracted with 30 mL of water at the temperature of 99 ^{o}C for 10 min with intermittent shaking. The operations were repeated two times. Total extracts were combined in a 100-mL volumetric flask, which was filled up to the calibration mark with water. The extracts were then filtered through a 0.5 μm Millipore filter. An aliquot of 5 μL solution was injected for HPLC-DAD-MS analysis.

2.4. Assay validation and sample determination

MS was used for identification in positive ion mode, and DAD was used for quantification at wavelength of 210 nm. Linearity for standards was determined with seven data points over the concentration range of the working solutions. Repeatability was evaluated by six

injections of the sample solution (GT1) within one day. Reproducibility was evaluated in intra- and inter-day assays of the tea sample GT1. The stability test was performed by analyzing the sample solution (GT1) over period of 24 h. The relative standard deviation (RSD) was taken as the measures of precision, repeatability and stability. To determine the recoveries, sample GT1 spiked with standards at low, middle and high concentration levels in three replicates were extracted and analyzed. Recovery was calculated by dividing the amount of analyte found in the spiked sample by the sum of the amount originally found in the sample plus the amount spiked. All tea samples were analyzed using the described method.

2.5. Data analysis

The mean value and standard deviation (SD) of analytes was calculated from the experimental data. The significance ($P < 0.05$) between two sets of data was determined by unpaired *t*-test using the software package Prism version 5.01 (GraphPad Software, Inc., La Jolla, CA, USA). To classify and discriminate between the tea samples, principal component analysis (PCA) and hierarchical cluster analysis (HCA) was performed with SPSS for Windows version 20.0 (SPSS, Chicago, IL, USA).

3. Results and discussion

3.1. Optimization of the sample extraction

Brewing tea with hot water for a short while is the most popular way of tea drinking. Thus, extraction of tea with hot water was chosen in this study. Infusion period was chosen from 3 to 30 min, and the results showed that the maximum release of analytes reached at 10 min of

infusion, followed by a constant decrease due to the thermal instability of catechins. Extraction times was further optimized, the results showed that that exhaustive extraction could be achieved when 0.5 g tea sample powder was extracted with 30 mL water brewing for 10 min with intermittent shaking, three times (Figure S1).

3.2. Optimization of the analysis conditions

By comparing the HPLC chromatograms of tea extracts acquired at different wavelengths within the range 190–500 nm, and the corresponding UV absorption maxima for each standard compound, it was found that 210 nm was more sensitive with lower interference. Therefore, 210 nm was chosen as the determination wavelength. Different ratios of acetonitrile and water were further tried, until satisfactory resolutions for the analytes within 30 min were obtained. Compared to the existing reports (Stodt et al., 2013), the present gradient elution condition presented the shortest analysis time for separation of ten analytes in tea by using HPLC. The typical chromatograms of seven teas at 210 nm are shown in Figure 2A.

*Insert **Figure 2** here*

In order to further obtain a comprehensive view on chemical constituents in seven types of tea, the mass spectrometric conditions were optimized in both positive and negative ion modes. Results revealed that the positive ion mode was more sensitive. The typical total ion chromatograms (TICs) of seven types of tea are shown in Figure 2B. The results show that unambiguous identification of analytes under the optimized conditions was achieved (Figure S2).

3.3. Validation of the analysis method

The validity of the method was well assessed, and the results are summarized in Table 1 (details are listed in Table S2 to Table S4 of the Supporting data). For all analytes, a good linearity with R^2> 0.99 was achieved. Based on visual evaluation with a signal-to-noise ratio of about 3:1 and 10:1, the LOD and LOQ of the analytes were found to be less than 1.3 and less than 3.8 ng, which were lower than the existing reports (Rahim et al., 2014). The RSD values of reproducibility were reported within the range of 0.68 and 2.45% for intra-day assays and 0.75 to 3.37% for inter-day assays. The average recovery of the ten analytes ranged from 91.63% to 105.28%. The results of stability test suggested that the tea extract sample was stable in the experiment (RSD < 1.35%). Based on these results we concluded that the overall analytical procedure is sensitive, precise and accurate, which is considered suitable for determination of tea samples.

*Insert **Table 1** here*

3.4. Comparison of the 10 components in 7 types of processed tea

The 74 tea samples, representing 7 processing methods, were analyzed using the present method, and the results are summarized in Table 2. To clarify the differences between the various teas, we describe the characteristics of these processing methods, and then correlate the key process with the chemical composition of each type of tea.

*Insert **Table 2** here*

Green Tea (GT). The first step of processing GT is called green-killing (“Shaqing” in Chinese), which is also the key process (Diagram 1). Green-killing refers to quick application

of heat, either with steam, or by parching in hot pans, to halt oxidation and fix most of the chemical constituents of the tea leaves (Lu et al., 2012; Zhou et al., 2013). As shown in Table 2, it was found that EGCG (54.06 mg/g) and CAF (34.86 mg/g) were the two most abundant of the ten components in GT, and in fact GT possessed the most abundant EGCG and CAF of all the other types of tea. This finding may correlate with the short processing period of GT, and the degradation of catechins in tea was inhibited.

Yellow Tea (YT). Yellowing process is unique to YT. Tea leaves after green-killing are allowed to be lightly heated (c.a. 37 ^{o}C) in a closed humid container for appropriate time, which causes a distinctive yellowish-green hue of the tea leaves (Zhou et al., 2013). And then, the other steps are as same as those of GT. As shown in Table 2, there was no significance in the contents of ten analytes between YT and GT ($p > 0.05$); the content of total catechins was 118.55 mg/g, which was comparable to the content of total catechins of 112.72 mg/g in GT. It was revealed that the similarity of chemical composition between GT and YT may be related to their similar processing methods (Diagram 1).

White Tea (WT). WT is prepared from the leaves of albino tea tree, and the leaves harvested are white. They are allowed to wilt for a brief time, and then baked dry. During the wilting process, the tea leaves are shielded from sunlight to keep them white and prevent the formation of pigments. As shown in Table 2, the contents of four components in WT, namely GA, GC, EGC and EC, were comparable to those in GT ($p > 0.05$); while the contents of other six components were significantly lower than those in GT (TBM, **, $p < 0.01$; C, CAF, EGCG, GCG and ECG, ***, $p < 0.001$). Overall, the total content of the ten components in WT was still lower than that in GT.

Oolong Tea (OT). OT is a type of a partially oxidized tea, produced by a unique process called blue-making ("Zuoqing" in Chinese, Diagram 1). Blue-making includes repeatedly tossing the leaves in baskets and stacking them indoors. The degree of oxidation of OT can be adjusted by increasing or reducing the cycles of blue-making (Lu et al., 2012; Zhou et al., 2013). Compared to the other teas (Table 2), OT has two special characteristics in chemical composition. Firstly, OT possesses the highest content of EGC (26.89 mg/g) and lowest content of CAF (19.67 mg/g) of all the teas. Secondly, the ratio of total catechins/ total alkaloids in OT is the highest (up to 3.75). These results indicate that blue-making can reduce levels of alkaloids and improve the content of EGC.

Black Tea (BT). BT is a completely oxidized tea. The withered tea leaves undergo bruising through crushing or cutting to disrupt leaf cell structures, fully releasing the leaf juices and enzymes that activate complete oxidation (Diagram 1). As shown in Table 2, the contents of all seven catechins (GC, EGC, C, EC, EGCG, GCG and ECG) in BT is significantly decreased compared to those in GT (***, $p < 0.001$). Although the content of CAF also decreased to 28.54 mg/g (**, $p < 0.01$), CAF is still the most abundant component in BT. Interestingly, the content of GA in BT (4.43 mg/g) is significantly increased compared to that in GT (2.01 mg/g; ***, $p < 0.001$). The increased GA may be generated from the galloyl moiety of EGCG, GCG and ECG during the bruising stage of the processing.

Aged Pu-erh Tea (APT). APT derives from GT that has undergone a natural aging process during storage at room temperature in normal humidity for a period of years (Zhou et al., 2013). Compared to the GT, APT has a longer storage time before consumption. As shown in Table 2, storage did not affect the contents of alkaloids, TBM (1.67 mg/g) and CAF (33.94

mg/g) in APT were almost equivalent to those in GT (1.29 mg/g for TBM and 34.86 mg/g for CAF), respectively. Compared to the catechins in GT, three catechins in APT were decreased, namely EGC (7.58 mg/g; *, $p < 0.05$), EGCG (28.19 mg/g; ***, $p < 0.001$) and GCG (3.87 mg/g; ***, $p < 0.001$), while three catechins were increased, namely C (7.43 mg/g; **, $p < 0.01$), EC (12.32 mg/g; ***, $p < 0.001$) and ECG (30.60 mg/g; ***, $p < 0.001$), and one catechin was unchanged, namely GC (2.75 mg/g, $p > 0.05$). As with BT, the content of GA (4.31 mg/g) in APT was significantly increased compared to that in GT (2.01 mg/g; ***, $p < 0.001$). It is noteworthy that, the content (30.60 mg/g) of ECG in APT was the highest among all the types of teas; thus ECG is a unique component in the chemical composition of APT.

Ripened Pu-erh Tea (RPT). RPT has undergone an accelerated oxidation process known as wet piling ("Wodui" in Chinese, Diagram 1). Freshly picked tea leaves are spread on trays, allowed to wilt, and then, sprayed with water. The trays are stacked, and stored in a controlled environment of kept at 40-60 ^{o}C by adjusting the humidity. Under these conditions, the speed and degree of oxidation is higher than APT. As shown in Table 2, we found that all catechins in RPT were significantly reduced (***, $p < 0.001$) compared to those in GT, and the contents of alkaloids in RPT was not affected by the process of wet piling ($p > 0.05$). The ratio of total catechins/ total alkaloids in RPT dropped to 0.19, which was comparable to BT (0.24; $p > 0.05$). This finding indicates that RPT is the most oxidized of all the seven types of tea. In terms of other parameters, the contents as well as the ratio of the ten components in RPT were similar to those in BT.

3.5. Classification of the 7 types of processed tea

Principal component analysis (PCA) and hierarchical cluster analysis (HCA) are two main

approaches in chemometrics, and they are widely used for the classification study in the field of food research (Yu, 2005). PCA is a statistical data reduction method. It transforms the original set of variables to a new set of uncorrelated variables called principal components (PCs). By plotting the PCA scores, it is possible to visually assess similarities between samples and determine whether samples can be grouped. In our study, the initial eigenvalues were generated by inputting the contents of ten determined components in the 74 tea samples to SPSS software. The cumulative percent variance (CPV) of three principal variables was found to be 86.62 % of the total variance, which meets the general requirements of CPV > 70%~85% for PCA analysis (Liu, 2007). Thus, the resulting data was plotted to produce a three-dimensional (3D) graphic of PCA scores shown in Figure 3.

*Insert **Figure 3** here*

Hierarchical cluster analysis (HCA) involves a measurement of the similarity between objects to be clustered, and samples with the maximum similarities were clustered preferentially (Yi et al., 2013). In this study, the ten determined components of the 74 tea samples was inputted into SPSS as variables, between group average linkage method was applied to sort tea samples into groups, and rescaled distance was selected as measurement to obtain a HCA dendrogram shown in Figure 4.

*Insert **Figure 4** here*

As shown in the PCA graphic and HCA dendrogram, the 74 tea samples representing the 7 types of tea processing methods were clearly clustered in four main groups. From the results, it was shown that BT and RPT, the two completed oxidized teas, were clustered into group I. This finding revealed that both bruising for BT and wet piling for RPT during

processing leads to the completed oxidization, which contribute to the global similarity in the chemical composition of BT and RPT. The second group (group II) was constituted by the two unoxidized teas, YT and GT. The contents of the major constituents in the two types of teas are similar due to their similar processing methods. The third group (group III) is composed of OT and WT, the two partially oxidized teas. The key process, wilting in shield for WT and blue-making for OT, makes tea leaves slightly oxidized. Moreover, all of the OT samples and half of WT samples (WT1, WT4, WT5 and WT8) originated in Fujian province of China. These reasons perhaps explain why OT and WT were clustered into a group in the PCA and HCA graphics. The fourth group (group IV) includes only APT. In the PCA and HCA graphics, all the APT samples were clustered away from other types of tea, visually representing the change in the chemical composition after processing (i.e., half of catechins reduced, the other half increased, and alkaloids unchanged). This change decided the chemical composition of APT was distinctive from other teas. Overall, the 7 types of processed tea were successfully divided into four categories based on our determination and chemometrics analysis.

4. Conclusion

In conclusion, a highly precise and accurate HPLC-DAD-MS method was developed to determine the 10 major components of 74 tea samples, which represented 7 types of tea processing methods in China. Our present method is adaptable for the quality evaluation of tea for tea producers and regulatory authorities. By comparing the contents of the major components and correlation analysis, the unique aspects of each type of tea processing were

described systematically and correlated with the characteristics in the chemical composition. The 7 types of tea processing methods were clearly clustered in four main groups. This study not only provides scientific information for consumers to distinguish different teas, but also advanced our knowledge about the effect of processing on the composition of tea.

References

Chan, P.T., Fong, W.P., Cheung, Y.L., Huang, Y., Ho, W.K., & Chen, Z.Y. (1999). Jasmine green tea epicatechins are hypolipidemic in hamsters (*Mesocricetus auratus*) fed a high fat diet. *Journal of Nutrition, 129,* 1094–1101.

Cimpoiu, C., Hosu, A., Seserman, L., Sandru, M., & Miclaus, V. (2010). Simultaneous determination of methylxanthines in different types of tea by a newly developed and validated TLC method. *Journal of Separation Science, 33,* 3794–3799.

Da Silva Pinto, M. (2013). Tea: A new perspective on health benefits. *Food Research International*, 53, 558–567.

Dong, L.L., Zhu, J.L., Li, X.P., & Li, J.R. (2013). Effect of tea polyphenols on the physical and chemical characteristics of dried-seasoned squid (*Dosidicus gigas*) during storage. *Food Control, 31*, 586–592.

El-Shahawi, M.S., Hamza, A., Bahaffi, S.O., Al-Sibaai, A.A., & Abduljabbar, T.N. (2012). Analysis of some selected catechins and caffeine in green tea by high performance liquid chromatography. *Food Chemistry, 134,* 2268–2275.

Fraser, K., Lanea, G.A., Ottera, D.E., Hemarb, Y., Quekb, S.Y., Harrisona, S.J., et al. (2013). Analysis of metabolic markers of tea origin by UHPLC and high resolution mass spectrometry. *Food Research International*, *53*, 827–835.

He, Y.H., & Shahidi, F. (1997). Antioxidant activity of green tea and its catechins in a fish meat model system. *Journal of Agricultural and Food Chemistry, 45*, 4262–4266.

Liu, R.Q. (2007). *SPSS statistical software.* (1st ed.). Beijing: China Press of Traditional Chinese Medicine, (Chapter 14).

Lu, J.X., & Shen, Z.D. (2012). A comparative study of tea customs. *Cross-Cultural Communication, 8,* 128–133.

Oh, J., Jo, H., Cho, A.R., Kim, S.J., & Han, J. (2013). Antioxidant and antimicrobial activities of various leafy herbal teas. *Food Control, 31,* 403–409.

Pongsuwan, W., Bamba, T., Harada, K., Yonetani, T., Kobayashi, A., & Fukusaki, E. (2008). High-throughput technique for comprehensive analysis of Japanese green tea quality assessment using ultra-performance liquid chromatography with time-of-flight mass spectrometry (UPLC/TOF MS). *Journal of Agricultural and Food Chemistry, 56,* 10705–10708.

Rahim, A.A., Nofrizal, S., & Saad, B. (2014). Rapid tea catechins and caffeine determination by HPLC using microwave-assisted extraction and silica monolithic column. *Food Chemistry, 147*, 262–268.

Sergent, T., Vanderstraeten, J., Winand, J., Beguin, P., & Schneider, Y.J. (2012). Phenolic compounds and plant extracts as potential natural anti-obesity substances. *Food Chemistry, 135*, 68–73.

Shahidi, F. (1997). Natural antioxidants: chemistry, health effects, and applications. AOCS press, Champaign, Illinois, 213-223.

Song, M.T., Li, Q., Guan, X.Y., Wang, T.J., & Bi, K.S. (2013). Novel HPLC method to evaluate the quality and identify the origins of Longjing green tea. *Analytical Letters, 46,* 60–73.

Stodt, U., & Engelhardt, U.H. (2013). Progress in the analysis of selected tea constituents over the past 20 years. *Food Research International, 53*, 636–648.

Wang, H., Chen, L.G., Xu, Y., Zeng, Q.L., Zhang, X.P., Zhao,Q., et al. (2011). Dynamic microwave-assisted extraction coupled on-line with clean-up for determination of caffeine in tea. *LWT - Food Science and Technology, 44,* 1490–1495.

Wang, H.F., Helliwell, K., & You, X.Q. (2000). Isocratic elution system for the determination of catechins, caffeine and gallic acid in green tea using HPLC. *Food Chemistry, 68,* 115–121.

Wu, S.C., Yen, G.C., Wang, B.S., Chiu, C.K., Yen, W.J., Chang, L.W., et al. (2007). Antimutagenic and antimicrobial activities of pu-erh tea. *LWT - Food Science and Technology, 40,* 506–512.

Yi, T., Chen, Q.L., He, X.C., So, S.W., Lo, Y.L., Fan, L.L., et al. (2013). Chemical quantification and antioxidant assay of four active components in *Ficus hirta* root using UPLC-PAD-MS fingerprinting combined with cluster analysis. *Chemistry Central Journal, 7,* 115.

Yu, P.Q. (2005). Applications of hierarchical cluster analysis (CLA) and principal component analysis (PCA) in feed structure and feed molecular chemistry research, using synchrotron-based Fourier transform infrared (FTIR) microspectroscopy. *Journal of Agricultural and Food Chemistry, 53,* 7115–7127.

Zhong, Y., Chiou, Y.S., Pan, M.H., Ho, C.T., & Shahidi, F. (2012). Protective effects of epigallocatechin gallate (EGCG) derivatives on azoxymethane-induced colonic carcinogenesis in mice. *Journal of Functional Foods, 4,* 323–330.

Zhong, Y., Ma, C.M., & Shahidi, F. (2012). Antioxidant and antiviral activities of lipophilic epigallocatechin gallate (EGCG) derivatives. *Journal of Functional Foods, 4,* 87–93.

Zhou, Z.X., Duan, W.H., Wu, H.Y., & Si, Z.M. (2013). Investigation and analysis of consumptive request for Chinese premium teas. *Journal of Zhejiang A & F University, 30*, 412–416.

Legends for Tables

Table 1 Linearity, sensitivity, precision, accuracy and stability of the method.

Table 2 The contents of the ten components in seven types of tea.

Table 1 Linearity, sensitivity, precision, accuracy and stability of the method.

Peak No.	RT (min)	Components	Linearity			LOD (ng)	LOQ (ng)	Repeatability (RSD, %, *n* =6)	Reproducibility (RSD, %, *n* =3)		Recovery (mean, %, *n* =3)				Stability (RSD, %, *n* =6)
			Range (mg /L)	Equation	R^2				Intra-day	Inter-day	Low	Middle	High	Average	
1	10.0	GA	0.5-25	y = 31.47 x - 6.69	0.9998	1.2	3.5	0.47	1.85	3.35	92.73	93.65	96.83	94.40	0.89
2	13.7	TBM	0.2-10	y = 31.65 x + 1.12	0.9995	0.5	1.6	0.35	2.36	3.10	91.56	96.12	90.29	92.66	1.26
3	14.1	GC	1-50	y = 39.08 x - 8.62	0.9980	1.1	3.0	0.18	1.92	1.54	99.32	92.47	94.76	95.52	0.65
4	17.7	EGC	2-100	y = 48.31 x - 8.51	0.9982	0.5	1.5	0.63	2.45	0.92	100.36	103.68	103.18	102.41	1.28
5	19.4	C	1-50	y = 39.19 x - 0.71	0.9999	1.3	3.8	0.56	1.79	2.84	101.44	96.92	95.42	97.93	0.39
6	20.2	CAF	2-100	y = 31.51 x + 22.11	0.9994	1.0	3.1	0.48	0.68	0.75	107.67	108.59	99.57	105.28	0.63
7	22.0	EC	1-50	y = 44.73 x + 12.46	0.9998	0.9	2.9	0.31	1.76	2.32	96.69	97.17	101.39	98.42	0.95
8	22.8	EGCG	2-100	y = 48.40 x - 5.23	0.9991	0.6	1.7	0.24	1.03	1.53	95.62	92.72	86.56	91.63	0.79
9	24.4	GCG	2-100	y = 33.96 x - 15.78	0.9999	0.8	2.2	0.31	2.15	3.37	104.61	95.75	101.28	100.55	1.35
10	27.6	ECG	2-100	y = 44.41 x + 35.22	0.9990	1.2	3.5	0.53	1.68	2.25	92.30	95.66	91.62	93.19	0.58

Table 2 The contents of the ten components in seven types of tea.

Component	Contents [a] (mg/g)						
	GT (17 [b])	YT (7)	WT (8)	OT (13)	BT (8)	RPT (11)	APT (10)
GA	2.01 ± 0.92	1.56 ± 1.22	2.33 ± 1.20	0.69 ± 0.71*** [c]	4.43 ± 1.47***	3.09 ± 1.99	4.31 ± 1.41***
TBM	1.29 ± 0.60	0.91 ± 0.45	0.52 ± 0.42**	0.37 ± 0.13***	1.06 ± 0.62	1.42 ± 0.45	1.67 ± 0.49
GC	4.02 ± 2.19	5.85 ± 2.82	2.54 ± 1.45	5.02 ± 1.10	0.14 ± 0.30***	0.40 ± 0.36***	2.75 ± 1.15
EGC	15.48 ± 9.02	20.83 ± 8.48	9.29 ± 4.68	26.89 ± 5.63***	0.78 ± 0.58***	0.75 ± 0.78***	7.58 ± 3.79*
C	5.37 ± 1.73	4.37 ± 1.63	2.05 ± 0.90***	1.01 ± 0.28***	0.52 ± 0.77***	0.56 ± 0.39***	7.43 ± 1.46**
CAF	34.86 ± 4.32	33.32 ± 7.10	27.17 ± 5.37***	19.67 ± 2.95***	28.54 ± 3.68**	31.78 ± 4.94	33.94 ± 3.68
EC	7.25 ± 2.26	8.14 ± 3.48	5.11 ± 3.88	7.00 ± 1.24	0.71 ± 0.57***	1.19 ± 0.85***	12.32 ± 3.86***
EGCG	54.06 ± 6.83	53.96 ± 8.69	23.73 ± 4.19***	27.44 ± 3.66***	2.19 ± 2.40***	1.43 ± 1.74***	28.19 ± 6.50***
GCG	9.44 ± 1.97	9.17 ± 3.01	3.71 ± 1.84***	2.70 ± 0.74***	0.23 ± 0.46***	0.35 ± 0.43***	3.87 ± 1.24***
ECG	17.10 ± 3.34	16.23 ± 7.01	8.12 ± 3.05***	5.09 ± 1.64***	2.65 ± 2.25***	1.57 ± 2.46***	30.60 ± 4.18***
Total alkaloids (TA)	36.15	34.23	27.69	20.04	29.6	33.2	35.61
Total catechins (TC)	112.72	118.55	54.55	75.15	7.22	6.25	92.74
Ratio of TC/ TA	3.12	3.46	1.97	3.75	0.24	0.19	2.60

a) The value is mean ± S.D. of samples from the same type of tea.

b) The number of samples for each type of tea.

c) *, $p < 0.05$; **, $p < 0.01$ and ***, $p < 0.001$ with respect to GT group.

Legends for Figures

Diagram 1. Illustration of the preparation of the seven types of Chinese tea (★ the key process).

Fig. 1. Chemical structures of the ten main components in tea.

Fig. 2. Typical (**A**) UV chromatograms at 210 nm and (**B**) TIC mass spectra in positive ion mode of seven processed Chinese teas and reference standards. (1, GA; 2, TBM; 3, GC; 4, EGC; 5, C; 6, CAF; 7, EC; 8, EGCG; 9, GCG; 10, ECG; STs, reference standards).

Fig. 3. 3D graphic of PCA scores by the ten quantified components in 74 tea samples (7 GT samples, 7 YT samples, 8 WT samples, 13 OT samples, 8 BT samples, 10 APT samples and 11 RPT samples).

Fig. 4. HCA dendrogram of 74 tea samples. (7 GT samples, 7 YT samples, 8 WT samples, 13 OT samples, 8 BT samples, 10 APT samples and 11 RPT samples).

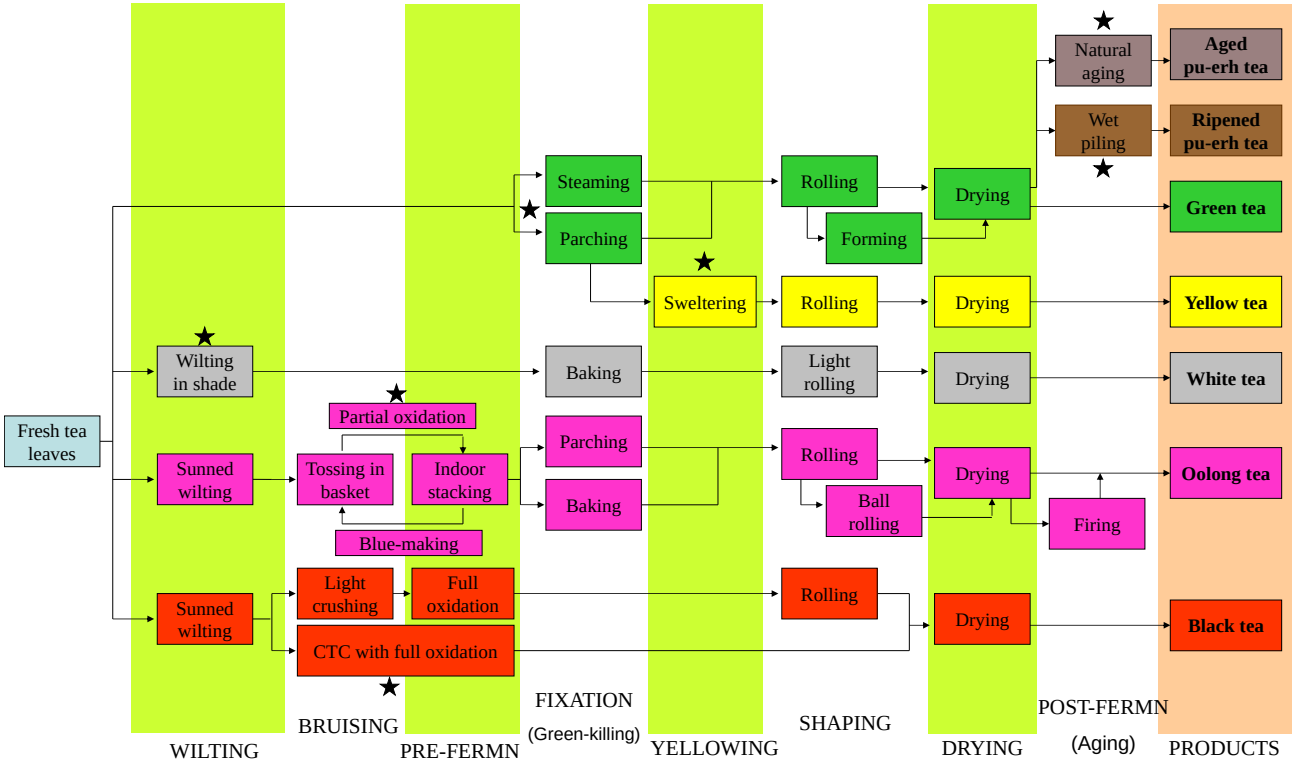


Diagram 1. Illustration of the preparation of the seven types of Chinese tea (★ the key process).

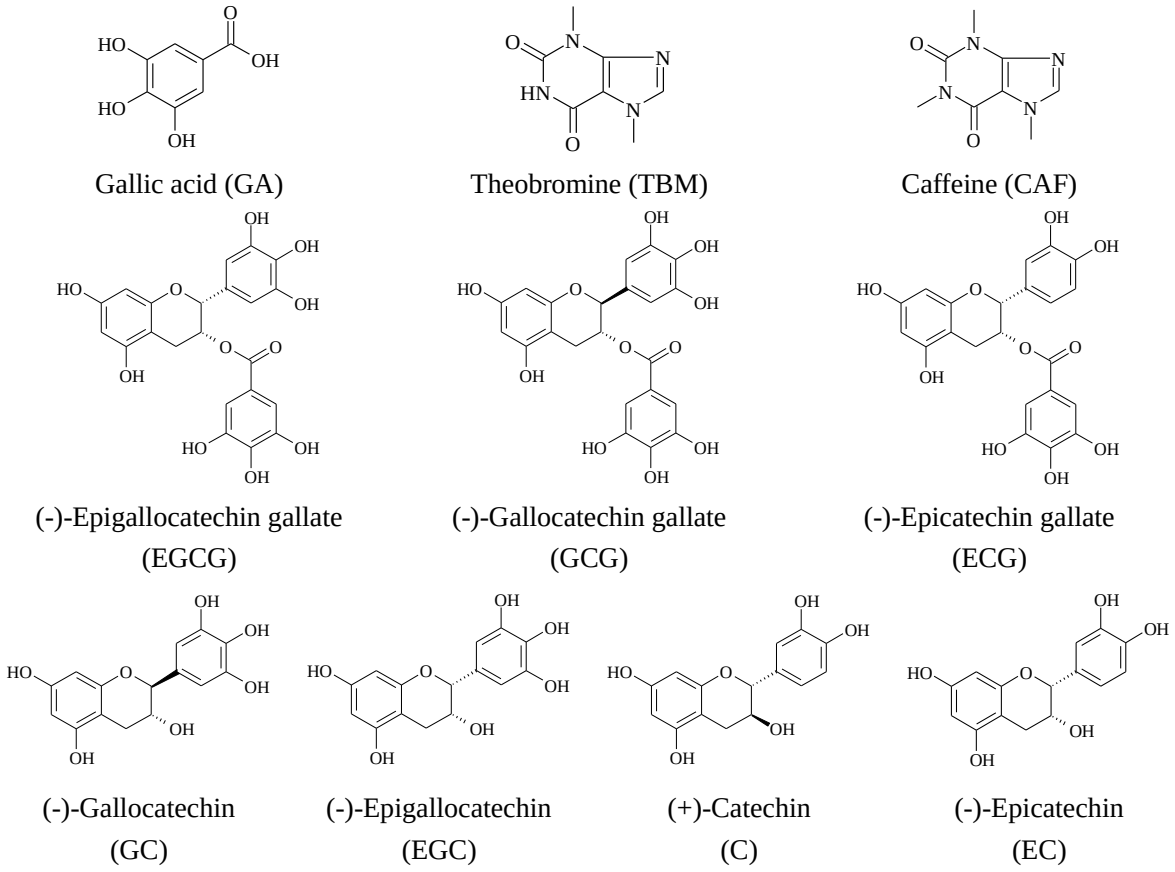


Fig. 1. Chemical structures of the ten main components in tea.

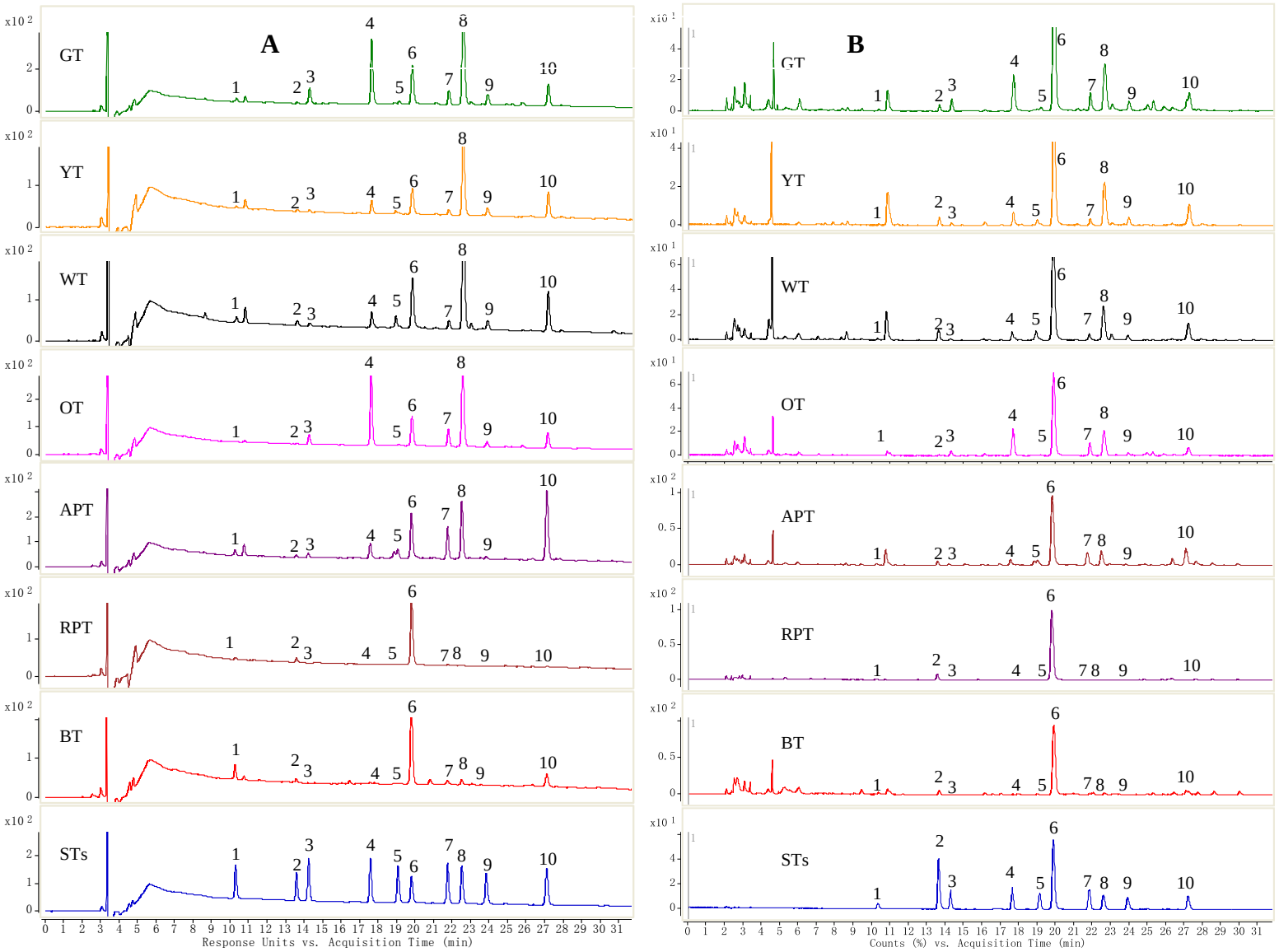


Fig. 2. Typical (**A**) UV chromatograms at 210 nm and (**B**) TIC mass spectra in positive ion mode of seven processed Chinese teas and reference standards. (1, GA; 2, TBM; 3, GC; 4, EGC; 5, C; 6, CAF; 7, EC; 8, EGCG; 9, GCG; 10, ECG; STs, reference standards).

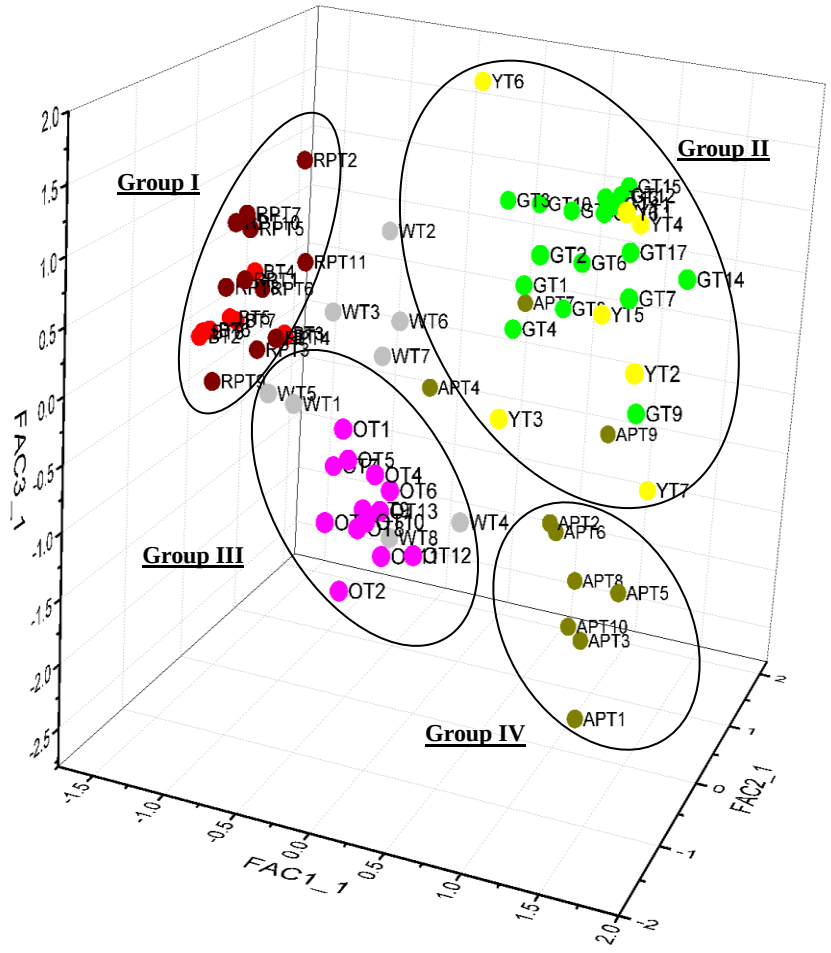


Fig. 3. 3D graphic of PCA scores by the ten quantified components in 74 tea samples (7 GT samples, 7 YT samples, 8 WT samples, 13 OT samples, 8 BT samples, 10 APT samples and 11 RPT samples).

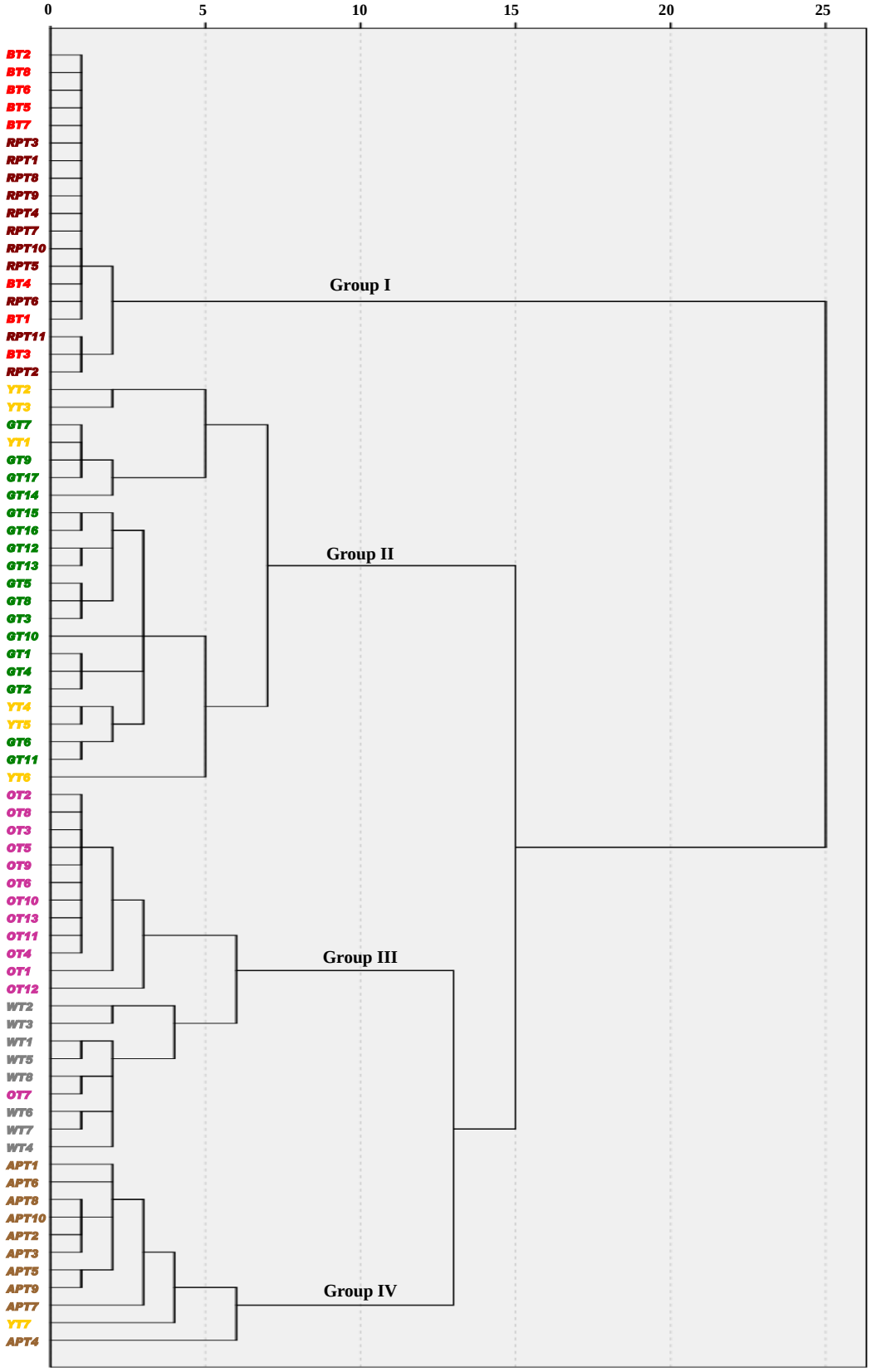


Fig. 4. HCA dendrogram of 74 tea samples. (7 GT samples, 7 YT samples, 8 WT samples, 13 OT samples, 8 BT samples, 10 APT samples and 11 RPT samples).

Legends for Supporting Data

Table S1. The detail information of 74 tea samples (types, brands, production places and contents of ten components).

Table S2. Linearity calibration curve factors, LOD and LOQ of the ten components.

Table S3. Repeatability, reproducibility and stability of the method.

Table S4. Recovery of the method

Fig. S1. Typical HPLC chromatograms of tea sample (GT1) of (A) the combined extracts by the 1^{st} + 2^{nd} + 3^{rd} times and (B) the extracts by the 4^{th} time.

Fig. S2. The MS^2 spectra and elemental composition of the ten components in positive ion mode.

Table S1. The detail information of 74 tea samples (types, brands, production places and contents of 10 components).

Type	No.	Brand name	Production place	Contents of ten components (mg/g, $n=3$)									
				GA	TBM	GC	EGC	C	CAF	EC	EGCG	GCG	ECG
Green tea (17)	GT1	Xihu Lungching	Hangzhou, Zhejiang province	1.52	1.28	3.32	13.37	4.89	29.71	6.28	47.67	8.10	13.74
	GT2	Lungching	Hangzhou, Zhejiang province	1.27	0.89	6.06	18.39	2.69	27.44	6.76	48.47	11.61	9.99
	GT3	Maofeng	Emei, Sicuan province	2.76	2.73	1.52	5.63	5.09	31.79	3.61	47.34	6.20	17.13
	GT4	Biluochun	Suzhou, Jiangsu province	1.61	1.31	2.60	14.34	5.79	31.21	6.77	46.72	5.43	14.02
	GT5	Yulu	Enshi, Hubei province	2.35	1.63	2.00	6.88	5.81	35.03	5.32	51.43	9.81	21.96
	GT6	Green tea	Anhui province	1.83	0.67	3.86	18.32	6.15	39.20	8.15	50.28	8.68	12.70
	GT7	Green tea	Guangdong province	1.09	1.07	8.88	27.89	3.59	32.58	8.68	56.71	9.97	14.67
	GT8	Maofeng	Fenggang, Guizou province	3.51	0.67	1.89	5.92	8.52	34.42	5.60	51.53	8.24	20.59
	GT9	Maojian	Guizou province	0.70	1.07	6.61	29.62	3.84	31.46	13.02	58.40	8.67	18.28
	GT10	Zhuyeqing	Emei, Sicuan province	3.18	2.00	1.70	4.47	5.83	39.35	4.80	42.30	7.19	20.31
	GT11	Queshe	Guizou province	1.92	0.42	4.90	21.72	3.33	40.77	8.73	56.67	11.69	15.55
	GT12	Xihu Lungching	Zhejiang province	2.33	1.71	3.72	12.83	6.27	34.38	7.19	56.14	12.27	16.84
	GT13	Xihu Lungching	Zhejiang province	2.36	2.05	3.40	9.35	6.28	33.13	6.46	54.30	11.44	18.29
	GT14	Biluochun	Jiangsu province	0.88	1.08	7.32	32.15	4.86	37.12	10.42	68.66	10.06	17.09
	GT15	Zhuyeqing	Emei, Sicuan province	3.49	1.42	1.94	6.94	9.12	42.34	5.50	58.52	9.69	21.91
	GT16	Green tea	Qianwei, Sicuan province	2.56	0.60	3.03	10.75	5.77	40.49	7.13	59.15	10.28	19.68
	GT17	Lungching	Hangzhou, Zhejiang province	0.76	1.25	5.53	24.65	3.44	32.14	8.87	64.67	11.10	17.98
Yellow tea (7)	YT1	Junshan Yinzhen	Yueyang, Hunan province	0.78	0.62	7.04	28.21	3.15	33.27	8.35	59.80	13.11	10.69
	YT2	Junshan Yinzhen	Yueyang, Hunan province	1.00	0.93	10.71	32.54	4.43	25.53	9.45	48.95	12.09	9.92
	YT3	Junshan Yinzhen	Yueyang, Hunan province	0.84	0.78	5.45	25.13	2.72	22.43	8.24	46.18	7.39	11.79
	YT4	Yin Zhen	Yueyang, Hunan province	1.77	1.88	6.10	18.53	4.43	35.36	7.89	54.31	11.37	21.11
	YT5	Huoshan Huangya	Huoshan, Anhui province	1.46	0.64	3.78	17.53	5.65	36.76	8.46	60.74	7.65	21.36

	YT6	Huangya	Anhui province	4.21	0.95	1.60	7.10	3.00	43.05	1.41	65.87	5.02	11.15
	YT7	Huoshan Huangya	Huoshan, Anhui province	0.87	0.56	6.29	16.79	7.22	36.83	13.20	41.89	7.56	27.56
White tea (8)	WT1	Baihao Yinzhen	Fuding, Fujian province	2.75	0.34	3.01	8.94	1.03	22.24	2.72	19.73	2.15	4.30
	WT2	White tea	Anji, Zhejiang province	4.24	0.85	0.82	3.92	3.10	39.20	2.44	27.49	3.22	9.47
	WT3	White tea	Zhenan, Guizou province	3.35	0.40	0.74	2.55	2.83	28.54	1.34	24.89	3.18	8.32
	WT4	Mudanwang	Fujian province	1.53	0.00	4.74	14.32	3.23	24.53	10.42	27.54	6.73	13.93
	WT5	Tianding Mudan	Fujian province	1.56	0.25	1.23	6.37	0.90	22.48	2.48	17.75	1.47	4.58
	WT6	Shoumeiwang	Fuding, Fujian province	2.91	0.80	3.49	12.09	1.76	27.58	4.43	29.01	5.97	8.29
	WT7	Shoumei	Fujian province	1.76	1.27	3.34	10.99	1.87	25.80	5.44	23.26	4.31	6.98
	WT8	Baimudan	Fuding, Fujian province	0.50	0.22	2.98	15.16	1.69	26.96	11.63	20.14	2.61	9.11
Oolong tea (13)	OT1	Tieguanyin	Anxi, Fujian province	0.27	0.40	4.59	23.34	0.82	16.34	5.26	34.65	4.35	-
	OT2	Malaocai	Wuyi, Fujian province	0.42	0.26	4.15	25.90	0.83	16.13	8.66	22.51	2.18	5.92
	OT3	Tieguanyin	Anxi, Fujian province	1.05	0.21	5.19	22.83	0.91	16.16	5.08	27.83	1.81	4.93
	OT4	Tieguanyin	Anxi, Fujian province	0.45	0.38	4.80	27.20	0.83	20.57	6.47	32.70	3.04	6.47
	OT5	Tieguanyin	Anxi, Fujian province	0.43	0.39	4.30	22.00	0.79	21.25	5.91	27.39	2.52	5.33
	OT6	Dahongpao	Wuyi, Fujian province	0.65	0.33	6.86	26.33	1.41	24.14	7.12	25.23	2.49	5.08
	OT7	Dahongpao	Wuyi, Fujian province	2.85	0.53	2.79	17.08	1.29	23.56	6.25	21.49	1.87	5.42
	OT8	Dahongpao	Wuyi, Fujian province	0.88	0.56	6.01	26.22	1.04	15.63	6.58	24.67	3.47	4.42
	OT9	Tieguanyin	Anxi, Fujian province	0.59	0.33	4.18	25.92	0.97	21.44	7.63	28.31	2.09	6.17
	OT10	Tieguanyin	Anxi, Fujian province	0	0.23	5.33	30.32	0.70	18.77	7.07	29.44	2.80	5.56
	OT11	Oolong tea	Taiwan	0.24	0.21	4.93	32.98	0.97	20.42	8.82	29.14	2.35	6.19
	OT12	Oolong tea	Nantou, Taiwan	0.83	0.35	6.66	40.02	1.68	22.55	8.72	27.06	2.55	4.80
	OT13	Tieguanyin	Anxi, Fujian province	0.36	0.61	5.46	29.45	0.92	18.80	7.47	26.31	3.54	5.86
Aged pu-erh tea (10)	APT1	Qingpuer tea	Puer, Yunnan province	2.20	1.04	4.21	13.40	8.36	27.09	17.27	29.45	2.77	28.58
	APT2	Puer tea	Puer, Yunnan province	3.76	1.44	2.01	5.72	7.74	34.04	11.40	29.27	3.65	31.35
	APT3	Shengbing tea	Puer, Yunnan province	4.35	1.57	3.86	9.81	8.62	31.70	15.01	28.31	2.62	32.36
	APT4	Shengbing tea	Lincang, Yunnan province	3.17	2.11	0.65	1.77	4.38	33.22	5.50	14.03	1.94	24.20
	APT5	Qingbing tea	Puer, Yunnan province	3.06	1.48	3.70	10.78	8.25	33.17	15.45	35.34	4.92	33.91

	APT6	Qingpuer tea	Puer, Yunnan province	6.78	1.59	3.80	12.13	6.38	34.72	13.08	32.25	4.12	25.91
	APT7	Puer tea	Puer, Yunnan province	5.87	2.54	1.77	3.95	5.65	40.04	6.45	28.35	5.12	25.59
	APT8	Puer tea	Puer, Yunnan province	5.38	1.86	2.00	5.33	8.75	35.16	13.93	25.58	3.09	35.20
	APT9	Qingpuer tea	Kunming, Yunnan province	4.78	2.08	2.78	7.33	8.17	38.78	10.62	36.67	5.40	34.15
	APT10	Qingpuer tea	Dali, Yunnan province	3.76	0.96	2.72	5.56	7.95	31.46	14.53	22.63	5.11	34.72
Ripened pu-erh tea (11)	RPT1	Shupuer tea	Puer, Yunnan province	2.30	1.40	0.47	0.47	0.56	29.28	1.11	-	0.70	0.08
	RPT2	Shutuo tea	Puer, Yunnan province	3.66	2.12	0.67	1.87	0.51	41.14	1.11	3.62	0.80	1.24
	RPT3	Shubing tea	Lincang, Yunnan province	5.44	1.28	0.82	1.35	0.95	27.68	2.26	1.18	0.87	1.25
	RPT4	Shupuer tea	Puer, Yunnan province	2.00	0.87	1.01	2.32	1.03	29.40	2.85	5.10	1.05	3.86
	RPT5	Shupuer tea	Puer, Yunnan province	1.27	1.59	-	0.29	0.38	33.31	0.67	0.41	-	0.66
	RPT6	Shubing tea	Puer, Yunnan province	7.32	1.69	0.49	0.76	0.58	33.76	1.48	0.54	-	0.57
	RPT7	Shubing tea	Lincang, Yunnan province	0.57	1.46	-	-	0.36	34.22	0.47	-	-	0.15
	RPT8	Shupuer tea	Puer, Yunnan province	3.40	1.07	0.51	-	-	30.25	0.47	-	-	-
	RPT9	Shuzhuan tea	Kunming, Yunnan province	4.14	0.87	0.47	0.46	0.59	21.78	0.84	1.33	0.43	0.73
	RPT10	Shupuer tea	Dali, Yunnan province	1.41	1.13	-	-	-	34.36	-	0.38	-	0.47
	RPT11	Shuzhuan tea	Puer, Yunnan province	2.45	2.17	-	0.74	1.23	34.35	1.85	3.13	-	8.25
Black tea (8)	BT1	Qimen black tea	Qimen, Anhui province	5.01	2.36	0.31	0.65	1.41	24.10	1.50	3.04	1.25	7.20
	BT2	Lizhi black tea	Yingde,Guangdong province	3.23	0.48	-	0.41	-	26.36	0.07	0.52	-	0.47
	BT3	Taicha	Xinbei, Taiwan province	7.02	0.71	0.84	2.13	1.93	34.40	1.35	7.74	-	4.42
	BT4	Douji black tea	Fengqing, Yunnan province	3.04	1.21	-	0.24	0.78	33.76	1.12	0.48	-	1.93
	BT5	Tongmuguan	Wuyi, Fujian province	4.30	1.05	-	0.74	-	29.02	0.73	1.16	-	2.58
	BT6	Jinjunmei	Wuyi, Fujian province	3.86	0.68	-	0.53	-	27.06	0.17	1.40	-	1.18
	BT7	Black tea	Lincang, Yunnan province	5.97	1.38	-	0.93	-	27.28	0.56	2.13	0.56	2.74
	BT8	Lizhi black tea	Yingde, Guangdong province	2.99	0.57	-	0.58	-	26.33	0.14	1.05	-	0.64

-, not detected.

Table S2. Linearity calibration curve factors, LOD and LOQ of the ten components.

Peak No.	RT (min)	Component	Range (mg /L)	Equation	R^2	LOD (ng)	LOQ (ng)
1	10.0	GA	0.5-25	y = 31.47 x - 6.69	0.9998	1.2	3.5
2	13.7	TBM	0.2-10	y = 31.65 x + 1.12	0.9995	0.5	1.6
3	14.1	GC	1-50	y = 39.08 x - 8.62	0.9980	1.1	3.0
4	17.7	EGC	2-100	y = 48.31 x - 8.51	0.9982	0.5	1.5
5	19.4	C	1-50	y = 39.19 x - 0.71	0.9999	0.6	1.9
6	20.2	CAF	2-100	y = 31.51 x + 22.11	0.9994	1.1	3.2
7	22.0	EC	1-50	y = 44.73 x + 12.46	0.9998	0.4	1.3
8	22.8	EGCG	2-100	y = 48.40 x - 5.23	0.9991	0.6	1.7
9	24.4	GCG	2-100	y = 33.96 x - 15.78	0.9999	0.8	2.2
10	27.6	ECG	2-100	y = 44.41 x + 35.22	0.9990	0.9	2.5

Table S3. Repeatability, reproducibility and stability of the method.

Component	Repeatability RSD (%, n = 6)	Reproducibility (n = 3)							Stability RSD (%, n = 6)
		First day		Third day		Fifth day		Interdays RSD (%)	
		Calculated content (mg/g)	RSD (%)	Calculated content (mg/g)	RSD (%)	Calculated content (mg/g)	RSD (%)		
GA	0.47	1.52 ± 0.03 [a]	1.85	1.49 ± 0.03	2.26	1.59 ± 0.03	2.02	3.35	0.89
TBM	0.35	1.28 ± 0.03	2.36	1.35 ± 0.02	1.85	1.28 ± 0.03	2.17	3.10	1.26
GC	0.18	3.32 ± 0.06	1.92	3.26 ± 0.05	1.63	3.22 ± 0.05	1.63	1.54	0.65
EGC	0.63	13.37 ± 0.33	2.45	13.21 ± 0.23	1.72	13.45 ± 0.31	2.32	0.92	1.28
C	0.56	4.89 ± 0.09	1.79	4.68 ± 0.06	1.21	4.64 ± 0.06	1.35	2.84	0.39
CAF	0.48	29.71 ± 0.20	0.68	29.42 ± 0.22	0.75	29.86 ± 0.35	1.16	0.75	0.63
EC	0.31	6.28 ± 0.11	1.76	6.31 ± 0.10	1.47	6.55 ± 0.13	1.82	2.32	0.95
EGCG	0.24	47.67 ± 0.49	1.03	46.26 ± 0.99	2.13	46.72 ± 0.60	1.29	1.53	0.79
GCG	0.31	8.10 ± 0.17	2.15	7.93 ± 0.10	1.28	7.58 ± 0.18	2.36	3.37	1.35
ECG	0.53	13.74 ± 0.23	1.68	14.02 ± 0.24	1.73	14.37 ± 0.28	1.98	2.25	0.58

[a] The value is collected from the green tea sample of GT1, and expressed as mean ± S.D. (n = 3).

Table S4. Recovery of the method.

Component	Added amount (mg) and recovery (%) at three spike levels									Average	
	50%			100%			200%				
	Added (mg)	Recovery (%)	RSD (%)	Added (mg)	Recovery (%)	RSD (%)	Added (mg)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
GA	0.40	92.73 ± 2.74 [a]	2.95	0.8	93.65 ± 1.23	1.31	1.6	96.83 ± 2.78	2.87	94.40 ± 2.15	2.28
TBM	0.30	91.56 ± 2.86	3.12	0.6	96.12 ± 4.15	4.32	1.2	90.29 ± 3.28	3.63	92.66 ± 3.07	3.31
GC	0.85	99.32 ± 2.55	2.57	1.7	92.47 ± 1.47	1.59	3.4	94.76 ± 2.48	2.62	95.52 ± 3.49	3.65
EGC	3.35	100.36 ± 3.24	3.23	6.7	103.68 ± 2.85	2.75	13.4	103.18 ± 2.28	2.21	102.41 ± 1.79	1.75
C	1.20	101.44 ± 2.60	2.56	2.4	96.92 ± 1.97	2.03	4.8	95.42 ± 1.57	1.65	97.93 ± 3.13	3.20
CAF	7.50	107.67 ± 1.57	1.46	15.0	108.59 ± 2.88	2.65	30.0	99.57 ± 1.10	1.10	105.28 ± 4.96	4.71
EC	1.55	96.69 ± 3.80	3.93	3.1	97.17 ± 2.64	2.72	6.2	101.39 ± 2.42	2.39	98.42 ± 2.59	2.63
EGCG	12.0	95.62 ± 3.47	3.63	24.0	92.72 ± 2.19	2.36	48.0	86.56 ± 2.54	2.94	91.63 ± 4.63	5.05
GCG	2.05	104.61 ± 1.53	1.46	4.1	95.75 ± 2.57	2.68	8.2	101.28 ± 2.91	2.87	100.55 ± 4.48	4.45
ECG	3.45	92.30 ± 3.02	3.27	6.9	95.66 ± 1.81	1.89	13.8	91.62 ± 3.34	3.65	93.19 ± 2.16	2.32

[a] The value is collected from the green tea sample of GT1, and expressed as mean ± S.D. (n = 3).

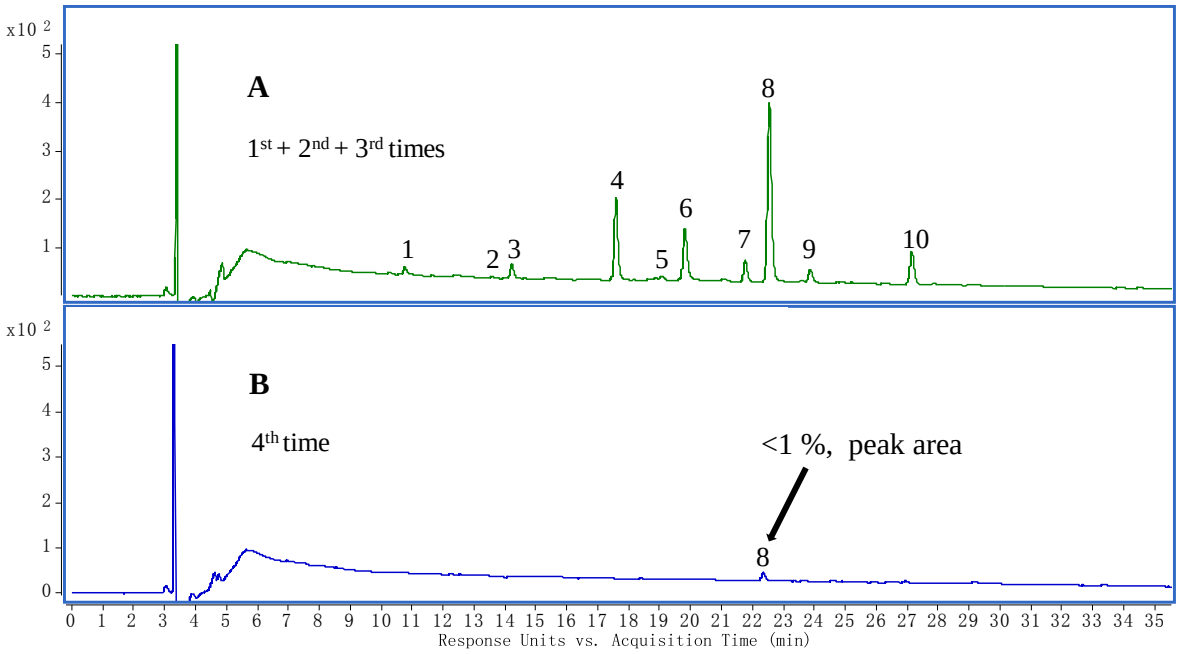
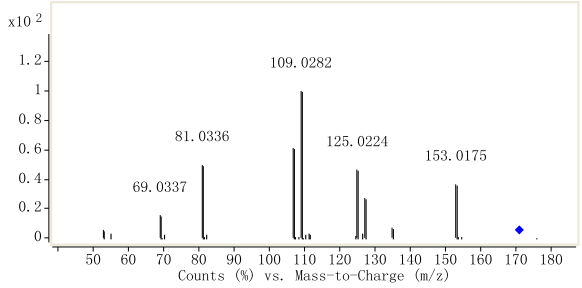
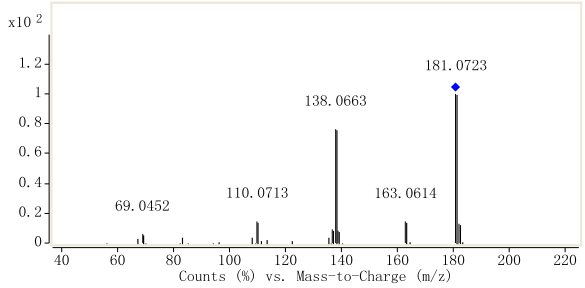
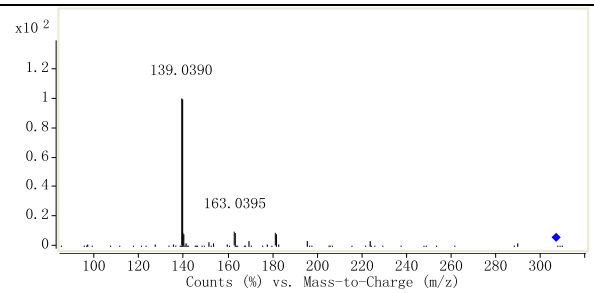
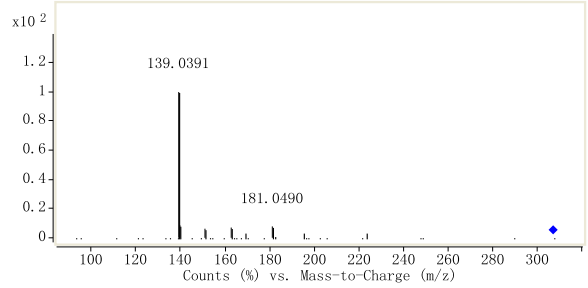
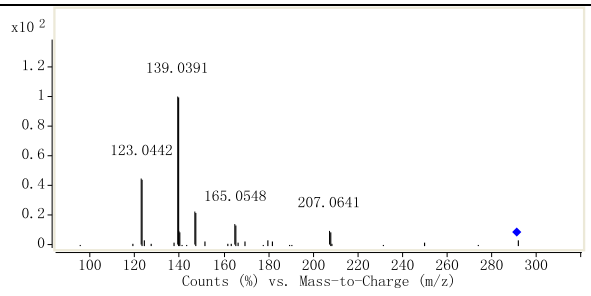


Fig. S1. Typical HPLC chromatograms of tea sample (GT1) of (A) the combined extracts by the 1st + 2nd + 3rd times and (B) the extracts by the 4th time.

Notes:

Brewing tea with hot water for a short while is the most popular way of tea drinking. Brewing period and brewing times were optimized, the results showed that that exhaustive extraction could be achieved when 0.5 g tea sample powder was extracted with 30 mL hot water at 99 ^{o}C for 10 min with intermittent shaking, three times (Figure S1).

Components	MS^2 spectra	Elemental composition			
		Formula	Calculated (m/z)	Observed (m/z)	Error (mDa)
GA		$C_7H_7O_5$	171.0293	171.0283	-1.0
		$C_7H_5O_4$	153.0188	153.0175	-1.3
		$C_6H_5O_3$	125.0239	125.0224	-1.5
		$C_6H_5O_2$	109.0290	109.0282	-0.8
		C_5H_5O	81.0340	81.0336	-0.4
		C_4H_5O	69.0340	69.0337	-0.3
TBM		$C_7H_9N_4O_2$	181.0726	181.0723	-0.3
		$C_7H_7N_4O$	163.062	163.0614	-0.6
		$C_6H_8N_3O$	138.0667	138.0663	-0.4
		$C_5H_8N_3$	110.0718	110.0713	-0.5
		$C_3H_5N_2$	69.0453	69.0452	-0.1
GC		$C_{15}H_{15}O_7$	307.0818	307.0819	0.1
		$C_9H_7O_3$	163.0395	163.0395	0.0
		$C_7H_7O_3$	139.0395	139.0390	-0.5
		-	-	-	-
		-	-	-	-
EGC		$C_{15}H_{15}O_7$	307.0818	307.0820	0.2
		$C_5H_9O_7$	181.0348	181.0490	14.2
		$C_7H_7O_3$	139.0395	139.0391	-0.4
		-	-	-	-
		-	-	-	-
C		$C_{15}H_{15}O_6$	291.0869	291.0870	0.1
		$C_{11}H_{11}O_4$	207.0657	207.0641	-1.6
		$C_9H_9O_3$	165.0552	165.0548	-0.4
		$C_7H_7O_3$	139.0395	139.0391	-0.4
		$C_7H_7O_2$	123.0446	123.0442	-0.4

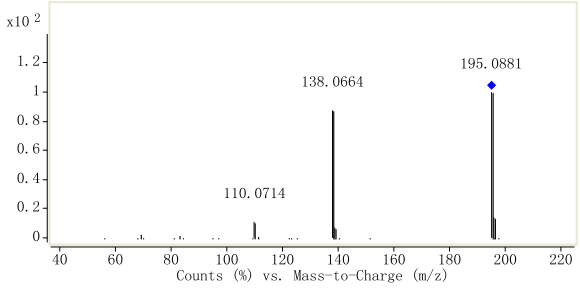
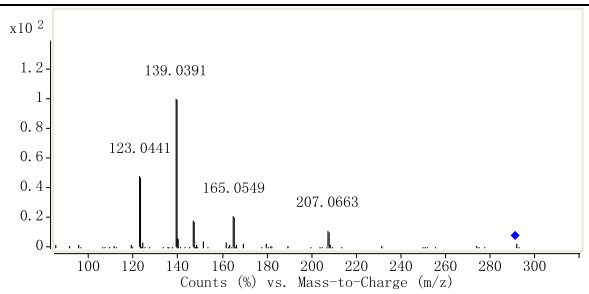
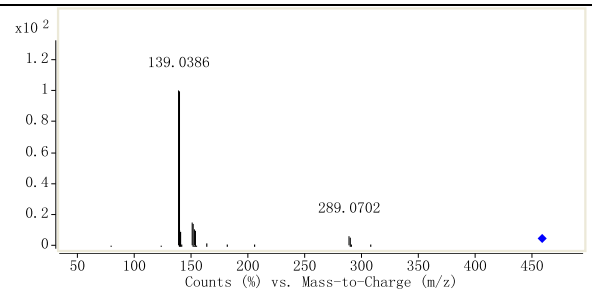
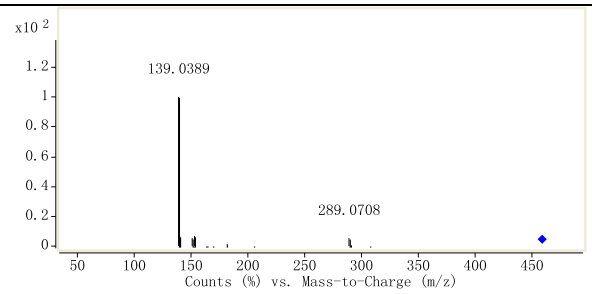
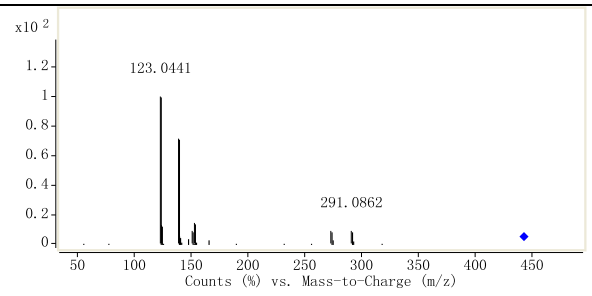
Components	MS^2 spectra	Elemental composition			
		Formula	Calculated (m/z)	Observed (m/z)	Error (mDa)
CAF		$C_8H_{11}N_4O_2$	195.0882	195.0881	-0.1
		$C_6H_8N_3O$	138.0667	138.0664	-0.3
		$C_5H_8N_3$	110.0718	110.0714	-0.4
EC		$C_{15}H_{15}O_6$	291.0869	291.0877	0.8
		$C_{11}H_{11}O_4$	207.0657	207.0663	0.6
		$C_9H_9O_3$	165.0552	165.0549	-0.3
		$C_7H_7O_3$	139.0395	139.0391	-0.4
		$C_7H_7O_2$	123.0446	123.0441	-0.5
EGCG		$C_{22}H_{19}O_{11}$	459.0927	459.0922	-0.5
		$C_{15}H_{13}O_6$	289.0712	289.0702	-1.0
		$C_7H_7O_3$	139.0395	139.0386	-0.9
		-	-	-	-
GCG		$C_{22}H_{19}O_{11}$	459.0927	459.0933	0.6
		$C_{15}H_{13}O_6$	289.0712	289.0708	-0.4
		$C_7H_7O_3$	139.0395	139.0389	-0.6
		-	-	-	-
ECG		$C_{22}H_{19}O_{10}$	443.0978	443.0980	0.2
		$C_{15}H_{15}O_6$	291.0869	291.0862	-0.7
		$C_7H_7O_2$	123.0446	123.0441	-0.5
		-	-	-	-

Fig. S2. The MS^2 spectra and elemental composition of the ten components in positive ion mode.