Survey of Catechins, Gallic Acid, and Methylxanthines in Green, Oolong, Pu-erh, and Black Teas

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An isocratic HPLC procedure was developed for simultaneous determination of six catechins, gallic acid, and three methylxanthines in tea water extract. A baseline separation was achieved on a Cosmosil C18-MS packed column with a solvent mixture of methanol/doubly distilled water/formic acid (19.5:80.2:0.3, v/v/v) as mobile phase. A gradient HPLC procedure was also provided for the separation of these tea components. The contents of catechins, gallic acid, and methylxanthines have been measured in infusions of a range of green tea, oolong tea, and pu-erh tea products sold and consumed in the China, Japan, and Taiwan. When 15 Chinese green tea and 13 Japanese green tea products were analyzed by the HPLC method, the mean levels of the total catechins, (−)-epigallocatechin 3-gallate, (+)-catechin, and caffeine were found to be very similar in these two groups, but other minor catechins such as (−)-epigallocatechin, (−)-epicatechin, and (−)-gallocatechin 3-gallate were found to be higher in Japanese green tea products, whereas (−)-epicatechin 3-gallate, gallic acid, theophylline, and theobromine were found to be higher in Chinese green tea products. Oolong tea products possessed lower levels of catechins, whereas pu-erh tea products contained negligible amounts of these constituents. The new HPLC method is rapid, reliable, and reproducible and should be highly recommended to tea industries for routine analysis of commercial tea samples.

Keywords: *Catechins; gallic acid; caffeine; theophylline; theobromine; green tea; oolong tea; paochong tea; pu-erh tea; black tea; isocratic HPLC*

INTRODUCTION

Tea plants are widely cultivated in Southeast Asia, including China, India, Japan, Taiwan, Sri Lanka, and Indonesia, and in central African countries. Tea is one of the most popular beverages in the world because of its attractive aroma, taste, and healthy effects. Hundreds of teas are now produced; generally, they are classified into three major categories: the nonfermented green tea, the partially fermented oolong or paochong tea, and the fully fermented black or pu-erh tea.

The composition of tea varies with species, season, age of the leaf (plucking position), climate, and horticultural practices (Lin et al., 1996). Green tea contains polyphenols, which include flavanols, flavadiols, flavonoids (Hertog et al., 1993), and phenolic acids; these compounds may account for up to 30% of the dry weight. The polyphenols are the most biologically active group of the tea components, especially certain catechins. The major tea catechins are (−)-epigallocatechin 3-gallate (EGCG), (−)-epigallocatechin (EGC), (−)-epicatechin (EC), (−)-gallocatechin (GC), and (+)-catechin (C). In the manufacturing of black tea, the monomeric flavan-3-ols undergo polyphenol oxidase-dependent oxidative polymerization leading to the formation of bisflavanols, theaflavins, thearubigins. and other oligomers in the process commonly known as fermentation.

Many biological functions of tea polyphenols have been studied (Yang and Wang, 1993; Lin et al., 1996, 1997) including antioxidative activity (Ho et al., 1992), antimutagenic effects (Shiraki et al., 1994), and anticarcinogenic effects (Oguni et al., 1988; Mukhtar et al., 1992; Katiyar et al., 1993; Lin et al., 1997) in several systems.

The tea alkaloids comprise several methylxanthines including caffeine, theophylline, and theobromine. Black tea contains normally 1.5−4% caffeine, 0.2−0.4% theobromine, and ∼0.02% theophylline. The different health effects of caffeine have been described by James (1991).

Numerous HPLC methods for the detection of tea alkaloids have been published (Dulitzky et al., 1984; Hoefler and Coggon, 1976; Naik et al., 1997), but in most countries the official methods are still nonchromatographic, including gravimetric determination and sodium hydrosulfite titration.

In most studies, the tea catechins and tea alkaloids are determined separately by HPLC (Dalluge et al., 1998) or other nonchromatographic methods. The major objectives of chemical analysis of tea cover the following important areas: (a) to find a constituent or a group of tea components that are a measure of tea quality; (b) to optimize the degree of fermentation that offers the best quality of tea; (c) to correlate the health effects of tea with certain tea components. Because both tea catechins and alkaloids have been demonstrated to exert their significant healthy effects to humans, it is important to develop a reliable and rapid HPLC method for the simultaneous determination of the levels of these two groups of constituents in a given tea sample. The composition of tea catechins and tea alkaloids in com-

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mercial tea samples varies with species, season, horticultural conditions (soil, water, minerals, fertilizers, etc.), and degree of fermentation during the manufacturing process. Therefore, a reliable method for systematic chemical analysis of tea composition is urgently needed.

In the present study, we have developed a simple and precise isocratic HPLC procedure for the determination of the composition of tea catechins and methylxanthines in various tea extracts. We have investigated the levels of tea catechins, gallic acid, and methylxanthines in the samples of green teas from China and Japan, paochong and oolong from Taiwan and China, and pu-erh teas from Yun-nan, China, by the newly developed HPLC method.

MATERIALS AND METHODS

Chemicals. Theophylline (1,3-dimethylxanthine), theobromine (3,7-dimethylxanthine), GCG, (−)-catechin 3-gallate (CG), and gallic acid (GA) were purchased from the Sigma Chemical Co. (St. Louis, MO). Caffeine (1,3,7-trimethylxanthine), methanol, and formic acid were purchased from E. Merck Co. (Darmstadt, Germany). (+)-Catechin (C) and EC were purchased from Aldrich Chemical Co. (Milwaukee, WI). EGCG standard was obtained from Wako Pure Chemical Industries (Osaka, Japan). EGCG, (−)-epicatechin 3-gallate (ECG), and EGC were isolated from longjing tea in this laboratory as described previously (Lin et al., 1996).

Tea Samples. In this study, several commercial tea samples including green, paochong, oolong, pu-erh, and black teas from different cities located in China, Japan, and Taiwan were analyzed. These tea samples were collected in the local markets when the authors were traveling in these cities. The chemical compositions of these tea samples varied with their species, cultivation conditions such as weather, temperature, moisture, latitude, and season, and process of manufacturing. It has been established that the levels of polyphenols, especially catechins, are remarkably affected by the production process.

Tea Varieties and Their Production Processes. It is well-known that green tea is nonfermented tea, whereas paochong and oolong teas are semifermented or partially fermented ones. The black and pu-erh teas are fully fermented teas. The production processes of these teas comprise a series of operations including withering, rolling, fermentation, and drying (Juan, 1993). Several terms that in the description of tea manufacturing need further elaboration. Solar withering, or hot-air withering, is the first step in tea manufacturing. Sun- or hot-air-drying ferments the tea leaves by activating chemical reactions within the cells. Indoor withering is an important step for partial fermentation. It allows fermentation to continue so that the tea leaf will release a special aroma when it is served by decocting or percolating in hot water. Tea leaves are stirred periodically by hand to expedite fermentation. A good tea maker knows how to control the degree of fermentation to produce the highest quality tea.

The purpose of panning is to block enzyme activity and then stop tea fermentation. Tea leaves lose most of the water that remains in the cells, turn soft, and then are ready for rolling and drying. Rolling has two major functions, which are to twist tea leaves into defined and beautiful shapes and damage cell structures. Cellular fluid is squeezed out and spread on the leaf surface to ensure better taste of the tea leaves. Mass-breaking is required to unknot tea leaves after rolling and make them easier to dry. Drying is the last, but not the least, important step in tea processing; the high heat disrupts the activity of enzymes such as polyphenol oxidase and glycosidases and thus completely stops the processes of fermentation.

Preparation of Tea Water Extract. The different varieties of tea samples were dried in an electric oven at 80−90 °C overnight and weighed. Each of the dry tea leaves (10 g) was added to 100 mL of boiling water and steeped for 10 min. The infusion was cooled to room temperature and then filtered. An aliquot of filtrate was diluted with water (×60 dilution) to give a 0.16% tea water extract (TWE) and then analyzed by HPLC as described below.

HPLC Analysis of Tea Extract. The composition of tea catechins, gallic acid, and methylxanthines in tea extract was determined by HPLC analysis using a Waters 600E system controller. The Waters 484 turnable absorbance detector was used to detect tea constituents at 280 nm, and all peaks were plotted and integrated by a Waters 745 data module. The HPLC method used a Cosmosil C18-MS packed column (5 μm, 46 mm i.d. × 250 mm) (Nacalai Tesque, Inc., Kyoto, Japan). The tea extract was filtered through a 0.45 μm filter disk, and then 20 μL was injected into the column. The concentrations of authentic catechin, gallic acid, and methylxanthine working solutions were 15 μg/mL. The amount of each authentic standard compound injected was 300 ng. The mobile phase was methanol/doubly distilled water/formic acid (19.5:80.2:0.3, v/v/v) and run by an isocratical elution at a flow rate of 1.0 mL/min. For the gradient elution, the following solvent systems were used: mobile phase A, methanol/formic acid/water (20:0.3:79.7, v/v/v); mobile phase B, methanol/formic acid (99.7:0.3, v/v). The gradient HPLC was performed as follows: 100% A for 10 min, to 90% A and 10% B for 15 min, and to 70% A and 30% B for 35 min in a linear gradient mode; elution was continued for 15 min. In all cases, the flow rate was 1.0 mL/min and both mobile phase flasks were degassed by continuous bubbling with helium gas. Identification of the individual catechin, gallic acid, and methylxanthine derivative was based on the comparison of the retention times of unknown peaks to those of reference authentic standards. The amount of each constituent in the tea extracts was estimated by the integrated datum provided by the Waters data module.

RESULTS

Separation of Authentic Catechins and Methylxanthines by Isocratic HPLC. A mixture of six catechins including EGC, C, EGCG, EC, GCG, and ECG, and gallic acid (GA) and three methylxanthines including theobromine, theophylline, and caffeine was separated by an isocratic HPLC as described above, and a baseline resolution was achieved (Figure 1A). The HPLC separation of these tea constituents (300 ng each) was accomplished in 60 min. The sensitivity of this HPLC analysis on tea catechins and xanthine alkaloids approached 30 ng per injection. The molar absorbency of EGC is rather low as compared to other catechins as indicated by their peak heights.

Separation of Authentic Catechins and Methylxanthines by Gradient HPLC. A mixture of seven catechins including EGC, C, EGCG, EC, GCG, ECG, and CG, gallic acid, and three methylxanthines was separated by a gradient HPLC as illustrated in Figure 2. The HPLC separation of these tea constituents (230 ng each) was accomplished in 36 min, whereas the whole gradient elution operation required 70 min as described above. An additional 20−30 min was required to bring the column to the initial conditions for the next sample injection. It seems that the gradient HPLC procedure is more time-consuming as compared to the isocratic one. However, the gradient HPLC has stronger elution power than the isocratic HPLC for the resolution of some less polar catechins such as CG (Figure 2). CG was found to stay on the column after 90 min of isocratic elution.

HPLC Separation of Catechins and Methylxanthines in Green Tea. Longjing tea and Mo-Li-Hua tea are classified as green tea in Taiwan and China, whereas decocted tea is regarded as green tea in Japan.

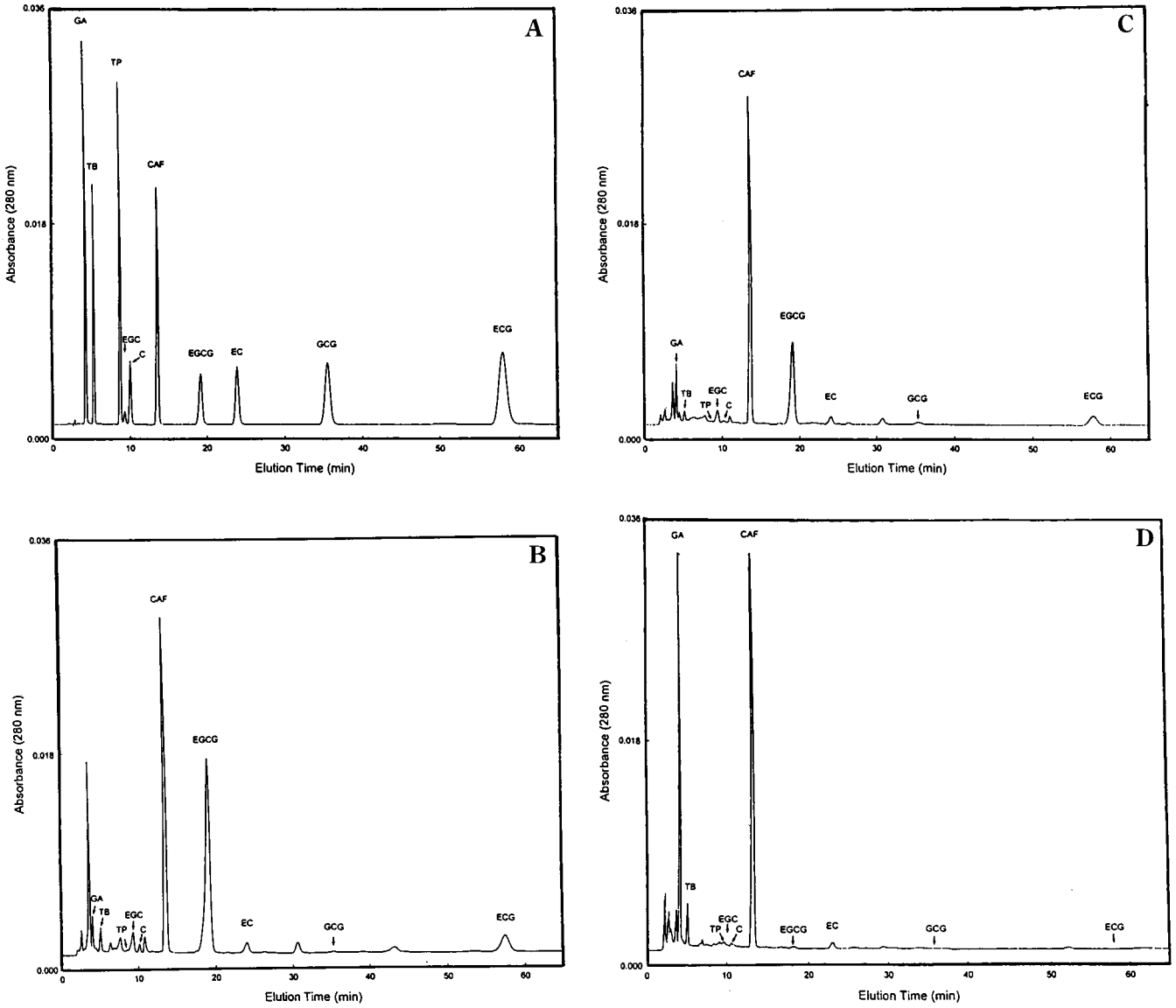

Figure 1. Isocratic HPLC separation of catechins, gallic acid, and alkaloids in tea water extracts. The chromatographic conditions were described under Materials and Methods. The HPLC method used a Cosmosil C18-MS packed column (5 μm, 46 mm i.d. × 250 mm). The TWE was filtered through a 0.45 μm filter disk, and a 20 μL aliquot of each sample solution was injected. The mobile phase was methanol/doubly distilled water/formic acid (19.5:80.2:0.3, v/v/v) run by an isocratic elution at a flow rate of 1.0 mL/min. The following sample solutions were analyzed: (A) mixture of authentic standard compounds (the amount of each compound in the injected solution is 300 ng); (B) Taiwan longjing tea, 0.16% TWE; (C) Fu-Chien oolong tea, 0.16% TWE; (D) Yun-Nan pu-erh tea, 0.16% TWE.

The representative HPLC patterns of longjing tea are illustrated in Figure 1B. The qualitative composition of longjing tea, Beijing green tea, and Kyoto green tea are very similar, but with some variations. It seems that the levels of EGC, EC, and caffeine are higher in Kyoto green tea as compared with that in Beijing green tea and Taiwan longjing tea. On the other hand, Beijing green tea and Taiwan longjing tea are higher in GA.

HPLC Separation of Catechins and Methylxanthines in Oolong Tea. Oolong tea is the most popular tea consumed in Taiwan and southern China because of its attractive aroma and taste. A representative HPLC pattern of Fu-Chien oolong tea is given in Figure 1C. It appears that the levels of catechins including EGCG, EC, ECG, EGC, and C are reduced after partial fermentation of tea (cf. Figure 1B). On the other hand, the levels of GA and caffeine are elevated after fermentation. The mechanisms of these elevations deserve further study.

HPLC Separation of Catechins and Methylxanthines in Black and Pu-erh Teas. Both black and pu-erh teas are classified as fully fermented teas. The representative HPLC patterns of Yun-Nan pu-erh tea are illustrated in Figure 1D. Both Lipton black tea and pu-erh tea contain very low levels of catechins; pu-erh tea, especially, contains only a scarce amount of catechins (Figure 1D). Both teas contain extremely high levels of GA and theobromine.

Determination of Catechins and Methylxanthines in Commercial Green Tea Products. The commercial Chinese green teas have many trade names, namely, Longjing, Mao-Feng, Mao-Chien, Yun-Woo, Pi-Luo-Chun, and Mo-Li-Hua (jasmine) teas (Table 1). The commercial Japanese green teas also have several trade names, namely, Sen-Cha, Mat-Cha, and decocted teas (Table 2). Fifteen Chinese green tea and 13 Japanese green tea products were analyzed by the HPLC method as described above, and the levels of their catechins and

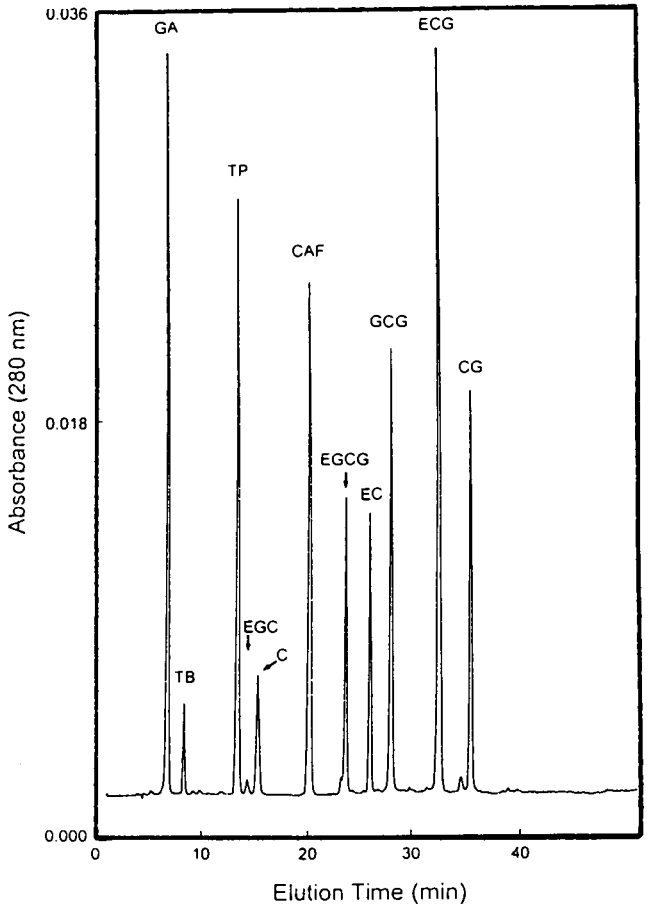

Figure 2. Gradient HPLC separation of catechins, gallic acid, and methylxanthines. The chromatographic conditions were described under Materials and Methods. A Cosmosil C18-MS packed column (5 μm, 46 mm i.d. × 250 mm) was used. A mixture of authentic standard compounds as described in Figure 1 plus (−)-CG was analyzed by the gradient elution procedure as described under Materials and Methods. The amount of each compound in the injected solution is 230 ng.

methylxanthines were estimated and are summarized in Tables 1 and 2, respectively. Their contents varied in different samples collected from different cities in China and Japan. When the mean values were calculated from these two groups, the levels of total catechins, EGCG, C, and caffeine were 17.86 and 17.80; 13.37 and 13.74; 0.02 and 0.02; and 7.73 and 7.68/100 mg, respectively (Tables 1 and 2). It is surprising to find that the mean levels of these four components are nearly identical. On the other hand, the levels of EGC, EC, and GCG were higher in Japanese green teas, whereas those of ECG, GA, TP, and TB were higher in Chinese green teas. The levels of catechins were found to be extremely high in green tea powder (Table 1, no. 16) as compared to other traditional green tea products. The green tea powder is a new type of commercial green tea product and is manufactured from fresh tea leaves, which are cultivated during the summer. It has been demonstrated that the tea catechins are higher in the tea leaves grown in summer (Lin et al., 1996). This finding suggests that green tea powder could be the product of choice for human consumption in the future.

Determination of Catechins and Methylxanthines in Commercial Oolong and Paochong Tea Products. It is estimated that ~80% of harvested tea is consumed as oolong tea in Taiwan. In the Taiwan local markets, both paochong and tieh-kuan-yin are

Table 1. Composition of Various Commercial Chinese Green Tea Products[a,b]

Chinese green tea[c]	total catechins	catechins							alkaloids		
		EGCG	EGC	ECG	EC	C	GCG	GA	caffeine	TP	TB
1, Fu-Chien Mao-Feng	20.20 ± 0.28	14.58 ± 0.18	0.54 ± 0.001	4.14 ± 0.08	0.67 ± 0.01	0.01 ± 0.002	0.27 ± 0.02	0.59 ± 0.04	9.70 ± 0.13	0.13 ± 0.01	0.93 ± 0.11
2, He-Nan Hsin-Yang Mao-Chien	22.66 ± 0.41	17.69 ± 0.28	0.58 ± 0.03	3.39 ± 0.12	0.62 ± 0.02	0.02 ± 0.001	0.37 ± 0.02	0.43 ± 0.01	7.47 ± 0.04	0.04 ± 0.01	0.41 ± 0.03
3, Hu-Pei He-Feng Fu-Hsi	20.74 ± 0.23	16.46 ± 0.21	0.46 ± 0.01	3.10 ± 0.07	0.47 ± 0.01	0.03 ± 0.002	0.21 ± 0.02	0.59 ± 0.01	9.83 ± 0.16	0.05 ± 0.01	0.52 ± 0.03
4, Chue-Chiag longjing	18.90 ± 1.32	16.48 ± 1.16	30 ± 0.19	17 ± 0.27	68 ± 0.30	01 ± 0.01	0.35 ± 0.04	0.60 ± 0.03	17 ± 0.59	06 ± 0.11	92 ± 0.09
5, Hu-Pei San-Hsia Mao-Chien	15.75 ± 0.01	12.28 ± 0.05	0.29 ± 0.02	2.64 ± 0.03	0.34 ± 0.01	0.02 ± 0.01	0.19 ± 0.02	0.47 ± 0.02	7.73 ± 0.25	0.12 ± 0.01	0.96 ± 0.01
6, Shan-Hsi Tsu-Yang	17.45 ± 0.81	13.87 ± 0.57	0.40 ± 0.01	2.53 ± 0.20	0.39 ± 0.08	0.01 ± 0.001	0.24 ± 0.01	0.34 ± 0.004	6.42 ± 0.15	0.09 ± 0.02	0.49 ± 0.02
7, He-Nan Hsin-Yang Yun-Woo	17.37 ± 0.38	13.58 ± 0.48	0.44 ± 0.02	2.75 ± 0.15	0.39 ± 0.06	0.02 ± 0.001	0.29 ± 0.04	0.42 ± 0.02	7.19 ± 0.17	0.13 ± 0.01	0.55 ± 0.02
8, Che-Chiang Hang-Chou longjing	18.16 ± 0.49	12.41 ± 0.31	0.31 ± 0.18	4.63 ± 0.18	0.47 ± 0.02	0.02 ± 0.01	0.32 ± 0.02	0.60 ± 0.07	8.91 ± 0.25	0.08 ± 0.03	0.46 ± 0.06
9, An-Hui Huang-Shan Yun-Woo	11.61 ± 0.15	8.87 ± 0.11	0.10 ± 0.06	2.25 ± 0.02	0.23 ± 0.02	0.1 ± 0.01	0.16 ± 0.04	0.65 ± 0.04	8.69 ± 0.13	0.10 ± 0.003	0.68 ± 0.04
10, An-Hui Pi-Luo-Chun	12.38 ± 0.14	8.65 ± 0.08	0.24 ± 0.05	2.96 ± 0.23	0.35 ± 0.01	0.01 ± 0.002	0.17 ± 0.01	0.62 ± 0.11	7.89 ± 0.26	0.07 ± 0.02	0.38 ± 0.08
11, Bei-Jing Mo-Li-Hua	22.18 ± 0.97	16.45 ± 0.80	0.78 ± 0.06	3.63 ± 0.09	0.88 ± 0.13	0.01 ± 0.01	0.43 ± 0.03	0.68 ± 0.03	8.67 ± 0.48	0.11 ± 0.004	0.80 ± 0.08
12, Fu-Chien Hu-Chiu Mo-Li-Hua	16.22 ± 0.77	12.74 ± 0.55	0.51 ± 0.18	2.24 ± 0.05	0.43 ± 0.04	0.02 ± 0.004	0.29 ± 0.07	0.48 ± 0.02	6.86 ± 0.50	0.07 ± 0.01	0.49 ± 0.03
13, Ssu-Chuan Cheng-Tou Mo-Li-Hua	13.11 ± 0.10	10.00 ± 0.13	0.39 ± 0.03	2.10 ± 0.15	0.41 ± 0.06	0.03 ± 0.002	0.19 ± 0.01	0.55 ± 0.01	5.19 ± 0.59	0.06 ± 0.002	0.39 ± 0.10
14, Ssu-Chuan Yung-Chuan Mo-Li-Hua	18.58 ± 0.38	13.60 ± 0.28	0.60 ± 0.01	3.32 ± 0.07	0.79 ± 0.06	0.05 ± 0.001	0.22 ± 0.03	0.70 ± 0.01	7.44 ± 0.15	0.07 ± 0.003	0.56 ± 0.02
15, San-Shya, Taipei longjing	22.61 ± 3.86	12.82 ± 1.86	0.59 ± 0.04	2.83 ± 0.25	1.14 ± 0.02	0.03 ± 0.002	0.24 ± 0.01	0.04 ± 0.01	5.76 ± 0.21	0.05 ± 0.01	0.49 ± 0.06
mean ± SE (n = 15)	17.86 ± 0.69	13.37 ± 0.47	0.44 ± 0.06	2.91 ± 0.13	0.55 ± 0.06	0.02 ± 0.005	0.26 ± 0.03	0.52 ± 0.03	7.73 ± 0.27	0.08 ± 0.02	0.60 ± 0.05
16, Tien-Fu, Taipei, powder[d]	26.03 ± 1.25	20.32 ± 0.78	0.83 ± 0.01	3.02 ± 0.11	1.10 ± 0.38	0.05 ± 0.01	0.71 ± 0.17	0.25 ± 0.09	7.11 ± 0.48	0.04 ± 0.01	0.44 ± 0.06

[a] Samples were prepared as described under Materials and Methods. [b] The values are averages from triplicate samples and are given in mg/100 mg dry tea leaves or %, w/w, mean ± SE. [c] The commercial Chinese green tea products comprise several varieties including longjing tea, Mo-Li-Hua tea, Pi-Luo-Chun tea, Yun-Woo tea, Tsu-Yang tea, Mao-Chien tea, and Mao-Feng tea. All these green teas are produced in different locations as indicated. [d] Green tea powder, a new preparative form of longjing tea, is made from dried fresh tea leaves by pulverization.

Table 2. Composition of Various Commercial Japanese Green Tea Products[a,b]

Japanese green tea[c]	total catechins	catechins							alkaloids		
		EGCG	EGC	ECG	EC	C	GCG	GA	caffeine	TP	TB
1, Osaka-Fu	8.92 ± 0.86	5.73 ± 0.67	0.57 ± 0.21	1.05 ± 0.07	0.19 ± 0.08	ND[d]	1.38 ± 0.08	1.23 ± 0.20	7.46 ± 0.31	0.15 ± 0.02	0.37 ± 0.17
2, Aichi-Ken	16.36 ± 0.74	13.61 ± 0.51	0.72 ± 0.09	1.23 ± 0.07	0.57 ± 0.13	0.02 ± 0.004	0.22 ± 0.03	0.12 ± 0.05	6.99 ± 0.18	0.05 ± 0.01	0.22 ± 0.01
3, Kyoto Mat-Cha	21.59 ± 0.55	16.83 ± 0.55	0.90 ± 0.13	2.56 ± 0.16	0.93 ± 0.11	0.03 ± 0.002	0.35 ± 0.03	0.23 ± 0.01	8.08 ± 0.23	0.08 ± 0.01	0.44 ± 0.02
4, Shizuoka 1	15.89 ± 0.32	11.61 ± 0.22	0.65 ± 0.09	2.50 ± 0.18	0.90 ± 0.04	0.03 ± 0.01	0.21 ± 0.01	0.15 ± 0.01	7.16 ± 0.35	0.05 ± 0.01	0.49 ± 0.004
5, Shizuoka 2	21.23 ± 0.40	16.75 ± 0.35	0.91 ± 0.02	2.32 ± 0.04	1.01 ± 0.06	0.02 ± 0.01	0.22 ± 0.04	0.12 ± 0.004	8.37 ± 0.13	0.04 ± 0.003	0.35 ± 0.02
6, Kyoto Sen-Cha	21.10 ± 1.13	16.19 ± 0.86	1.05 ± 0.03	2.46 ± 0.16	1.19 ± 0.23	0.02 ± 0.001	0.18 ± 0.06	0.19 ± 0.02	8.20 ± 0.40	0.07 ± 0.01	0.34 ± 0.05
7, Kyoto	14.39 ± 0.12	10.99 ± 0.18	0.70 ± 0.01	1.39 ± 0.08	0.70 ± 0.12	0.02 ± 0.001	0.58 ± 0.04	0.28 ± 0.01	6.94 ± 0.03	0.09 ± 0.02	0.28 ± 0.01
8, Fukuoka-Ken	17.81 ± 1.12	14.22 ± 1.12	0.70 ± 0.04	1.77 ± 0.05	0.87 ± 0.07	0.03 ± 0.004	0.22 ± 0.01	0.18 ± 0.01	9.57 ± 0.64	0.17 ± 0.02	0.30 ± 0.05
9, Tokyo decocted 1	18.13 ± 0.53	13.77 ± 0.69	1.17 ± 0.09	1.84 ± 0.11	1.06 ± 0.16	0.02 ± 0.01	0.26 ± 0.04	0.08 ± 0.02	6.72 ± 0.55	0.06 ± 0.004	0.31 ± 0.03
10, Tokyo decocted 2	24.11 ± 1.11	18.81 ± 1.19	1.19 ± 0.09	2.59 ± 0.10	1.15 ± 0.09	0.02 ± 0.01	0.56 ± 0.05	0.12 ± 0.002	8.62 ± 0.66	ND	0.26 ± 0.04
11, Tokyo decocted 3	19.13 ± 1.33	14.88 ± 1.19	0.95 ± 0.11	2.04 ± 0.10	0.92 ± 0.13	0.02 ± 0.001	0.33 ± 0.03	0.10 ± 0.04	6.90 ± 1.11	ND	0.26 ± 0.05
12, Tokyo Yn-Loo	18.88 ± 3.26	14.69 ± 2.65	0.93 ± 0.02	1.95 ± 0.36	1.05 ± 0.28	0.03 ± 0.02	0.24 ± 0.08	0.12 ± 0.02	7.80 ± 0.74	0.04 ± 0.02	0.28 ± 0.06
13, Tokyo Thu-Ya	13.69 ± 0.28	10.50 ± 0.46	0.64 ± 0.01	1.61 ± 0.24	0.76 ± 0.07	0.02 ± 0.001	0.16 ± 0.04	0.12 ± 0.001	7.06 ± 0.45	0.02 ± 0.001	0.29 ± 0.008
mean ± SE (n = 13)	17.80 ± 0.90	13.74 ± 0.82	0.88 ± 0.07	1.95 ± 0.13	0.87 ± 0.12	0.02 ± 0.006	0.38 ± 0.04	0.23 ± 0.03	7.68 ± 0.44	0.06 ± 0.01	0.32 ± 0.04

[a,b] As described in Table 1. [c] The Japanese green tea products comprise a wide variety of names including Mat-Cha, Sen-Cha, decocted tea, and others. The green tea samples are purchased from different locations as indicated. [d] Not detectable.

recognized as lighter oolong teas. In this study, nine commercial Chinese oolong tea products were analyzed by the HPLC method as described above. The composition of catechins and alkaloids is summarized in Table 3. The levels of total catechins, EGCG, ECG, and GCG were down to one-third of those found in Chinese green teas (Table 1). The levels of caffeine, TP, and TB were also reduced.

Determination of Catechins and Methylxanthines in Commercial Pu-erh Tea Products. Pu-erh tea is manufactured according to a special two-step procedure. The first step is similar to that of longjing green tea, whereas the second step is natural postoxidation by incubating the above intermediate products (from step 1) in a closed storage chamber for a defined period of time. The moisture of the storage chamber will profoundly determine the quality of pu-erh tea. A wet storage chamber (with high air moisture) can shorten the manufacturing time but give a poor-quality tea product, whereas a dry storage chamber (with reasonably low air moisture) can prolong the incubating time but give a high-quality pu-erh tea. It is believed that some unidentified fungus species may be involved in the natural postoxidation processes.

Seven commercial pu-erh tea products were analyzed by the HPLC method as described above. The levels of catechins and alkaloids are summarized in Table 4. All catechin components in pu-erh tea were dramatically reduced as compared with those in green and oolong teas, but the level of GA is remarkably increased.

Effect of Fermentation on the Levels of Tea Catechins and Alkaloids in TTE 12 Tea Leaves. From the aforementioned data (Tables 1−4), it seems that the degree of fermentation has a profound effect on the levels of catechins and alkaloids. Because samples of commercial teas have been obtained from different locations, the origins and manufacturing processes of these tea samples are completely unclear. Therefore, we decided to use one species of fresh tea leaves, namely, TTE 12, to study the effect of fermentation process on the levels of these constituents. The results are illustrated in Table 5. The reasons for selecting TTE 12 as starting material are as follows: The tea plant TTE 12 was derived from *Camellia sinensis* by hybridization at Taiwan Tea Experiment Station in 1981. This hybrid tea species is highly resistant to various insect infections and very productive in leaf yield with moderate tea polyphenol contents; therefore, TTE 12 is now widely cultivated in many tea gardens in Taiwan. In this study, the fermentation processes are carried out by tea-making experts in the Taiwan Tea Experiment Station. The resulting tea products are classified A, B, C, and D according to the degree of fermentation, namely, 0, 10, 25, and 85%, correspondingly. These four classes of tea products are equivalent to the commercial longjing, paochong, oolong, and black teas, respectively. The levels of all catechins including EGCG, EGC, ECG, EC, C, and GCG are progressively reduced during the fermentation process, whereas the level of GA is elevated (Table 5). The levels of both TP and TB are also progressively reduced, whereas that of caffeine is not changed in the low degree (10−25%) of fermentation but is significantly elevated in the high degree (85%) of fermentation up to 16 mg/100 mg (Table 5). The biochemical mechanism of this elevation is interesting and deserves further investigation.

Table 3. Composition of Various Commercial Chinese Oolong and Paochong Tea Products[a,b]

Chinese oolong and paochong tea[c]	total catechins	catechins							alkaloids		
		EGCG	EGC	ECG	EC	C	GCG	GA	caffeine	TP	TB
1, Fu-Chien An-Hsi Tieh-Kuan-Yin	7.18 ± 0.97	5.48 ± 0.68	0.39 ± 0.05	0.96 ± 0.16	0.32 ± 0.10	0.01 ± 0.001	0.05 ± 0.01	0.40 ± 0.05	6.02 ± 0.66	0.03 ± 0.001	0.19 ± 0.03
2, Fu-Chien (Shen-Chun) An-Hsi Tieh-Kuan-Yin	8.19 ± 0.03	6.34 ± 0.03	0.45 ± 0.05	1.00 ± 0.01	0.30 ± 0.04	0.01 ± 0.004	0.09 ± 0.01	0.34 ± 0.01	5.75 ± 0.07	0.04 ± 0.001	0.26 ± 0.01
3, Fu-Chien Yu-Shan Tieh-Kuan-Yin	5.93 ± 0.53	4.69 ± 0.51	0.30 ± 0.01	0.69 ± 0.01	0.17 ± 0.02	0.01 ± 0.001	0.07 ± 0.003	0.22 ± 0.01	5.45 ± 0.23	0.03 ± 0.01	0.15 ± 0.005
4, Fu-Chien oolong	8.87 ± 1.07	7.11 ± 1.11	0.39 ± 0.02	97 ± 0.05	0.26 ± 0.03	0.02 ± 0.001	0.13 ± 0.03	0.58 ± 0.03	6.88 ± 0.37	0.01 ± 0.001	0.29 ± 0.02
5, Fu-Chien Wun-Yi oolong	5.51 ± 0.93	4.34 ± 0.95	0.21 ± 0.05	0.64 ± 0.01	0.18 ± 0.05	0.01 ± 0.003	0.13 ± 0.06	0.56 ± 0.02	6.11 ± 0.46	0.07 ± 0.001	0.21 ± 0.01
6, Fu-Chien An-Chi Tieh Kuan-Yin	5.37 ± 0.39	4.12 ± 0.30	0.22 ± 0.04	0.66 ± 0.04	0.20 ± 0.02	0.01 ± 0.001	0.16 ± 0.06	0.57 ± 0.02	6.55 ± 0.20	0.06 ± 0.05	0.30 ± 0.06
7, An-Hui Huang-Shan Mao-Feng	5.24 ± 0.93	3.88 ± 0.42	0.18 ± 0.07	1.21 ± 0.04	0.24 ± 0.02	0.01 ± 0.001	0.06 ± 0.01	0.38 ± 0.03	4.54 ± 0.12	0.03 ± 0.01	0.22 ± 0.07
8, Ping-Lin, Taipei, paochong	7.12 ± 0.18	5.08 ± 0.12	0.59 ± 0.03	0.82 ± 0.04	0.45 ± 0.02	ND[d]	0.04 ± 0.01	0.42 ± 0.11	7.78 ± 0.24	0.05 ± 0.04	0.45 ± 0.05
9, Ping-Lin, Taipei, oolong	5.20 ± 0.12	3.68 ± 0.11	0.39 ± 0.05	0.65 ± 0.01	0.35 ± 0.01	ND	0.11 ± 0.02	0.58 ± 0.08	8.32 ± 0.09	0.02 ± 0.01	0.47 ± 0.09
mean ± SE (n = 9)	6.51 ± 0.57	4.97 ± 0.47	0.35 ± 0.04	0.84 ± 0.04	0.27 ± 0.03	0.009 ± 0.001	0.09 ± 0.02	45 ± 0.04	6.38 ± 0.27	0.04 ± 0.01	0.28 ± 0.04

[a,b]As described in Table 2. [c] Both paochong and tieh-kuan-yin teas are classified in the same category with 10−25% fermentation, whereas oolong tea is moderately fermented with a wide range of fermentation (25−60%). In the Taiwan local markets, both paochong and tieh-kuan-yin are regarded as lighter oolong teas. [d] Not detectable.

Table 4. Composition of Various Commercial Pu-erh Tea Products[a,b]

Pu-Erh tea[c]	total catechins	catechins							alkaloids		
		EGCG	EGC	ECG	EC	C	GCG	GA	caffeine	TP	TB
1, Yun-Nan Co.	0.34 ± 0.05	0.09 ± 0.01	0.23 ± 0.05	ND[d]	0.02 ± 0.001	ND	ND	1.25 ± 0.06	7.20 ± 0.38	0.06 ± 0.06	0.64 ± 0.01
2, Tien-Jen-Ming-Cha	0.14 ± 0.12	0.04 ± 0.07	0.06 ± 0.02	ND	0.04 ± 0.04	ND	ND	0.78 ± 0.03	5.64 ± 0.09	ND	0.50 ± 0.24
3, I-Pin-Tang Cha-Chuang	0.72 ± 0.11	0.10 ± 0.07	0.26 ± 0.09	0.12 ± 0.01	0.25 ± 0.05	ND	ND	2.01 ± 0.03	8.62 ± 0.58	0.06 ± 0.05	0.89 ± 0.30
4, China Native & Animal I&E Corp.	0.51 ± 0.10	0.25 ± 0.07	0.21 ± 0.01	ND	0.05 ± 0.02	ND	ND	1.88 ± 0.04	8.63 ± 0.16	ND	0.63 ± 0.02
5, San-Hua Cha-Hang	0.16 ± 0.01	ND	0.08 ± 0.01	01 ± 0.01	0.07 ± 0.001	ND	ND	0.97 ± 0.02	6.71 ± 0.11	ND	0.40 ± 0.02
6, Tein-Hsiang-Ming-Cha	0.94 ± 0.10	0.19 ± 0.16	0.40 ± 0.05	0.09 ± 0.08	0.22 ± 0.02	ND	0.04 ± 0.07	1.04 ± 0.05	9.93 ± 0.20	ND	0.82 ± 0.06
7, Shang-Mei Cha-Chuang	0.64 ± 0.03	0.37 ± 0.02	0.10 ± 0.07	0.12 ± 0.01	ND	ND	0.05 ± 0.05	2.50 ± 0.03	7.20 ± 0.08	0.10 ± 0.05	0.56 ± 0.01
mean ± SE (n = 7)	0.49 ± 0.07	0.15 ± 0.06	0.19 ± 0.04	0.05 ± 0.01	0.09 ± 0.02	ND	0.01 ± 0.02	1.49 ± 0.04	7.70 ± 0.23	0.03 ± 0.02	0.63 ± 0.09

[a,b]As described in Table 2. [c] Most Pu-Erh teas were produced in Yun-Nan, China. The names of suppliers are given. [d] Not detectable.

Table 5. Effects of Fermentation on the Levels of Tea Catechins and Alkaloids in TTE 12 Species[a,b]

classification	degree (%) of fermentation	commercial name	total catechins	catechins							alkaloids		
				EGCG	EGC	ECG	EC	C	GCG	GA	caffeine	TP	TB
A	0	longjing tea	8.85 ± 0.17	6.46 ± 0.17	0.72 ± 0.04	1.02 ± 0.04	0.54 ± 0.02	0.04 ± 0.03	0.09 ± 0.02	0.20 ± 0.07	8.69 ± 0.28	0.06 ± 0.02	0.47 ± 0.09
B	10	paochong tea	7.00 ± 0.19	5.08 ± 0.15	0.59 ± 0.03	0.82 ± 0.04	0.45 ± 0.02	ND[c]	0.06 ± 0.01	0.42 ± 0.11	7.72 ± 0.23	0.05 ± 0.04	0.45 ± 0.05
C	25	oolong tea	5.09 ± 0.10	3.62 ± 0.10	0.38 ± 0.05	0.63 ± 0.01	0.34 ± 0.01	ND	0.11 ± 0.02	0.58 ± 0.08	8.25 ± 0.10	0.02 ± 0.02	0.47 ± 0.09
D	85	black tea	0.52 ± 0.08	0.30 ± 0.10	0.19 ± 0.02	ND	0.04 ± 0.03	ND	ND	1.83 ± 0.52	16.03 ± 0.75	ND	0.26 ± 0.06

[a] Samples were prepared as described under Materials and Methods. The tea plant of TTE 12 was derived from *Camellia sinensis* by hybridization and developed by Taiwan Tea Experiment Station in 1981. This tea species is resistant to various worm infections and very productive in leaf yield. Therefore, TTE 12 is widely cultivated in Taiwan tea gardens. [b] The values are averages from six samples and are given in mg/100 mg of dry tea leaves, or % (w/w). [c] ND, not detectable.

DISCUSSION

It has been demonstrated that polyphenols are the most abundant group of constituents in the tea leaf. Among these, the catechins constitute the major components, with up to 26−30% of the dry matter of the fresh leaf. Catechins make an important contribution, especially to the bitter and astringent taste of green tea. Tea has been considered as a crude medicine in China for more than 4000 years. Different kinds of pharmacological effects such as protection of blood vessels, reduction of serum cholesterol levels, and prevention of arteriosclerosis were reported as integrated effects (Cheng et al., 1988). The major active principles of tea have been identified as catechins. Their action mechanisms of anticarcinogenesis have been attributed to the competitive inhibition of cytochrome P450 involved in the bioactivation of various carcinogens, as well as to antioxidant properties as scavengers of reactive oxygen species (Katiyar et al., 1993).

Recent studies have shown that tea catechins cause increases in the activities of phase II detoxifying enzymes (Lee et al., 1995), suppression of extracellular signals and cell proliferation by tea extract and EGCG (Liang et al., 1997), and inhibition of the induction of nitric oxide synthase by EGCG (Lin and Lin, 1997). Investigations from this laboratory and others have indicated that modulation of mitotic signal transduction by these catechins may be attributed, in part, to the molecular mechanisms of these cancer chemopreventive agents.

The constitutents of green and black teas have been the subject of intensive investigations for a long time (Hoefler and Coggon, 1976; Treutter, 1989; Opie et al., 1991). The first objective following the identification of the main catechins of tea is to determine their distribution in different parts of the tea plant (Lin et al., 1996) and their fate during the processing of the black tea (Roberts et al., 1957; Bhatia and Ullah, 1968). During the manufacture of oolong and black tea, the catechins (flavanols) are easily oxidized by polyphenol oxidase, and further polymerizations lead to theaflavins, thearubigins, and compounds of higher molecular mass. The amount and proportion of various catechins, depending on the leaf age and degree of fermentation (Tables 1−3), are directly correlated with the quality of the final beverage. It is generally believed that the finest teas are made from young tea shoots containing the highest catechin levels (Thanaraj and Seshadri, 1990).

Gallic acid is the most important phenolic acid in tea. The amount of gallic acid increases during the fermentation owing to its liberation from catechin gallates (Table 5). The antioxidant property has been described in many studies. Several studies on the anticarcinogenic effect of gallic acid are now in progress.

It is apparent that catechins, methylxanthines, and gallic acid are three major groups of biologically active principles in tea. HPLC analysis of methylxanthines and gallic acid with different mobile systems has been described (Hoefler and Coggon, 1976). These authors also developed a mobile system for analyzing theaflavins, but not for catechins. In the present study, we have developed an isocratic HPLC procedure for simultaneous determination of catechins, methylxanthines, and gallic acid in tea water extract. The applicability and reproducibility of this newly developed method have been evaluated and assessed by the estimations of 28 commercial green tea products (Tables 1 and 2), 9 commercial oolong tea products (Table 3), and 7 commercial pu-erh tea products (Table 4). The analytical data seem to be reliable and reproducible. Therefore, the new method is highly recommendable for routine analysis of commercial tea samples.

The preference of individual people for various kinds of tea is rather different in different countries. Most American and European people prefer black tea, whereas most Japanese and northern Chinese prefer green tea. On the other hand, oolong and paochong teas are consumed by most people living in Taiwan and southern China. According to the processes of manufacturing, oolong and paochong teas require more tedious procedures and specific techniques to control the degree of fermentation. These hand-operated processes will determine the aroma and taste of the final tea products. The aroma and taste of infusions made from oolong and paochong tea are quite unique and different from those of green or black tea. The aroma and taste of green tea is rather mild and astringent; this situation is improved in Chinese green tea by aromatized with jasmine flower during its manufacturing processes. Therefore, most Chinese green teas in the local market are sold as jasmine (Mo-Li-Hua) green tea (Table 1).

It is estimated that >70% of manufactured tea is consumed as black tea worldwide. However, this is not the case in Taiwan, where >80% of cultivated tea is consumed as oolong and paochong tea. The anticarcinogenic effects of green and black tea have been intensively studied (Huang et al., 1992; Wang et al., 1995; Lin et al., 1997). Rather little attention has been paid to the biological effects of oolong and paochong tea. The health effects of the active principle in oolong tea are now under investigation in our laboratory.

ABBREVIATIONS USED

C, (+)-catechin; EC, (−)-epicatechin; CG, (−)-catechin 3-gallate; ECG, (−)-epicatechin 3-gallate; EGC, (−)-epigallocatechin; EGCG, (−)-epigallocatechin 3-gallate; GCG, (−)-gallocatechin 3-gallate; GA, gallic acid; CAF, caffeine; TB, theobromine; TP, theophylline; TWE, tea water extract; HPLC, high-performance liquid chromatography.

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