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ORIGINAL PAPER

Effects of temperature and ultrasonic scaler on the infusion process of green tea leaves and catechins stability under ultrasonic vibration

Yanfeng Wang[1] · Qiao Yuan[1] · Jiajun Zeng[1] · Yu Cai[1] · Qingxian Luan[1]

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Abstract

This study was aimed to investigate the effects of temperature and ultrasonic scaler on infusion process of green tea leaves, and catechins stability under ultrasonic vibration in green tea infusion (GTI). (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG), (−)-catechin (C), (+)-epicatechin (EC), (−)-epigallocatechin gallate (EGCG) and (−)-gallocatechin gallate (GCG) were measured with ultra-high performance liquid chromatography (UHPLC). Green tea leaves were infused with distilled water (1:50 w/v) for 0.5–24 h at 25 °C, and 15 min at 65 °C respectively. Meanwhile, the infusion process of same ratio was conducted for 2 h at 25 °C, with or without assistance of ultrasonic scaler. After filtration of tea leaves, the tip of ultrasonic scaler was immersed into GTI and vibrated at 33 kHz for 2 h. More amounts of EGC, ECG, C, EC could be obtained in GTI (24 h, 25 °C) compared to the contrast (15 min, 65 °C), meanwhile, the concentration of EGCG could achieve 966.5 ± 2.9 μg/mL which accounted for 97.6% of that in the contrast. During the infusion process for 2 h, catechins amount could be improved by 20.8–31.2% with the ultrasonic assistance. After filtration of tea leaves, the amount of total catechins in GTI could be increased by 4.7% under ultrasonic vibration. Our results indicate that a high-quality GTI could be prepared at 25 °C and the level of catechins achieve a plateau phase after 3 h. The ultrasonic scaler could accelerate the infusion process, and didn't affect the catechins stability in GTI at 25 °C.

Keywords Tea infusion · Catechins · Temperature · Ultrasound · Ultra-high performance liquid chromatography

Introduction

Tea is one of the most popular beverages in the world and data from the FAO questionnaire (International Tea Committee) shows that annual consumption of tea and tea products is approximately 3 million tons, worldwide, just after water [1]. Drinking tea has potential effects on reducing risks for cardiovascular diseases and cancers, as well as losing body weight, which is attributed to the main composition in tea, catechins, including (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG), (−)-catechin (C), (+)-epicatechin (EC), (−)-epigallocatechin gallate (EGCG) and (−)-gallocatechin gallate (GCG), and unfermented green tea contains more catechin ingredients than fermented black tea and semi-fermented oolong tea [2, 3]. Characterized by presence of benzopyran structure bearing at least one aromatic ring in molecular structures, tea catechins could exert potential health benefits as anti-inflammatory, antioxidant and antibacterial agents [3–5]. As the most abundant catechin in green tea, EGCG is also known for excellent biological activities derived from hydroxyl groups and a gallate moiety

✉ Qingxian Luan
kqluanqx@126.com

Yanfeng Wang
kqnickwang@163.com

Qiao Yuan
zahuazahua@126.com

Jiajun Zeng
zjjpanda1993@163.com

Yu Cai
jessonjesson@hotmail.com

[1] National Engineering Laboratory for Digital and Material Technology of Stomatology, Beijing Key Laboratory of Digital Stomatology, Department of Periodontology, Peking University School and Hospital of Stomatology, No. 22, Zhongguancun South Avenue, Haidian District, Beijing 100081, China

esterified particularly [6–8]. Although catechins possess a considerable biological activity, their relatively low stability and high sensitivity to environment cause the rapid degradation influenced by ambient conditions such as temperature, pH, oxygen, light, ions and initial concentration [9–11]. The degradation of catechins is mainly due to auto-oxidation and epimerization. In the former, catechins lose hydrogen atoms and generate superoxide and oxidized products, dimers and polymers, most of which are unfavorable products leading to side-effects [12–14]. The epimerization in catechins leads to transformation between epicatechins (*cis*-catechins) to non-epicatechins (*trans*-catechins), such as EGCG and GCG, but this epimerization between pair catechins is reversible and isomeric pairs possess similar biological activities [8, 15, 16].

As polar compounds, catechins in green tea leaves could solubilize into polar organic solvents such as ethanol, methanol, acetone and water. Green tea leaves are usually infused with water as the conventional tea beverage, accompanied with catechin ingredients releasing. Previous studies have identified factors influencing catechin amounts in green tea infusion using water, while processing temperature and time are regarded to be the most impactful factors [14, 17]. Perva-Uzunalic et al. [17] reported that when using water as solvent to extract major catechins in green tea, the catechins content reached a maximum after 20 min at 80 °C and after 10 min at 95 °C, however, with prolonged extraction time it decreased due to degradation. Jin et al. [18] also showed that the total catechin level peaked at 10 min at 95 °C brewing with water, then decreased. Generally, high infusion temperature contributes to a more rapid extraction of catechins from tea leaves, while excess thermal condition could accelerate synthesis of more complex and oxidized compounds derived from catechins, impacting biological activities [18]. Aimed at achieving the maximal efficiency, infusion temperature higher than 60 °C is usually selected for catechin extraction and purification in tea industry. In daily life, brewing with boiling water is the most traditional and popular way to make tea beverage, while excess processing time could reduce the amount of health-prompting catechins, which is difficult to avoid. Compared with relatively high temperature, the room temperature of constant 25 °C is more helpful for catechins stability in aqueous systems [9], however, the feature of infusion process at 25 °C was barely discussed before.

Ultrasound is found to accelerate the extraction process of catechins from tea leaves by cavitation phenomena generating high pressure and temperature towards tea surface, thus accelerating mass transfer into solvents [19–22]. Two kinds of ultrasound devices are commonly applied in tea industry, ultrasonic bath and ultrasonic probe or horn system, while both are too large and expensive to apply in domestic fields. Based on the same ultrasound mechanism, ultrasonic scaler is usually applied for periodontal treatment, which is much more available and has the potential to be modified to a household appliance for tea infusion, while no previous study has elucidated its effect on tea infusion process yet. Ultrasound could also form free radicals and amplified the reaction energy of solution systems to degrade antioxidants [22]. As described above, stability of catechins is variable under different ambient conditions, therefore, the effect of ultrasonic scaler should be evaluated. Zeng et al. [23] has proved that atomization of ultrasonic scaler could accelerate the degradation of EGCG solution via firm contact with oxygen and light, but the ultrasonic vibration alone might not be responsible for this effect. The atomization merely allowed a relatively short function time and was affected by light and oxygen, therefore, in this study we improved the method to immerse the scaler tip into green tea infusion (GTI) after filtration to allow a more sufficient and longer-time function of ultrasonic vibration in the absence of light and oxygen.

In brief, this study was aimed to investigate the effects of temperature and ultrasonic scaler on infusion process of green tea leaves, and catechins stability under ultrasonic vibration in GTI. The results might suggest a simplified method to obtain high-quality GTI via available ultrasonic device at room temperature, which is appropriate for daily drinking and potential medical application.

Materials and methods

Materials

The following catechin standards were purchased from Yuanye Biotechnology Co. Ltd. (Shanghai, China): EGC, ECG, C, EC, EGCG and GCG (≥ 98%). Acetic acid, Ethylenediamineletraacetic acid disodium salt dehydrate (EDTA-Na_2) and acetonitrile of HPLC grade were purchased from Merck (Germany). Water of HPLC grade was produced by a purification system (Millipore Direct-Q, USA). Syringe filters (0.22 μm GHP) were purchased from Waters (USA). A piezoelectric ultrasonic dental scaler (SKL A7 with P3 tip) was sponsored by SKL Medical Instrument Co. Ltd. (Guangdong, China). Screen mesh with size of 300 was purchased from Lvruo Co. Ltd. (Hebei, China). Laboratory films were PARAFILM "M" from Bemis (USA).

Nature Chinese green tea leaves of same batch were purchased from Longmenxinzhu Co. Ltd (Mingqian organic green tea, Leshan, Sichuan, China). The major catechins content in this green tea was estimated as following: EGCG 52 mg/g, GCG 2 mg/g, EGC 13.4 mg/g, ECG 15.7 mg/g, EC 10.6 mg/g, C 2.4 mg/g.

Ultra-high performance liquid chromatography (UHPLC) analysis

UHPLC analysis was conducted on a Waters ACQUITY Arc systems (Waters Technologies Shanghai Limited, Shanghai, China) equipped with a Waters XSelect HSS C18 column (3.0 mm × 150 mm, 2.5 μm). The flow rate was 0.8 mL/min at a column temperature of 35 °C, and the UV detection wavelength was 278 nm. Two mobile phases were applied for gradient elution. Mobile phase A: distilled H_2O, acetonitrile, acetic acid, and EDTA-Na_2 (888/90/20/2, v/v/v/v) and mobile phase B: distilled H_2O, acetonitrile, acetic acid, and EDTA-Na_2 (178/800/20/2, v/v/v/v). The initial composition was 100% A (0–7.97 min). Subsequently, it was changed to 68% A and 32% B (7.97–12.76 min) and returned to 100% A (12.76–22 min). The injection volume was 3 μL. Membrane syringe filters (0.22 μm) were applied before injection.

As catechins were sensitive to light and oxygen, sampling of tea infusion was conducted as rapid as possible by syringes (1 mL) and fine needles (Outer diameter: 0.45 mm) through laboratory film which sealed opening of lightproof containers previously, while only a narrow gap was allowed for fine needles when opening container caps in a dim lighting working environment. All samples were taken in three replications. During infusion process, exact 0.5 mL infusion was taken out every sampling following by adding distilled water of equal volume.

Evaluation of catechins calibration curve

A stabilizing buffer was prepared by mixing 25 mL EDTA-Na_2 solution (10 mg/mL), 25 mL ascorbic acid solution (10 mg/mL), 50 mL acetonitrile and adding distilled water to 500 mL in a volumetric flask. The individual catechin standard was dissolved at 0.5 mg/mL with stabilizing buffer to decide retention time by UHPLC method. EGCG, GCG, EGC, ECG, EC and C were well-distinguished without interference from each other. The standard stock solution of mixed catechins was prepared with stabilizing buffer at following concentration: EGCG 1 mg/mL, EGC 1 mg/mL, EC 0.5 mg/mL, ECG 0.5 mg/mL, C 0.5 mg/mL and GCG 0.5 mg/mL. Then, the stock solution was separated into seven portions and diluted by certain ratios. A series of mixed catethins aqueous solution were prepared at gradient concentrations of EGCG (0.01–1 mg/mL), EGC (0.01–1 mg/mL), EC (0.005–0.5 mg/mL), ECG (0.005–0.5 mg/mL), C (0.005–0.5 mg/mL) and GCG (0.005–0.5 mg/mL), which were conserved at 4 °C in the absence of light and oxygen. The calibration curves of six catechins were evaluated and determination coefficient (R^2) was calculated by means of least-square analysis.

Effect of temperature on infusion process of green tea leaves

One gram of green tea leaves was weighed and infused with 50 mL distilled water (1:50 w/v) at 25 °C in a lightproof container (50 mL), and then the cap was tightened constantly to avoid oxygen interference. The container was stored at 25 °C (RT). The concentrations of EGCG, GCG, EGC, ECG, EC and C were measured at the point of 0.5 h, 1 h, 1.5 h, 2 h, 3 h, 6 h, 12 h and 24 h. As the contrast, one gram of green tea leaves was weighed and infused with 50 mL distilled water at 65 °C in a lightproof container (50 mL) with the cap tightened constantly. The container was stored in a hot water bath (65 °C) for 15 min, then it was cooled in an ice-water bath till temperature dropped to 25 °C and the concentrations of catechins above were measured with UHPLC.

Ultrasonic vibration effect on infusion process of green tea leaves

Two groups were designed in this part and both were not exposed to light and oxygen. For each group, one gram of green tea leaves was weighed and infused with 50 mL distilled water at 25 °C in a lightproof container as preparation. In control group, the preparation was stored at 25 °C with the cap tightened. Meanwhile, the preparation of test group was stored in a water bath (25 °C) with the container opening sealed by laboratory film. The volume of lightproof container is 60 mL to avoid liquid overflow after ultrasonic handpiece inserting. Ultrasonic handpiece with P3 tip penetrated seal film, immersed into the preparation and vibrated at a frequency of 33 kHz without cooling water. The vibration was paused for 10 min every 20 min to protect the handpiece from overheat. The temperature of preparations was monitored and stabled within a 25 °C water bath. After 30 min, 60 min, 90 min and 120 min, the concentrations of catechins were measured with UHPLC for each group.

The specific effect of the ultrasonic dental scaler on infusion process were evaluated by the D-value:

$$\text{D-value} = (\text{Test group concentration} - \text{Control group concentration})/\text{Control group concentration} \times 100\%.$$

Effect of ultrasonic vibration on stability of catechins in green tea infusion

The green tea infusion was prepared as above at 25 °C. After 3 h, the infusion was filtered quickly by screen mesh with size of 300, which is specialized for tea leaves screening,

then divided into two portions. In the control group, the infusion was reserved in a lightproof container (25 mL) at 25 °C with the cap tightened. The test group was stored in a water bath (25 °C) with the container opening sealed by laboratory film. Ultrasonic handpiece with P3 tip penetrated the seal film and immersed into the infusion and vibrated at a frequency of 33 kHz without cooling water. The vibration was paused for 10 min every 20 min to protect the handpiece from overheat. After 2 h, the concentrations of catechins were measured with UHPLC for each group. The specific effect of ultrasonic vibration on catechins stability in GTI were evaluated by the D-value between groups.

Statistical analysis

Data are expressed as the mean value ± standard deviation (mean ± SD) (n = 3). A Student's t-test was used for comparison of independent samples, and a general linear model was used for comparison of continuous samples. A p-value < 0.05 was accepted as significant. Statistical differences were determined in Statistical Product and Service Solutions (SPSS, v.26, IBM, USA).

Results

Method validation

Selectivity and specificity

Six catechins were well distinguished and no endogenous interfering peak was observed in the mixed catechins standard as shown in Fig. 1a. In Fig. 1b, peaks of six catechins were discerned in the chromatogram of nature tea infusion, coinciding with the graph of mixed standards. The sequence of retention time was EGC, C, EGCG, EC, GCG and ECG.

Linearity and lower limit of quantification (LLOQ)

The calibration curve, correlation coefficient and LLOQ were shown in Table 1. The calibration curves of six catechins were linear over a certain range with a correlation coefficient (R^2) larger than 0.99. The LLOQ was set to the lowest concentration on the corresponding calibration curve.

Precision, accuracy and recovery rates

Intra-day accuracies and precisions were evaluated by measuring samples of mixed standard at 1 concentration level in triplicates during a single day, and inter-day accuracies and precisions were measured in duplicate over 2 consecutive days. For catechins analyzed, the intra-day accuracies were 98.27% to 98.95%, and the precisions were 0.52% to 1.27% (RSD). The inter-day accuracies were 97.06% and 98.02%, and the precisions were 0.57% and 1.13% (RSD) respectively, as shown in Table 2. To verify the precisions for green tea samples, concentrations of six catechins in GTI prepared for 3 h were measured in triplicates and the RSD was 1.73% to 2.82%. The recovery test was carried out by adding the standards solution into the GTI (final concentrations were equal to 100% of values measured beforehand), and the recovery rates were 98–103% for six catechins, as shown in Table 3.

Comparison of catechins concentration in green tea infusion prepared with water at 25 °C and 65 °C

Regarding to the 25 °C group, the amounts of total catechins and EGCG kept increasing in first 3 h, then achieved a plateau phase as showed in Fig. 2a. As for ECG, its kept increasing, but the first 3 h showed the most significant increment accounting for 86.7% of total increment in 24 h. As showed in Fig. 2b, EGC possessed a similar changing trend with EC that the concentration kept on increasing in first 3 h and achieved the peak, then decreased slightly with time till 24 h. Non-epicatechins, GCG and C, possessed the lowest concentrations which were stable between 30 and 40 μg/mL after 3 h. Except for EGCG and GCG, the concentration of individual catechins at 24 h has exceeded its corresponding concentrations in the high temperature group.

The order of catechins amount was: EGCG > ECG > EGC > EC > C > GCG/C. The most abundant ingredient is EGCG, which accounted for approximately 50–60% in total catachins. More amounts of EGC, ECG, C, EC could be obtained at 25 °C for 24 h, compared to the 65 °C group. As for EGCG, the concentration obtained at 25 °C for 24 h was up to 966.5 μg/mL that was close to 990.3 μg/mL obtained at 65 °C for 15 min and the ratio was 97.6%.

Ultrasonic vibration accelerating effect on infusion process of green tea leaves

The concentrations of catechins in the infusion process with or without ultrasonic assistance were summarized in Table 4. Catechin concentrations kept increasing in both groups during 2 h. Individual catechins except for C and total catechins had a significant difference between two groups. The D-values were 20.8–31.2% and decreasing smoothly with time. That meant that the ultrasonic vibration had an accelerating effect on the infusion process of green tea leaves and the effect was reducing smoothly in 2 h.

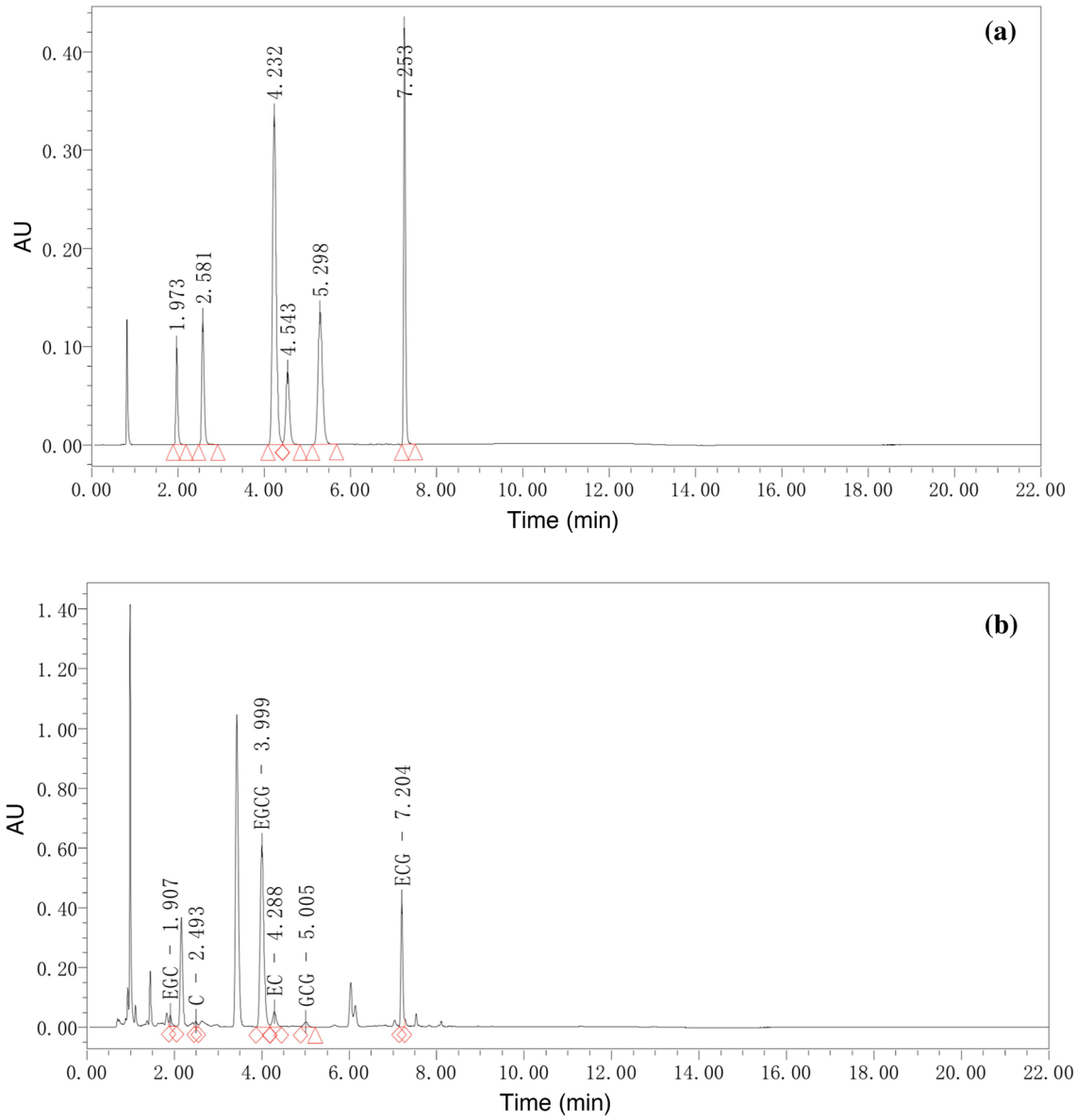

Fig. 1 The chromatograms of mixed catechins standard and green tea infusion (**a**) mixed catechins standard at the concentration: EGCG 0.5 mg/mL, EGC 0.5 mg/mL, EC 0.25 mg/mL, ECG 0.25 mg/mL, C 0.25 mg/mL and GCG 0.25 mg/mL, and (**b**) nature tea leaves infused with distilled water at room temperature (RT) for 3 h

Table 1 Standard curves, correlation coefficients, linear ranges and lower limit of quantification (LLOQ) of six catechin standards

Catechin	Calibration curve	R^2	Linear range (mg/mL)	LLOQ (mg/mL)
EGCG	Y = 4177.795X − 3760.068	0.99	0.01–1	0.01
GCG	Y = 4131.442X − 6781.306	0.99	0.005–0.5	0.005
EGC	Y = 636.2081X − 176.4714	0.99	0.01–1	0.01
ECG	Y = 4828.324X − 4142.338	0.99	0.005–0.5	0.005
EC	Y = 1857.755X + 250.8998	0.99	0.005–0.5	0.005
C	Y = 1952.850X + 163.3762	0.99	0.005–0.5	0.005

Table 2 Intra-day/inter-day accuracy and precision of six catechin standards

Analyte	Nominal concentration (μg/mL)	Intra-day			Inter-day		
		Measured concentration (μg/mL)	RSD (%)	Accuracy (%)	Measured concentration (μg/mL)	RSD (%)	Accuracy (%)
EGCG	500.7	493.4 ± 2.9	0.59	98.55	488.9 ± 3.1	0.64	97.65
GCG	250.7	248.0 ± 1.7	0.69	98.95	244.7 ± 1.5	0.62	97.62
EGC	504.0	496.2 ± 2.6	0.52	98.45	494.0 ± 2.8	0.57	98.02
ECG	250.7	246.4 ± 3.1	1.27	98.28	244.0 ± 2.8	1.13	97.35
EC	250.7	246.3 ± 2.8	1.12	98.27	243.3 ± 1.1	0.46	97.06
C	249.3	246.5 ± 2.2	0.88	98.85	243.1 ± 1.8	0.73	97.51

Table 3 The precision and recovery rates of green tea samples

Analytes	EGCG	GCG	EGC	ECG	EC	C
RSD (%)	1.78	2.82	1.99	2.11	1.73	1.99
Recovery (%)	103	99	102	98	102	99

Catechins stability of green tea infusion within ultrasonic vibration

The concentrations of catechins in GTI with or without ultrasonic processing were summarized in Table 5. Data showed that the concentrations of EGCG, EGC, ECG and C were significantly higher in the test group. The concentrations of EC and GCG were also higher, but the difference is not statistically significant. The total concentration of catechins was higher in the test group and the D-value was 4.7%. The D-value of EGCG was 4.0%. These implied that the ultrasonic vibration didn't have a reducing impact on catechins stability.

Discussion

The method we applied to analyze catechins in GTI is based on HPLC, which is proved to be most popular for tea catechins, gallic acid, purine alkaloids, theanine [24]. Furthermore, it is identical for both non-epi and epi forms of an isomeric pair. Conventional HPLC methods require average 30–40 min to separate catechins for per sample analyzing [25–27]. In this study, the UHPLC system with a UV-detector relying on columns packed with 2.5 μm particles costs 22 min to separate 6 catechins. A series of parameters were performed to evaluate the method validation including selectivity and specificity, precision and accuracy, recovery rate, linearity and LLOQ. Green tea samples were relatively stable under the preservation environment in UHPLC device. Catechins were well-identified without interference peak of other substance, and good linearity was obtained over the whole concentration range for analytes designed, with R^2 systematically higher than 0.99. As described in other studies, the UHPLC system provides an effective and accurate quantitative determination of tea catechins [28–30].

Infusion process of green tea leaves consists of two procedures, extraction and degradation. High temperature contributed to the extraction of catechins in GTI [18, 31], however, it's also an essential factor to accelerate catechins degradation in aqueous solution [10, 32] and 25 °C is more helpful for catechins stability in aqueous systems [9]. Jin et al. [18] reported that the highest level of total catechins was achieved at 95 °C for 10 min in tea infusion process, however, 60 °C required a longer time, 60 min to achieve the peak. In our study, the concentration of EGCG obtained at 25 °C for 24 h was up to 966.5 μg/mL, which is close to 990.3 μg/mL obtained at 65 °C for 15 min and the ratio was 97.6%. The GTI obtained at 3 h could achieve 1610.6 μg/mL of total catechins, which has proven to exert health-prompting effect in medical practice [33]. Except for GCG and EGCG, which are the most stable pair, other individual catechins were observed to possess higher concentrations at 25 °C. This might be attributed to the accelerating effect of high temperature on catechins degradation whose rates proceed faster with temperature increasing [34, 35], and catechins are rather unstable at low concentrations [9, 11, 23]. These caused that the total catechins concentration at 25 °C exceeded that in the 65 °C group at 3 h. During the whole infusion process, the most rapid increasing rates of individual catechins occurred in the first 3 h. After that, the amounts of EGCG, GCG, C were maintained stable as the plateau phase, which meant that the extraction and degradation had achieved a balance. The amount of ECG kept increasing in a relatively gentle trend. Meanwhile, the amounts of EGC and EC decreased slightly with time till 24 h. These different changing trends of individual catechins were due to the relationship between the amounts of extraction and degradation.

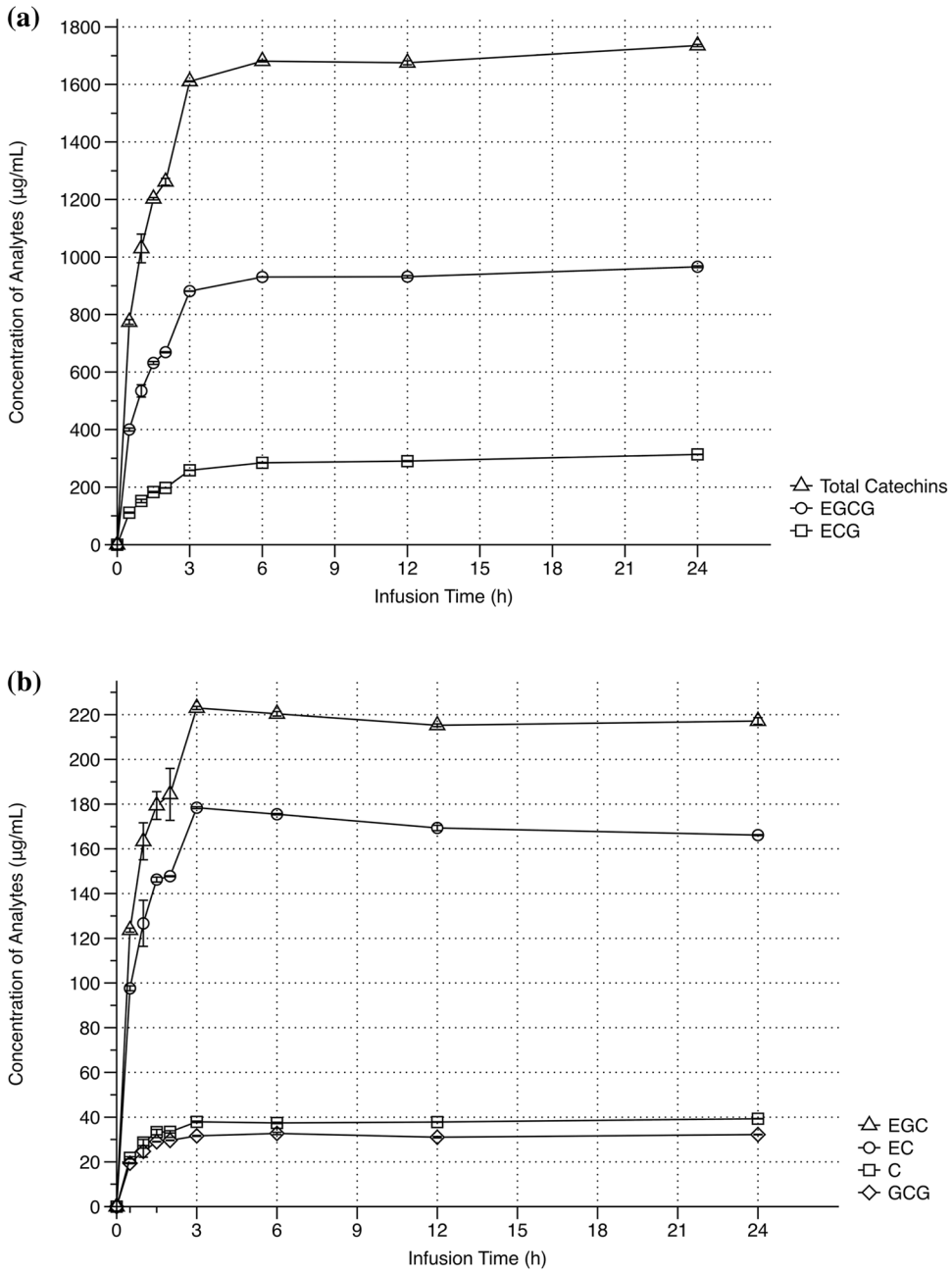

Fig. 2 The concentration of catechins in green tea infusion (GTI) at 25 °C. **a** Total catechins, EGCG and ECG, **b** EGC, EC, C and GCG

For instance, EGC and EC are relatively more feasible to degrade than EGCG and ECG at low concentrations [3]. The extraction of these catechins have achieved maximum at 3 h and the degradation started to predominate, therefore, the amount of these catechins decreased. But in general, the amount of total catechins remained stable after 3 h in GTI at 25 °C.

Ultrasound is found to accelerate extraction process of green tea leaves [19–22]. Both et al. [20] studied ultrasonic effects on infusion process of black tea and concluded that the application of ultrasonic intensification leads to an increase of catechins by approximately 15%. The essential mechanism of ultrasonic device is cavitation phenomena generating microbubbles [36, 37], which collapse close to the tea surface and generate microjets with high pressure and temperature towards the surface. Inducing micro-channels between solvent and tea samples, cavitation accelerates dispersion of organic compound in the plant body by breaking cell walls, which are beneficial for mass transfer during infusion [37]. Two kinds of ultrasound devices are commonly applied, ultrasonic bath and ultrasonic probe or horn system. Ultrasonic bath needs solid materials to be immersed into the solvent tank and ultrasonic probe is designed to be a vibrating horn diameter of 10–20 mm directly immersed

Table 4 The concentration of catechins in the infusion process with or without ultrasonic assistance

Analytes	Group	Time (min)				*p*-value
		30 min (μg/mL)	60 min (μg/mL)	90 min (μg/mL)	120 min (μg/mL)	
EGCG	Test	552.0 ± 1.2	742.0 ± 2.1	832.5 ± 1.6	877.1 ± 1.2	< 0.001*
	Control	400.2 ± 5.0	534.5 ± 21.3	631.5 ± 5.0	668.9 ± 1.8	
GCG	Test	24.7 ± 0.1	29.7 ± 0.2	31.1 ± 0.2	31.2 ± 0.2	0.002*
	Control	19.4 ± 0.2	24.7 ± 2.6	29.0 ± 0.1	29.6 ± 0.1	
EGC	Test	154.6 ± 0.7	182.6 ± 0.3	189.1 ± 0.6	187.5 ± 0.5	0.006*
	Control	123.7 ± 0.9	163.4 ± 8.3	179.4 ± 6.2	184.4 ± 11.6	
ECG	Test	144.2 ± 0.4	201.1 ± 0.6	231.4 ± 0.6	250.3 ± 0.6	< 0.001*
	Control	111.1 ± 1.6	152.0 ± 5.7	183.0 ± 2.0	197.3 ± 0.5	
EC	Test	115.7 ± 0.3	142.7 ± 0.6	148.5 ± 0.2	147.0 ± 0.3	0.008*
	Control	97.6 ± 1.1	126.7 ± 10.3	146.3 ± 1.1	147.8 ± 0.2	
C	Test	24.1 ± 0.2	29.6 ± 0.1	30.9 ± 0.2	30.7 ± 0.2	0.51
	Control	21.8 ± 0.5	28.6 ± 1.4	33.3 ± 1.2	33.4 ± 0.5	
Total	Test	1015.4 ± 2.6	1328.6 ± 3.6	1463.5 ± 2.6	1523.8 ± 1.4	< 0.001*
	Control	773.9 ± 8.3	1030.0 ± 49.6	1202.6 ± 3.9	1261.6 ± 12.0	
D-value (%)		31.2%	29.0%	21.7%	20.8%	

*Significant difference between groups ($p < 0.05$)

Table 5 The concentration of catechins in GTI with or without ultrasonic processing

Analytes	Test group (μg/mL)	Control group (μg/mL)	*p*-value and D-value (%)
EGCG	765.8 ± 8.9	736.2 ± 0.7	0.005*
GCG	33.2 ± 0.3	29.9 ± 10.9	0.094
EGC	185.6 ± 2.2	176.1 ± 0.6	0.002*
ECG	228.4 ± 2.9	220.0 ± 0.8	0.009*
EC	150.8 ± 1.5	140.5 ± 6.2	0.096
C	32.6 ± 0.4	30.6 ± 0.5	0.006*
Total	1396.4 ± 16.3	1333.4 ± 6.3	0.003*
			D-value = 4.7%

*Significant difference between groups ($p < 0.05$)

into the solvent with raw materials [22]. However, there is no previous study related to the ultrasonic effect of dental scaler on tea infusion process. The piezoelectric dental scaler applied in this study worked at 33 kHz with a P3 tip specialized for dental treatment. As results, the D-values were 20.8–31.2% between two groups, representing ultrasonic effect to accelerate the infusion process. The group with assistance of the dental scaler showed higher amounts in individual catechins except C. The mechanism of ultrasonic dental scaler is cavitation generating microbubbles in accordance with ultrasonic bath and probe, acoustic microstreaming and radiation pressure forces [38, 39]. The cavitation and shear stresses around work tip could bring out cell disruption and damage [40], which might be effective for plant cells in green tea leaves and contributes to catechins extraction. However, ultrasound could also form free radicals to degrade natural antioxidants and amplified the reaction energy of solution systems [22], therefore, the extraction with assistance of the dental scaler and the degradation happened in the same time. The concentration of catechin C didn't show a significant increase, however, which was significantly higher in the ultrasonic group during the first 60 min. It meant that the extraction prevailed in the first 60 min. However, during the second half, the extent of catechins extraction almost achieved the maximum and degradation of catechin C started to prevail. C and EC are the most unstable isomeric pair [15, 41] and catechin C is feasible to degradate under low concentration as 21.8–33.4 μg/mL [9]. As for D-values, the ultrasonic accelerating effect was decreasing smoothly, which might be also attributed to the extent of extraction. As showed in Fig. 2a, the curve slope of total catechins decreased slightly with time, which meant that the infusion process was closed to plateau phase, the balance of extraction and degradation. Even with ultrasonic assistance, the degradation would prevail when most of catechin ingredients were extracted into the solvent, therefore the accelerating effect of ultrasonic vibration on infusion process was not obvious gradually.

Zeng et al. [23] reported the ultrasonic atomization might not have an influence on EGCG aqueous solution in the absence of light and oxygen, but only temporary vibration effects could act on GTI by atomization. In our method, the dental scaler could form a cavitation field around the working tip [42, 43], which allowed a more sufficient and longer-time function of ultrasonic vibration on GTI. In this study, results showed that concentration of total catechins was increased by ultrasonic vibration with the D-value as 4.7% and that of EGCG was 4.0%. These implied that the

ultrasonic vibration didn't have a reducing impact on catechins stability. Opposite to the results, ultrasound field could form free radicals to degrade natural antioxidants and amplified the reaction energy of solution systems [22], which are unfavorable to catechins stability. Therefore, it was deduced that although screen mesh with size of 300 was specialized for tea products and commonly used in tea industry, invisible green tea fragments and cells could get through the screen into solutions, which were disrupted by cavitation of the dental scaler and continued to release catechins ingredient as mentioned before. Furthermore, the degradation of catechins could be concluded to two main reactions, epimerization and auto-oxidation and the epimerization leads to transformation between epicatechins (*cis*-catechins) to nonepicatechins (*trans*-catechins), like EGCG and GCG. This epimerization between pair catechins is common and reversible in an aqueous system [9, 44]. Wang et al. [44] reported that epimerization from nonepi- to epicatechin could occur between 44 and 98 °C. The ultrasonic dental scaler could cause a local high temperature by releasing energy, which may accelerate transformation from nonepicatchins to corresponding epi-forms, such as CG to ECG. These two aspects may contribute to the increasing amount of catechins within the ultrasonic vibration. The differences of GCG and EC were not statistically significant. This might attribute to low concentration of GCG and insufficient antioxidant of EC, which caused their degradation. Generally speaking, GTI is difficult to analyze because of its complicated composition and the degradation consists of auto-oxidation and epimerization among catechins. The specific effect of the ultrasonic vibration on catechins stability need further investigations based on purified catechin standards, but in this study, it didn't have a reducing impact on stability of six catechins in GTI at 25 °C.

Conclusions

When infused at 25 °C, a high-quality GTI could be prepared and catechins amount might achieve the plateau phase after 3 h. The ultrasonic scaler could accelerate the infusion process, and didn't affect the catechins stability in GTI at 25 °C.

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Declarations

Conflict of interest The authors declare no conflict of interest related to this study.

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