



Studies on the mechanism of efficient extraction of tea components by aqueous ethanol



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ABSTRACT

Effect of solvent on the extraction yield and its relevant mechanism have been studied in this paper. Compared with extraction by water, catechins and caffeine could be easily extracted from green tea by aqueous ethanol, but hardly at all by absolute ethanol. Results of the vacuum-assisted extraction, solubility determination of EGCG and caffeine, as well as swelling ratio analysis of the infused leaves, indicated that an excellent leaf-matrix-swelling effect and high solubility of tea components might be the key mechanisms for high extraction efficiency by the aqueous ethanol. These mechanisms were further confirmed by the pre-swelling extraction. This is a first report on the mechanism of efficient extraction by aqueous organic solvent. Application of pre-swelling extraction is also discussed.

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1. Introduction

Catechins and caffeine, categorized as polyphenols and an alkaloid respectively, are well-known and important components with many physiological activities, found in tea or tea extracts (Lu et al., 2010). Tea catechins mainly include epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), epicatechin (EC), catechin (C), gallic acid (GCG), gallic acid (GC) and catechin gallate (CG) (Liang, Liang, Dong, & Lu, 2007). Tea extracts can be easily and widely applied in nutraceuticals (Wang & Weller, 2006), functional food (Namal Senanayake, 2013) and pharmaceuticals (Hara, 2011) compared with normal tea. Extraction is the key procedure used to obtain active ingredients from raw plant materials (Pasrija & Anandharamakrishnan, 2015; Pinelo, Sineiro, & Núñez, 2006).

Extraction efficiency is usually influenced by solvent type, temperature, time and ratio of material to solvent (Choung & Lee, 2011; Choung et al., 2014; Perva-Uzunalić et al., 2006), of which the solvent greatly influences the extraction yield and ingredient proportion of the extracts (Nwaha, 2000; Perva-Uzunalić et al., 2006). After comparing the extraction efficiency of catechins and caffeine from green tea achieved with different solvents, including water, absolute organic solvent (acetone, ethanol, methanol and acetonitrile) and the corresponding aqueous solvents at boiling

point, Perva-Uzunalić and colleagues reported that the lowest yield of caffeine and catechins was obtained by extraction with absolute acetone and acetonitrile; whilst the highest yield of catechins was achieved by extraction with 50% aqueous acetone and acetonitrile as well as 80% ethanol (Perva-Uzunalić et al., 2006). Liang and colleagues further pointed out that catechins could efficiently be extracted by 30–70% aqueous ethanol from green tea and by 75% ethanol from fresh leaves at room temperature (Liang, Liang, Dong, & Lu, 2007). Studies also showed that the highest yield of polyphenols could be extracted by 50% aqueous organic solvents (acetone, dimethylformamide, ethanol and methanol) from black tea (Turkmen, Velioglu, Sari, & Polat, 2007). Rusak et al. also confirmed that the phenolics extraction from green tea and white tea depended strongly on the solvent type and 40% aqueous ethanol was the most efficient solvent (Rusak, Komes, Likić, Horžić, & Kovač, 2008). Similar findings were obtained by Choung et al. (Choung & Lee, 2011; Choung et al., 2014). Although high extraction efficiency of aqueous organic solvents has been observed by many independent studies, the investigation of the mechanism of this phenomenon has been quite limited until now. In the present study, experiments were carried out to illustrate the mechanism.

2. Materials and methods

2.1. Materials

Green tea was supplied by Cino Tea Co., Ltd. (Hangzhou, China). Reference compounds, including GC, EGC, C, EC, EGCG, GCG, ECG,

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CG and caffeine were Sigma products (Sigma–Aldrich, St. Louis, MO, USA). HPLC grade acetonitrile and methanol were supplied by Merck (Darmstadt, Germany). Ethanol and *n*-hexane were of analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). The Ultrapure water used was prepared by an EASY Pure II Water System (Barnstead International, Dubuque, IA, USA). Other reagents used were of analytical grade unless stated otherwise.

2.2. Extraction test with different concentrations of ethanol

Tea samples were ground and passed through 8, 45 and 100 mesh sieves, respectively. An amount of ground sample (1.000 g) was extracted in a HZ-9211KB shaker (Taicang Hualida Experimental Equipment Co. Ltd., Jiangsu, China) with 50 ml of different concentrations of ethanol (0, 25%, 50%, 75%, 100%, v/v) at 150 rpm and room temperature for 2.5 h. The mixture was filtered through No. 102 Whatman filter paper and then the filtrate was centrifuged (SIGMA 3–18 K, Osterode am Harz, Germany) at 12,000 rpm and 4 °C for 10 min. The supernatant was collected and adjusted to 50.0 ml for chemical analysis. Each test was repeated in triplicate.

2.3. Vacuum-assisted extraction test

Each sample was ground and passed through a 20 mesh sieve. Vacuum-assisted extraction was performed as follows: the ground sample (1.000 g) was mixed with 50.0 ml absolute ethanol and 50% ethanol respectively and pre-extracted 3 times under 0.01 MPa in a DZF-6030 vacuum chamber (Shanghai Jiecheng Experimental Instrument Co. Ltd., Shanghai, China) each for 5 min with another 5 min interval under normal atmosphere, and finally extracted at room temperature and 150 rpm for 120 min under normal atmospheric conditions. As a control group, normal extraction (1.000 g tea/50.0 ml solvents) was directly performed with the same solvents at room temperature and 150 rpm for 150 min under a normal atmosphere. After extraction, filtration and centrifugation were carried out for obtaining the supernatant as above. Each test was also repeated in triplicate.

2.4. EGCG and caffeine solubility test

Approximately 0.6 g EGCG (purity $\geq 95\%$, Siming Tea Biological Products Co. Ltd., Ningbo, China) and 0.2 g caffeine were dissolved in weighted ethanol solution of various concentrations (0, 25%, 50%, 75%, 100%, v/v) and incubated at 25 °C and 150 rpm for 2.5 h, and then the mixture was centrifuged at 25 °C and 12,000 rpm for 15 min. The supernatant was suitably diluted and used for estimating EGCG and caffeine concentration by HPLC. Each test was duplicated. Solubility of EGCG and caffeine was expressed as amount of solute (g) per 100 g solvent.

2.5. Swelling ratio measurement of the infused tea leaves

Four grams of ground tea (8 mesh) were extracted with 200 ml of various concentrations of ethanol (0, 25%, 50%, 75%, 100%, v/v) at room temperature and 150 rpm for 2.5 h. The infused leaves were separated after vacuum filtration and then put back into 25.0 ml of the corresponding filtrate. The incremental volume (V_1) was taken to be the volume of the infused leaves. The initial volume of tea sample (V_0) was recorded as the increased volume after putting 4.000 g ground sample (8 mesh) into 25.0 ml *n*-hexane. Here, *n*-hexane was used as a medium for measuring the initial volume of tea since it is a colorless liquid with a boiling point of 69 °C, and it is a largely unreactive non-polar solvent that can hardly extract components from tea and scarcely alters the volume.

Swelling ratio (SR) was calculated according to the following equation:

$$SR (\%) = (V_1 - V_0) / V_0 * 100.$$

2.6. Pre-swelling extraction test

In the energy research area, reports show that pre-swelling treatment with special solvents could enhance the extraction yield of oil–gas from coal (Shui, Wang, & Cao, 2008; Sonmez & Giray, 2011). In order to find out whether pre-swelling treatment would have an impact on the yield of tea components, an experiment was carried out in which one gram of ground tea (8, 45, 100 mesh) was mixed thoroughly with various amounts of water (1.00, 2.00, 3.00, 4.00, 5.00 g), pre-swollen at room temperature for 1 h, and then extracted with 39.00 g ethanol at room temperature and 150 rpm for another 1.5 h. As a control, sample (without pre-swelling) was directly extracted with a different mixture of water (1.00, 2.00, 3.00, 4.00, 5.00 g) and 39.00 g ethanol at room temperature and 150 rpm for 2.5 h. The supernatant was collected after filtration and centrifugation for chemical analysis. Each treatment was tested with 3 replicates.

2.7. Chemical analysis of the extracts

A model LC 20A HPLC (Shimadzu Co., Kyoto, Japan) coupled with a Zorbax 5 μ m TC-C₁₈(2) column (250 \times 4.6 mm, Agilent Technologies Inc., CA, USA) was used to estimate caffeine and catechins (EGC, GC, EC, C, EGCG, GCG, ECG and CG) in the test solutions. The HPLC conditions were as follows: injection volume, 10 μ l; oven temperature, 28 °C; mobile phase A, acetonitrile/acetic acid/water (3/0.5/96.5, v/v/v); mobile phase B, acetonitrile/acetic acid/water (30/0.5/69.5, v/v/v); gradient elution, 30% mobile phase B to 85% mobile phase B by linear gradient increasing during the early 35 min and holding at 85% mobile phase B for further 5 min; flow rate, 1 ml/min; detecting wavelength, 280 nm. Caffeine and catechins were identified and quantified by comparing with the retention time and peak area of authentic standards. Detailed operation was carried out as described in a previous paper (Dong et al., 2011).

2.8. Data analysis method

Statistics were carried out on software of the SAS System for Windows (v8.01; SAS Institute Inc. 1999–2000). Significant difference of the mean ($p < 0.05$) was performed using Duncan's multiple range test and indicated by different letters. Calculation of correlation coefficient and fitness of linear curve were performed using Microsoft Excel 2000 (Microsoft Corporation, Redmond, WA, USA).

3. Results and discussion

3.1. Effect of the solvent on extraction efficiency

When 8 mesh green tea samples were used, yield of caffeine, catechin monomers and total catechins changed remarkably along with ethanol concentration in extraction solvents. The aqueous ethanol could extract significantly more catechins and caffeine than water and absolute ethanol. Particularly, the highest yield was achieved when 50% ethanol was used whilst the lowest one was obtained when absolute ethanol was used (Table 1). Similar yield tendency was also observed when another two size samples (45 mesh and 100 mesh) were tested. This observation was consistent with previous findings (Choung et al., 2014; Liang et al., 2007;

Table 1
Effect of solvent and particle size on yield of catechins and caffeine (mg/g).^a

Particle (mesh)	Solvent	GC	EGC	C	EC	EGCG	GCG	ECG	CG	Total catechins	Caffeine
8	Water	1.13 ± 0.13b	22.37 ± 0.45d	0.09 ± 0.00ecd	10.11 ± 0.14b	24.29 ± 1.66f	0.40 ± 0.08e	6.12 ± 0.54e	0.69 ± 0.02d	65.21 ± 1.97f	16.00 ± 0.41g
	25% EtOH	1.85 ± 0.02a	23.64 ± 0.66cd	0.34 ± 0.01a	11.19 ± 0.51ab	60.28 ± 2.34d	0.84 ± 0.03d	15.10 ± 0.39d	0.99 ± 0.03c	114.23 ± 3.37d	22.21 ± 0.75e
	50% EtOH	0.69 ± 0.12de	24.19 ± 0.65cd	0.29 ± 0.19a	12.64 ± 1.30a	65.00 ± 1.54d	1.40 ± 0.25ab	19.20 ± 1.22c	1.04 ± 0.07c	124.44 ± 4.17c	24.96 ± 1.79c
	75% EtOH	0.40 ± 0.24f	16.86 ± 1.14e	0.24 ± 0.03ab	7.84 ± 0.57c	49.09 ± 3.53e	0.77 ± 0.07d	14.18 ± 1.64d	0.94 ± 0.08c	90.33 ± 6.82e	22.31 ± 1.22e
	Absolute EtOH	0.02 ± 0.01g	0.62 ± 0.08h	0.02 ± 0.01e	0.27 ± 0.01d	1.80 ± 0.13i	0.01 ± 0.00f	0.57 ± 0.01h	0.02 ± 0.00e	3.34 ± 0.24i	0.88 ± 0.02j
45	Water	0.89 ± 0.07c	22.02 ± 1.39d	0.17 ± 0.03bc	10.72 ± 0.80b	24.26 ± 2.20f	0.27 ± 0.09e	6.53 ± 0.60e	0.97 ± 0.09c	65.83 ± 1.80f	20.30 ± 1.12f
	25% EtOH	1.83 ± 0.05a	24.44 ± 0.62cd	0.39 ± 0.01a	11.78 ± 0.20a	68.95 ± 1.41c	1.11 ± 0.08c	18.00 ± 0.29c	0.99 ± 0.02c	127.49 ± 1.49c	23.78 ± 0.47d
	50% EtOH	0.76 ± 0.20cd	26.20 ± 0.66b	0.14 ± 0.03bcd	12.36 ± 0.12a	69.11 ± 1.75c	1.07 ± 0.36c	22.29 ± 1.04b	1.59 ± 0.21a	133.53 ± 2.21bc	28.66 ± 0.37b
	75% EtOH	0.63 ± 0.18e	25.87 ± 0.12b	0.05 ± 0.03de	10.92 ± 1.24b	64.67 ± 0.55d	1.16 ± 0.08c	19.04 ± 1.63c	1.07 ± 0.21c	123.40 ± 0.20cd	25.33 ± 0.14c
	Absolute EtOH	0.14 ± 0.02g	2.55 ± 0.91g	0.01 ± 0.01e	1.12 ± 0.28d	6.77 ± 0.53h	0.06 ± 0.02ef	2.08 ± 0.15g	0.02 ± 0.01e	12.75 ± 1.93h	3.19 ± 0.53i
100	Water	0.61 ± 0.01e	22.05 ± 1.19d	0.28 ± 0.02a	10.29 ± 0.31b	26.71 ± 1.49f	0.27 ± 0.06e	7.21 ± 0.61e	0.93 ± 0.08c	68.36 ± 0.64f	20.81 ± 0.59f
	25% EtOH	1.94 ± 0.05a	25.77 ± 1.36bc	0.38 ± 0.00a	12.21 ± 0.12a	69.76 ± 0.85c	1.12 ± 0.07c	17.96 ± 0.22c	1.13 ± 0.01c	130.26 ± 2.49bc	24.41 ± 0.30cd
	50% EtOH	0.84 ± 0.01cd	28.88 ± 0.44a	0.24 ± 0.04ab	12.40 ± 1.19a	77.64 ± 0.57a	1.54 ± 0.02a	24.64 ± 0.31a	1.51 ± 0.05a	147.68 ± 1.14a	30.78 ± 0.95a
	75% EtOH	0.73 ± 0.10de	25.64 ± 2.17bc	0.12 ± 0.01cd	12.44 ± 0.61a	73.85 ± 0.68b	1.23 ± 0.04bc	23.19 ± 0.51ab	1.32 ± 0.04b	138.50 ± 4.11b	29.28 ± 0.30b
	Absolute EtOH	0.14 ± 0.02g	4.41 ± 0.48f	0.01 ± 0.00e	2.23 ± 0.26d	14.51 ± 0.69g	0.12 ± 0.01ef	4.60 ± 0.21f	0.16 ± 0.03e	26.17 ± 1.46g	6.39 ± 0.39h

^a Sample (1.000 g) was extracted with different solvents (50.0 ml) at room temperature and 150 rpm for 2.5 h. The data are shown as mean ± standard deviation of triplicate tests, and significant difference ($p < 0.05$) was indicated by different letters in a same column.

Table 2
Comparison of vacuum-assisted extraction and normal extraction (mg/g).^a

Solvent	Extraction	GC	EGC	C	EC	EGCG	GCG	ECG	CG	Total catechins	Caffeine
Absolute EtOH	Vacuum-assisted	0.11 ± 0.02a	0.83 ± 0.09a	0.03 ± 0.00a	0.35 ± 0.02a	4.02 ± 0.28a	0.01 ± 0.00a	1.12 ± 0.11a	0.04 ± 0.00a	6.52 ± 0.48a	1.84 ± 0.12a
	Normal	0.12 ± 0.03a	0.81 ± 0.06a	0.03 ± 0.00a	0.34 ± 0.01a	4.00 ± 0.22a	0.01 ± 0.00a	1.09 ± 0.05a	0.04 ± 0.00a	6.44 ± 0.33a	1.81 ± 0.09a
50% EtOH	Vacuum-assisted	0.75 ± 0.05a	26.88 ± 1.87a	0.26 ± 0.01a	12.31 ± 0.17a	67.01 ± 1.30a	1.25 ± 0.02a	20.08 ± 0.81a	1.49 ± 0.04a	130.03 ± 3.80a	26.81 ± 0.42a
	Normal	0.79 ± 0.03a	24.73 ± 2.04a	0.26 ± 0.00a	12.60 ± 0.26a	68.01 ± 1.26a	1.24 ± 0.04a	20.27 ± 0.20a	1.48 ± 0.05a	129.38 ± 2.66a	26.34 ± 0.48a

^a Vacuum-assisted extraction was carried out in which ground tea (1.000 g, 20 mesh) was mixed with 50.0 ml absolute ethanol or 50% ethanol, and vacuum pretreatments performed 3-times (0.01 MPa) each for 5 min with a 5 min interval of normal atmosphere, and then extracted at room temperature and 150 rpm for another 120 min under normal atmospheric conditions; normal extraction (1.000 g ground tea/50.0 ml ethanol) was performed directly at room temperature and 150 rpm for 150 min without vacuum pretreatment. Significant difference ($p < 0.05$) between vacuum-assisted extraction and normal extraction with a same solvent is indicated by different letters.

Table 3
Solubility (g/100 g) of EGCG and caffeine in different solvents at 25 °C.*

	Water	25% EtOH	50% EtOH	75% EtOH	Absolute EtOH
EGCG	6.10 ± 0.06e	51.37 ± 0.85d	54.55 ± 1.29c	59.80 ± 0.82b	76.80 ± 1.73a
Caffeine	2.11 ± 0.05d	3.87 ± 0.03c	7.95 ± 0.71a	6.00 ± 0.04b	0.62 ± 0.01e

* Significant difference ($p < 0.05$) is indicated by different letters in the same row.

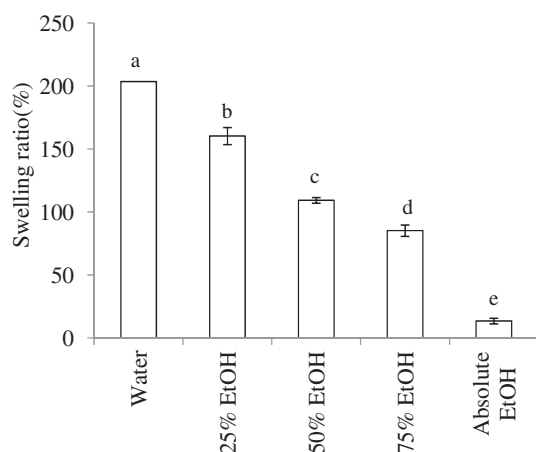


Fig. 1. Swelling ratio of tea leaves extracted with aqueous ethanol and water. The swelling ratio is expressed as percentage of increased volume after extraction vs. initial volume of tea sample. Significant difference ($p < 0.05$) is indicated by different letters. The bar shows the deviation of triple tests.

Perva-Uzunalić et al., 2006; Rusak et al., 2008). This result also showed that the yield of total catechins and caffeine increased significantly with decrease in sample particle size especially when absolute ethanol was used as solvent. This indicated that reducing sample size could also improve the extraction efficiency since diffusion path of solutes decreases with particle size (Gujar, Chattopadhyay, Wagh, & Gaikar, 2010; Spiro & Selwood, 1984). Statistics revealed that the effect of solvent type on the extraction yield is more pronounced than that of particle size (Supplement 1).

3.2. Comparison of vacuum-assistant extraction and normal extraction

In order to understand whether extraction efficiency was related to the penetration ability of the solvent, a vacuum-assisted extraction (20 mesh sample) was conducted using absolute ethanol and 50% aqueous ethanol as solvent. Compared with normal extraction, no significant increase in the yield of catechins and caffeine was observed in vacuum-assisted extraction (Table 2), although vacuum pretreatment could help the solvent penetrate into the sample matrix since a large number of bubbles appeared around the sample during the early period of vacuum treatment. This implied that a treatment which only promotes solvent exchange between the inside and outside of a sample might not significantly improve the extraction efficiency when extraction is performed for a long enough time period such as 2.5 h. Combined with the result of extraction with various solvents in previous tests, a significant linear relationship was obtained between extraction yield of catechins (Y_{TC1} for 50% ethanol and Y_{TC2} for absolute ethanol, mg/g) and caffeine (Y_{CAF1} for 50% ethanol and Y_{CAF2} for absolute ethanol, mg/g) and particle size (x , mesh), and higher yield could be achieved from samples with smaller particle size (Supplement 2); however, the yield was mainly subject to the solvent type rather than the particle size, as seen by a similar slope but greatly different intercept of the yield curves for the two solvents ($Y_{TC1} = 0.241x + 123.513$ & $Y_{TC2} = 0.248x + 1.464$, $p < 0.01$; $Y_{CAF1} = 0.061x + 25.053$ & $Y_{CAF2} = 0.059x + 0.518$, $p < 0.01$)

were observed. As predicted by the models, when absolute ethanol was used as the solvent, whether the vacuum-assisted treatment was performed or not, the particle size of the sample should be smaller than 500 mesh to obtain the yield of total catechins and caffeine that would be similar to the extraction of 100 mesh sample with 50% aqueous ethanol. This indicated that only the catechins and caffeine on, or near, the particle surface are extractable by the absolute ethanol. In other words, absolute ethanol cannot noticeably help the tea components diffuse in the material matrix, perhaps since it cannot alter the compact structure of the tea sample during extraction.

3.3. Effect of solute solubility on the extraction yield

To illustrate the effect of the solute solubility on the extraction yield, EGCG, accounting for approximately 50% of total catechins in green tea, was selected as an indicator of catechins and dissolved into different solvents. As shown in Table 3, solubility of EGCG in aqueous ethanol (51.37–59.80 g/100 g) was significantly higher than that in water (6.10 g/100 g), but lower than that in absolute ethanol (76.8 g/100 g) at 25 °C. This result agreed with the previous observation that greater amounts of catechins can dissolve in 95% ethanol than in pure water at 40 °C when tea extract (containing 60% catechins) is used as initial solute (Nwuha, Nakajima, Tong, & Ichikawa, 1999). Results also showed that the solubility of caffeine significantly increased along with increasing of ethanol level in solvent when the ethanol level was no more than 50%, and decreased markedly when the level further increased (Table 3). This was in accordance with previous findings (Shalmashi & Golmohammad, 2010). Obviously, the solubility of tea components could not entirely account for the extraction yield difference of the different solvents since the yield change was not always in line with the solubility variation and the increasing of ethanol level in the solvents.

3.4. Swelling ratio difference of the infused leaves

As shown in Fig. 1, swelling ratio of the infused tea leaves after being extracted with absolute ethanol was around 13.42%, significantly lower than that with 25–75% aqueous ethanol (85.30–160.38%) and water (203.51%). The relationship between swelling ratio (y , %) and ethanol level (x , %) in solvent could be fitted to the linear curve $y = -1.821x + 205.431$ ($R^2 = 0.981$, $p < 0.01$). The result clearly shows that tea particles were hardly swollen by the absolute ethanol, while easily swollen by water. Therefore, the structure of tea sample might remain compact and rigid throughout the extraction with absolute ethanol; however, it would become loose and extended during extraction with aqueous ethanol and water. Although molecule structure of the solutes might be influenced by ethanol concentration in the solvent (Nwuha, 2000), different efficiency in extraction with various concentrations of ethanol might be at least partially attributed to the structure difference of the tea sample after coming into contact with the solvent and, furthermore, matrix swelling might be one of the most important prerequisites for high extraction yield. Interestingly, many past findings showed that pre-swelling with suitable solvents could greatly enhance the extraction yield of

Table 4
Effect of pre-swelling treatment on the extraction yield (mg/g).^a

Pre-swelling	Extraction solvent	GC	EGC	C	EC	EGCG	GCG	ECG	CG	Total catechins	Caffeine
8 mesh sample (1.00 g)											
1.00 g water	39.00 g EtOH	0.33 ± 0.01ab	11.92 ± 0.85e	0.03 ± 0.02c	5.42 ± 0.06c	34.76 ± 1.81d	0.36 ± 0.07d	11.33 ± 0.18d	0.61 ± 0.04e	64.76 ± 2.76d	16.07 ± 0.69d
Without	1.00 g water + 39.00 g EtOH	0.04 ± 0.04d	0.88 ± 0.05f	0.05 ± 0.01c	0.37 ± 0.04d	2.29 ± 0.21g	0.01 ± 0.01e	0.68 ± 0.05f	0.04 ± 0.01f	4.35 ± 0.30f	1.03 ± 0.09e
2.00 g water	39.00 g EtOH	0.40 ± 0.05a	17.46 ± 2.86d	0.08 ± 0.09c	7.18 ± 0.11c	43.94 ± 2.19c	0.72 ± 0.29c	14.38 ± 0.78c	0.79 ± 0.07d	84.94 ± 6.06c	20.57 ± 2.48c
Without	2.00 g water + 39.00 g EtOH	0.04 ± 0.03d	0.90 ± 0.11e	0.02 ± 0.02c	0.42 ± 0.02d	2.97 ± 0.18fg	0.02 ± 0.00e	0.84 ± 0.01f	0.04 ± 0.01f	5.26 ± 0.23f	1.27 ± 0.04e
3.00 g water	39 g EtOH	0.29 ± 0.06bc	23.64 ± 0.21c	0.24 ± 0.07ab	9.55 ± 2.51b	58.21 ± 2.33b	1.19 ± 0.06b	17.88 ± 0.65b	1.07 ± 0.03c	112.07 ± 5.74b	25.85 ± 1.19b
Without	3.00 g water + 39.00 g EtOH	0.05 ± 0.04d	1.44 ± 0.07f	0.08 ± 0.01c	0.64 ± 0.05d	4.05 ± 0.28efg	0.02 ± 0.03e	1.13 ± 0.08f	0.06 ± 0.01f	7.47 ± 0.50ef	1.74 ± 0.11e
4.00 g water	39 g EtOH	0.23 ± 0.06c	26.02 ± 0.10b	0.30 ± 0.09a	10.79 ± 2.45ab	64.32 ± 0.54a	1.32 ± 0.02ab	19.87 ± 0.25a	1.18 ± 0.07b	124.03 ± 3.28a	27.88 ± 1.90a
Without	4.00 g water + 39.00 g EtOH	0.04 ± 0.04d	1.63 ± 0.05f	0.01 ± 0.00c	0.73 ± 0.04d	4.79 ± 0.39ef	0.06 ± 0.01e	1.37 ± 0.13e	0.08 ± 0.02f	8.72 ± 0.52ef	2.16 ± 0.15e
5.00 g water	39.00 g EtOH	0.31 ± 0.04b	28.59 ± 0.31a	0.21 ± 0.01b	12.71 ± 2.42a	64.50 ± 1.53a	1.39 ± 0.04a	20.22 ± 0.10a	1.30 ± 0.05a	129.23 ± 1.42a	28.53 ± 1.04a
Without	5.00 g water + 39.00 g EtOH	0.06 ± 0.04d	2.24 ± 0.17f	0.02 ± 0.00c	1.04 ± 0.11d	6.03 ± 0.66e	0.09 ± 0.01e	1.76 ± 0.19e	0.09 ± 0.02f	11.32 ± 1.10e	2.95 ± 0.32e
45 mesh sample (1.00 g)											
1.00 g water	39.00 g EtOH	0.26 ± 0.02b	15.87 ± 0.37d	0.15 ± 0.01b	5.43 ± 0.24d	44.41 ± 4.34d	0.85 ± 0.02c	13.20 ± 0.98d	0.44 ± 0.01d	80.62 ± 3.59d	19.02 ± 0.27d
Without	1.00 g water + 39.00 g EtOH	0.06 ± 0.05e	2.37 ± 0.26h	0.03 ± 0.00e	1.30 ± 0.10g	8.41 ± 0.28g	0.07 ± 0.03g	2.57 ± 0.09g	0.11 ± 0.01g	14.93 ± 0.76g	3.61 ± 0.18g
2.00 g water	39.00 g EtOH	0.32 ± 0.00a	20.48 ± 1.11c	0.17 ± 0.00a	6.33 ± 0.09c	54.92 ± 2.71c	0.61 ± 0.02d	17.74 ± 0.26c	0.67 ± 0.02c	101.24 ± 4.12c	24.54 ± 0.24c
Without	2.00 g water + 39.00 g EtOH	0.12 ± 0.04d	2.94 ± 0.45gh	0.03 ± 0.00de	1.57 ± 0.30g	10.20 ± 0.93fg	0.08 ± 0.06g	3.12 ± 0.33g	0.15 ± 0.02fg	18.21 ± 2.11g	4.41 ± 0.46g
3.00 g water	39 g EtOH	0.26 ± 0.02b	24.69 ± 0.76b	0.18 ± 0.03a	8.09 ± 0.37b	64.67 ± 1.66b	1.35 ± 0.03b	20.17 ± 0.56b	1.21 ± 0.04b	120.62 ± 3.44b	27.38 ± 0.72b
Without	3.00 g water + 39.00 g EtOH	0.14 ± 0.02 cd	4.00 ± 0.86fg	0.04 ± 0.01dc	2.20 ± 0.33f	13.83 ± 1.64ef	0.16 ± 0.06f	4.19 ± 0.53f	0.20 ± 0.04ef	24.76 ± 3.43f	5.91 ± 0.74f
4.00 g water	39 g EtOH	0.31 ± 0.02a	28.75 ± 0.74a	0.14 ± 0.00b	9.46 ± 0.34a	72.57 ± 1.66a	1.51 ± 0.02a	22.34 ± 0.59a	1.43 ± 0.04a	136.49 ± 3.39a	30.67 ± 0.67a
Without	4.00 g water + 39.00 g EtOH	0.16 ± 0.04c	5.08 ± 0.15ef	0.05 ± 0.00c	2.67 ± 0.03ef	15.99 ± 0.48e	0.20 ± 0.01ef	4.77 ± 0.16ef	0.24 ± 0.02e	29.16 ± 0.86ef	6.89 ± 0.19ef
5.00 g water	39.00 g EtOH	0.28 ± 0.02ab	29.16 ± 1.52a	0.14 ± 0.01b	9.16 ± 0.60a	72.64 ± 3.72a	1.56 ± 0.06a	22.14 ± 1.21a	1.45 ± 0.08a	136.53 ± 7.17a	30.59 ± 1.57a
Without	5.00 g water + 39.00 g EtOH	0.19 ± 0.02c	5.77 ± 0.35e	0.06 ± 0.00c	3.04 ± 0.18e	17.58 ± 0.90e	0.24 ± 0.01e	5.25 ± 0.27e	0.27 ± 0.01e	32.40 ± 1.73e	7.69 ± 0.41e
100 mesh sample (1.00 g)											
1.00 g water	39.00 g EtOH	0.24 ± 0.02c	20.44 ± 0.64b	0.06 ± 0.01c	6.60 ± 0.29b	46.30 ± 1.99d	1.02 ± 0.02c	14.94 ± 0.71d	0.88 ± 0.04c	90.47 ± 3.71d	21.86 ± 0.79c
Without	1.00 g water + 39.00 g EtOH	0.17 ± 0.00d	5.37 ± 0.05f	0.06 ± 0.01c	2.95 ± 0.06e	19.11 ± 0.27h	0.19 ± 0.05f	5.94 ± 0.08h	0.25 ± 0.01g	34.03 ± 0.22h	8.12 ± 0.10g
2.00 g water	39.00 g EtOH	0.26 ± 0.03b	22.95 ± 1.95b	0.07 ± 0.01c	10.65 ± 0.94a	54.48 ± 6.31c	1.10 ± 0.08c	16.93 ± 2.10c	0.97 ± 0.12c	107.40 ± 11.47c	21.89 ± 2.56c
Without	2.00 g water + 39.00 g EtOH	0.19 ± 0.01d	6.97 ± 0.36ef	0.08 ± 0.01c	3.84 ± 0.19de	25.08 ± 1.24g	0.17 ± 0.01f	7.50 ± 0.37g	0.34 ± 0.02fg	44.15 ± 2.19g	10.22 ± 0.50f
3.00 g water	39 g EtOH	0.33 ± 0.00a	29.54 ± 4.16a	0.08 ± 0.01c	11.70 ± 2.27a	66.32 ± 0.86b	1.32 ± 0.01b	20.57 ± 0.35b	1.25 ± 0.01b	131.10 ± 6.85b	27.06 ± 1.30b
Without	3.00 g water + 39.00 g EtOH	0.24 ± 0.01c	8.77 ± 0.28de	0.10 ± 0.01c	4.76 ± 0.16cd	30.35 ± 1.05f	0.22 ± 0.01f	8.96 ± 0.31f	0.41 ± 0.01ef	53.82 ± 1.80f	12.28 ± 0.44e
4.00 g water	39 g EtOH	0.34 ± 0.01a	29.53 ± 0.10a	0.15 ± 0.08ab	11.36 ± 1.72b	72.28 ± 1.74a	1.38 ± 0.10b	22.19 ± 0.70a	1.28 ± 0.11b	138.52 ± 1.17ab	29.60 ± 2.01a
Without	4.00 g water + 39.00 g EtOH	0.27 ± 0.01b	10.44 ± 0.12cd	0.12 ± 0.00bc	5.61 ± 0.06bc	34.27 ± 0.35ef	0.36 ± 0.09e	10.23 ± 0.09ef	0.49 ± 0.01de	61.80 ± 0.17e	14.13 ± 0.15de
5.00 g water	39.00 g EtOH	0.35 ± 0.01a	30.63 ± 0.71a	0.20 ± 0.01a	10.65 ± 0.31a	74.88 ± 2.18a	1.51 ± 0.03a	23.00 ± 0.67a	1.47 ± 0.02a	142.68 ± 3.93a	31.30 ± 0.76a
Without	5.00 g water + 39.00 g EtOH	0.32 ± 0.02a	11.79 ± 0.27c	0.12 ± 0.01cb	6.27 ± 0.16b	37.21 ± 0.81e	0.46 ± 0.01d	11.17 ± 0.24e	0.57 ± 0.04d	67.91 ± 1.55e	15.54 ± 0.36d

^a Sample (1.00 g) was mixed with various amounts of water (1.00–5.00 g) and pre-swollen for 1 h, then was extracted with 39.00 g ethanol at room temperature and 150 rpm for 1.5 h. As a control, sample was directly extracted with a mixture of corresponding water (1.00–5.00 g) and 39.00 g ethanol at room temperature and 150 rpm for 2.5 h. Data are expressed as mean ± standard deviation of triplicates. Significant difference ($p < 0.05$) of each component yield was compared between various treatments using same size sample, and is indicated by different letters in column.

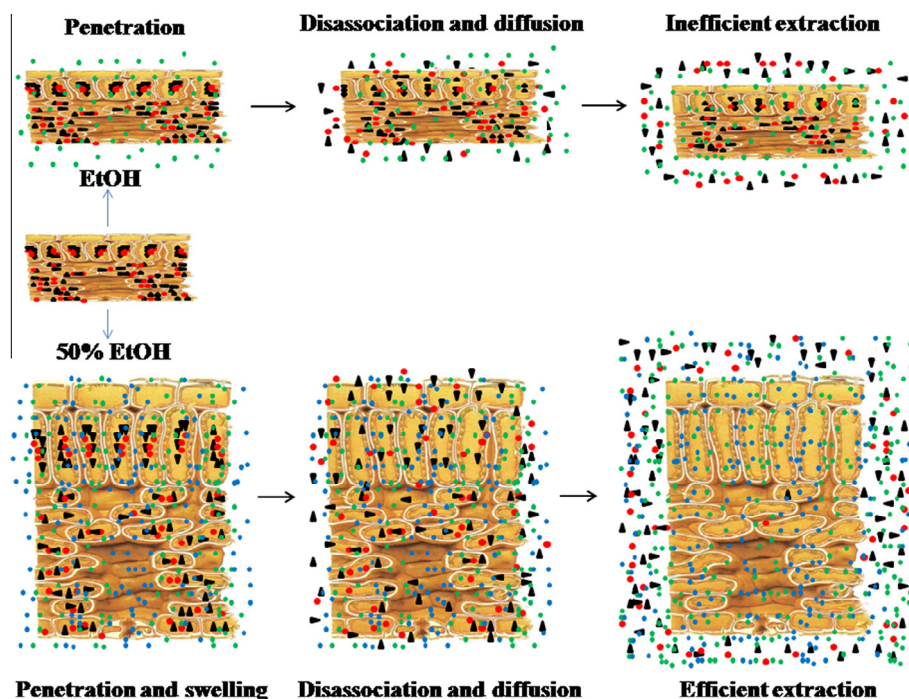


Fig. 2. The mechanism of high extraction efficiency by aqueous ethanol. Black triangle, red circle, green dot and blue dot indicate the catechins, caffeine, ethanol and water, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

oil–gas by changing the coal molecular structure (Shui et al., 2008; Sonmez & Giray, 2011).

3.5. Effect of pre-swelling on extraction yield

In order to verify the previous hypothesis about swelling effect, a pre-swelling extraction was conducted in which tea samples were firstly swollen with an amount of water and then extracted with absolute ethanol. The result is shown in Table 4. When 8 mesh samples were used, the extraction yield of catechins and caffeine increased markedly along with an increase in the amount of water for pre-swelling, and a significantly high yield in the pre-swelling extraction could be obtained compared with the corresponding control extraction performed with a mixture of water and ethanol as solvent, although the yield of the control extraction also increased with increasing of water amount in the solvent. A similar tendency was also observed when sample with smaller particle size (45 and 100 mesh) was used. According to the yield of catechins and caffeine, efficiency of pre-swelling extraction in which sample was swollen at a tea/water ratio of 1/4–5 (w/w) and then extracted by absolute ethanol, was equivalent to that of normal extraction with 50% ethanol (Tables 1 and 4). Furthermore, along with decrease in particle size of used sample, the yield of pre-swelling extraction increased significantly; however, the difference between pre-swelling extraction and the corresponding control extraction (using the same amount of water and ethanol) decreased slightly. These findings confirmed that pre-swelling the sample with a small amount of water can extremely enhance the extraction efficiency of absolute organic solvent, and a more significant efficiency promotion can be achieved, in particular for the samples with relatively large particle size. The extraction behavior can usually be described as three successive steps, i.e., penetration of the solvent into the solid matrix, disassociation and diffusion of the solute from matrix to the leaf/water interface and finally transferral of solute across the interface to the bulk fluid phase (Rodríguez-Jimenes et al., 2013; Ziaedini, Jafari, & Zakeri,

2010), of which the second step is the rate-determining process of the extraction (Pinelo, Sineiro, & Núñez, 2006; Price & Spitzer, 1994; Spiro & Jago, 1982). Our results highly agreed with these previous findings. More importantly, our study clearly illustrated that swelling or expansion of tea leaves might be the key factor in the rate-determining process during extraction since it was one of the most important prerequisites influencing the disassociation and diffusion of the solute from the matrix to the leaf/water interface. Combined with the results of the vacuum-assisted extraction test, solubility test as well as swelling ratio measurement, loose structure and relatively extended interface of tea sample after being swollen with water promote the disassociation and diffusion of components in tea leaf matrix, and high solubility of tea components in the organic solvent enhances the equilibrium concentration in extracts, thus, high efficiency in extraction with aqueous organic solvent can be achieved (Fig. 2).

Nowadays, water is commonly used as an extraction solvent for preparing tea polyphenols or tea extracts. Low yield and high energy consumption for removing solvent are the main obstacles of water extraction. Many technologies, including ultrasound-assisted extraction (Both, Chemat, & Strube, 2014; Xia, Shi, & Wan, 2006), microwave-assisted extraction (Nkhili et al., 2009; Pan, Niu, & Liu, 2003), enzyme-assisted extraction (Chandini, Jaganmohan Rao, Gowthaman, Haware, & Subramanian, 2011) and pressure-assisted extraction (Xi, He, & Yan, 2015; Xi, Xue, Xu, & Shen, 2013), have been developed to promote the yield during water extraction through changing the structure of leaf and/or characteristics of the solvent. However, these technologies might complicate the assembly line because of the introduction of additional equipment, and could not reduce the energy consumption for removing water from the extract. Pre-swelling the sample with a small amount of water followed by organic solvent (such as ethanol) extraction might be an alternative because of its high yield and easy recovery of the solvent, especially for large-scale tea extract production. Moreover, although this work mainly focused on illustrating the high yield mechanism of aqueous organic

solvent, pre-swelling treatment might also be suitable for brewing a cup of tea, with full health benefits, at home, however this should be studied further.

4. Conclusions

Extraction is the key step for the comprehensive utilization of tea components. Compared with extraction by water, catechins and caffeine can be easily extracted from green tea by aqueous ethanol (especially 50% ethanol), but hardly at all by absolute ethanol. Efficiency of extraction with absolute ethanol mainly depends on the particle size of the sample, and rarely on solvent penetration. Solubility of EGCG in aqueous ethanol increases with increasing ethanol level in solvent, and is significantly higher than that in water (6.10 g/100 g), but lower than that in absolute ethanol (76.8 g/100 g) at 25 °C. Solubility of caffeine increases markedly with an increase in ethanol level in solvent when the ethanol level is no more than 50%, and then decreases when the level further increases. Swelling ratio of the infused leaves decreases significantly along with an increase in ethanol level in the solvent. Pre-swelling extraction in which the tea sample was extracted with absolute ethanol after being pre-swollen with a small amount of water (4–5 g/g sample), can greatly improve the extraction efficiency and achieve as high yield of catechins and caffeine as extraction with 50% ethanol. These findings prove for the first time that the efficient extraction capability of an aqueous organic solvent for tea components is closely related to its good leaf-matrix-swelling effect and high solubility of catechins and caffeine, while the inefficiency of absolute organic solvent is mainly due to its weak swelling effect. For industrialized preparation of tea components, pre-swelling extraction might be regarded as an excellent alternative technology because of its high yield and low cost of solvent removal.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.foodchem.2015.08.029>.

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