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Preparation of Partially Decaffeinated Instant Green Tea

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The caffeine level of instant tea extracted from decaffeinated leaf tea with 4.0 mg g^{-1} caffeine is commonly above 10.0 mg g^{-1}, the maximum limit of caffeine for decaffeinated instant tea. Further removal of caffeine by active carbon (AC) from the green tea extract was investigated. It showed that the removal of caffeine from the tea extract solutions depended on the treatment time and tea extract concentration while the ethanol concentration and pH had little effect on the removal of caffeine. According to the removal of caffeine and the ratio of total catechins to caffeine in the tested samples, the optimum decaffeination conditions were determined to be as follows: tea extract concentration 15−30 g L^{-1} for common tea extract but higher for partially decaffeinated tea leaf extract; ratio of tea solution to AC, 100 mL:4 g; treatment time, 4 h; and natural tea extract pH. Instant tea powder extracted from partially decaffeinated leaf tea with a caffeine level of 4.03 mg g^{-1} and further decaffeinated by AC had a caffeine level of 7.81 mg g^{-1}, which was 31% lower than that without AC treatment.

KEYWORDS: ***Camellia sinensis*; instant tea; caffeine; catechins; EGCg; HPLC; active carbon**

INTRODUCTION

Tea consumption is increasing because of its healthy benefits (*1*−*3*). However, tea has 3−5% caffeine, and it poses problems for many tea lovers because it stimulates the cerebral cortex to induce excitation in the central nervous system (*4*), and it also causes irritation of the gastrointestinal tract and sleeplessness for certain people (*5*). Kaufman and Sachdeo showed that excessive caffeine ingestion from tea increased the seizure frequency of heart disease, and avoidance of excessive caffeine in patients with epilepsy was recommended (*6*). UVB-induced apoptosis in the epidermis cells was increased by caffeine (*7*). Chronic consumption of caffeinated liquids increased the risks of psychosocial stress as well as miscarriages (*8*, *9*). On the basis of product usage and available consumption data, Baronea and Roberts suggested a mean daily caffeine intake of 4 and 1 mg kg^{-1} body weight for U.S. adult consumers and children younger than 18 years of age, respectively (*10*). Decaffeinated tea is a choice for many tea lovers, and its demand is expanding.

Attempts, including the use of chloroform or methylene chloride and ethyl acetate, have been made to remove caffeine from tea so as to produce decaffeinated teas and tea extracts. However, it is not widely accepted by consumers because of toxicity from the chemical residues (*11*). Decaffeination by supercritical carbon dioxide is a safe method (*12*, *13*), but its production cost is expensive and not completely satisfied. Sakanaka confirmed that caffeine could be separated from tea extracts by sawdust lignocellulose columns (*11*). An inexpensive and safe method for the decaffeination of fresh green tea leaves by a hot water treatment was recently developed (*14*).

There are two kinds of decaffeinated tea products marketed, that is, decaffeinated leaf teas and decaffeinated instant teas. The maximum caffeine levels are always limited to 4 mg g^{-1} for leaf teas and 10 mg g^{-1} for instant teas. Instant tea is extracted from leaf teas, and about 3 kg of leaf tea is used to produce 1 kg of instant tea. The caffeine level of the instant tea will be above 10 mg g^{-1} if it is extracted from the decaffeinated leaf tea with a caffeine level of 4 mg g^{-1}. In the present work, active carbon (AC) was used to partially remove caffeine from tea extracts so as to find a method for preparing decaffeinated instant green tea with caffeine levels below 10 mg g^{-1} from the partially decaffeinated leaf teas.

MATERIALS AND METHODS

Materials. Two kinds of tea materials were used in the present study. Green tea extract supplied by Novanat Bioresources Co., Ltd. (Shanghai, China) was used for investigating decaffeination conditions, and partially decaffeinated green tea leaves prepared by the method described in our previous paper (*14*) was used for preparing decaffeinated instant green tea powder.

Granular AC (0.5−1 mm in diameter) and chemical purity grade ethanol were bought from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). High-performance liquid chromatography (HPLC) reference compounds including (+)-catechin (C), (−)-epicatechin (EC), (+)-gallocatechin (GC), (−)-epigallocatechin (EGC), (+)-catechin gallate (Cg), (−)-gallocatechin gallate (GCg), (−)-epicatechin gallate (ECg), and (−)-epigallocatechin gallate (EGCg) were provided by Dr. Takeda from the National Tea Research Institute Japan. Caffeine was a Sigma product (St. Louis, MO). The other chemical reagents used

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were of HPLC grade (Jinmei Biotech Corp., Tianjin, China) except where stated otherwise.

Pretreatment of AC. The AC was steeped in 2 volumes of 85% (v/v) ethanol solution for 24 h and then washed with distilled water after the ethanol solution was decanted. The washed AC was drenched in 2 volumes of 0.1 N NaOH overnight and washed to pH 7 with distilled water as before. The NaOH-treated AC was drenched in 2 volumes of 0.1 N HCl overnight, and finally, the HCl residue was washed with distilled water (pH 7). The prepared AC was naturally dried at room temperature.

Adsorption Time. Two grams of the tea extract was dissolved in 100 mL of distilled water in a 250 mL glass flask, and 4 g of the prepared AC was added. Thirty-nine flasks were prepared in total. The flasks with tea extract and AC were placed in a model H2S-H shaking water bath (Donglian Electronic Technology Co., Ltd., Harbin, China) at 25 °C and 130 rpm for 24 h. Three flasks were sampled from the water bath for HPLC analysis at designed time intervals of 5−800 min.

Concentration of Ethanol. Two grams of the tea extract was dissolved in 100 mL of ethanol solution of various concentrations (0, 5, 15, 25, 40, and 50%, v/v), and then, 4 g of the prepared AC was added. The shaking treatment was carried out for 4 h as described in the Adsorption Time section.

Concentration of Tea Extract. One hundred milliliters of distilled water was used to prepare tea extract solutions with tea extract concentrations 15−60 g L^{-1}, and the solutions were treated with 4 g of AC in a shaking water bath for 4 h as described in the Adsorption Time section.

Test of pH Effect. Four grams of the tea extract was dissolved in 90 mL of distilled water and adjusted to pH 3−9 with 0.1 N HCl or 0.1 N NaOH. The solution was then diluted to 100 mL with distilled water. The prepared solutions were shaken for 4 h as described in the Adsorption Time section.

Preparation of Decaffeinated Instant Tea with Partially Decaffeinated Leaf Tea. Five hundred grams of the partially decaffeinated green tea was extracted in 8 L reverse osmosis filtered water at 90 °C for 40 min. The extracted solution was separated from the leaves by filtration through two layers of gauze and then centrifuged at 5478*g* for 10 min. The tea-extracted solution was concentrated to 4 L (solids concentration was about 40 g L^{-1}) in a circumvolve evaporator at reduced pressure and 45 °C and then treated with 160 g of AC for 4 h as described in the Adsorption Time section.

The AC-treated solution was centrifuged at 5478*g* for 10 min. The supernatant was concentrated to 1 L in a circumvolve evaporator at reduced pressure and 45 °C. The concentrated tea extract was dried by an OPD-8 laboratory spray dryer (Ohkawara Dryers CO., Ltd., Shanghai, China) at an inlet temperature of 200 °C and an outlet temperature of 105 °C. The obtained instant green tea powder was kept in a desiccator containing silica gel at room temperature. Another 500 g of the partially decaffeinated green tea was used to prepare instant green tea as above method but without the AC treatment for control.

HPLC Analysis. Before HPLC, the instant tea powders (200 mg) were dissolved in 100 mL of 50% (v/v) ethanol. The partially decaffeinated tea leaf was ground and sifted through 0.45 mm sifter. The ground tea sample (300 mg) was extracted in 50 mL of 50% (v/v) ethanol solution for 45 min. The extract was filtered through "Double-Ring" 102 filter paper (Xinhua Paper Industrial Co. Ltd., Hangzhou, China).

The above solutions were centrifuged at 5478*g* and 20 °C for 15 min and filtered through a 0.22 μm Millipore filter before being injected into the HPLC. The HPLC was carried out as described in a previous paper (*14*).

Data Statistics. The tests in the present paper were in triplicate, and the mean values of the triplicate tests were presented. Statistics was carried out on Version 8.1 SAS System for Windows (SAS Institue Inc., Cary, NC) software.

RESULTS AND DISCUSSION

Effect of Adsorption Time on Levels of Caffeine and Catechins of Tea Extract. Figure 1 showed that levels of caffeine and total catechins in the tea extract solution decreased with an increase in AC treatment time. However, their decline rates differentiated, and the decline of caffeine was more quick than that of total catechins. At the fourth hour (240 min), about 80% caffeine and 32% total catechins were removed, respectively.

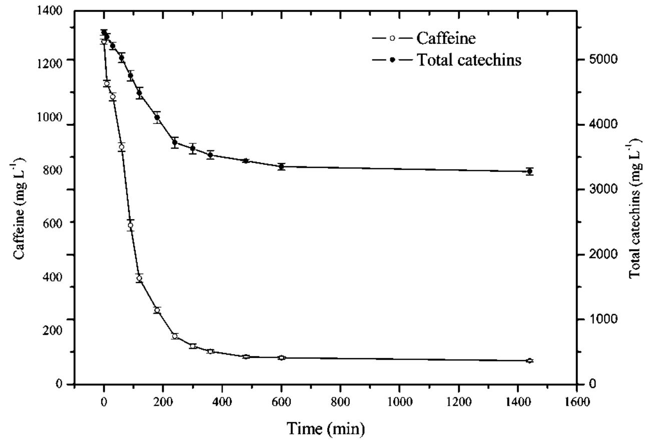

Figure 1. Effect of AC treatment time on levels of caffeine and total catechins.

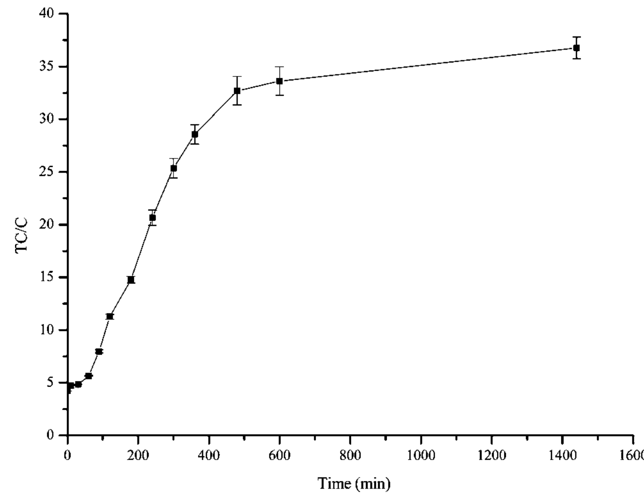

Figure 2. Effect of AC treatment on ratio of total catechins to caffeine (TC/C).

The differentiation of removal of caffeine and catechins can be judged by the ratio of total catechins to caffeine (TC/C). **Figure 2** showed that the ratio of TC/C increased till 500 min of AC treatment and then became more stable. It suggests that the removal of caffeine by AC was quicker than that of catechins if the treatment time was less than 500 min.

From a decaffeinated instant tea processing point of view, the perfect treatment time should be when as much as possible caffeine is removed while the catechins remain in the tea extract as much as possible. The treatment time might also depend on the initial caffeine level of the tea extract used. The total concentration of tea catechins in common instant green tea is always above 200 mg g^{-1}. If the caffeine requirement of decaffeinated instant tea is below 10 mg g^{-1}, the ratio of TC/C should be above 20 in decaffeinated instant green tea. On the basis of the results in **Figures 1** and **2** obtained from the tested tea extract and the maximum limit of caffeine level for decaffeinated instant tea, 4 h of AC treatment time will be a choice (**Figure 1**), at which the ratio of TC/C was 22 (**Figure 2**). If partially decaffeinated tea extract is used, the time should be shorter because its caffeine level is lower than the tested tea extract.

Table 1. Effect of Ethanol Concentration on the Adsorption of of Caffeine and Catechins (mg L^{-1})[a]

ethanol concn (%)	GC	EGC	C	EC	EGCg	GCg	ECg	Cg	total catechins	caffeine	TC/C[b]
control[c]	316.5 a	1119.2 a	151.2 a	506.2 a	2169.6 a	400.7 a	573.6 a	180.7 a	5417.7 a	1282.8 a	4.2 f
0	271.1 c	801.9 c	128.2 c	350.9 d	1393.4 e	270.2 d	372.3 e	110.2 e	3698.2 e	179.6 e	20.6 b
5	233.7 d	673.5 d	114.9 d	300.6 e	1342.3 f	258.6 e	352.1 f	101.4 f	3377.1 f	151.3 f	22.3 a
15	235.8 d	679.7 d	117.6 d	302.5 e	1346.6 f	260.5 e	358.1 f	105.8 ef	3406.6 f	154.1 f	22.1 a
25	281.0 bc	795.4 c	128.7 c	345.9 d	1715.1 d	301.6 c	407.8 d	123.1 d	4098.6 d	258.9 d	15.8 c
40	290.3 b	820.6 b	137.6 b	373.6 c	1748.3 c	320.7 b	438.3 c	140.3 c	4269.7 c	299.8 c	14.2 d
50	302.8 a	832.7 b	143.5 b	405.7 b	1761.7 b	342.2 b	459.1 b	159.8 b	4407.5 b	379.2 b	11.6 e

[a] Data with different alphabetic letters in a same column were significantly different at $p < 0.05$. [b] TC/C, ratio of total catechins to caffeine. [c] Control: 2 g of the tea extract was dissolved in 100 mL of water.

Table 2. Effect of Concentration of Tea Extract on Decaffeination (mg L^{-1})

concn[a] (g L^{-1})		GC	EGC	C	EC	EGCg	GCg	ECg	Cg	total catechins[b]	caffeine[b]	TC/C[c]
15	B	235.1 ± 14.1	835.8 ± 21.3	111.1 ± 9.8	382.6 ± 12.5	1628.5 ± 38.2	300.4 ± 4.0	430.3 ± 13.9	137.3 ± 7.2	4061.1 ± 12.4 (100%)	969.8 ± 11.2 (100%)	4.19 ± 0.06
	A	196.6 ± 10.2	526.1 ± 8.1	94.8 ± 3.9	236.8 ± 8.7	866.3 ± 20.1	174.5 ± 7.1	234.1 ± 5.1	72.0 ± 3.1	2401.2 ± 21.9 (59.1%)	101.6 ± 5.3 (10.5%)	23.63 ± 0.97
20	B	314.8 ± 12.1	1114.9 ± 16.3	149.3 ± 4.5	508.0 ± 19.3	2166.5 ± 34.8	396.8 ± 10.3	571.3 ± 10.1	182.4 ± 7.2	5404.0 ± 67.6 (100%)	1280.4 ± 13.6 (100%)	4.22 ± 0.01
	A	272.9 ± 10.6	800.1 ± 13.4	131.2 ± 7.1	357.1 ± 9.5	1397.4 ± 18.3	268.3 ± 8.6	368.5 ± 6.4	111.9 ± 3.2	3707.4 ± 36.9 (68.6%)	182.2 ± 2.1 (14.2%)	20.35 ± 0.43
30	B	462.4 ± 16.3	1665.3 ± 29.1	216.7 ± 6.0	752.2 ± 11.9	3235.1 ± 32.3	584.4 ± 9.6	844.6 ± 10.2	265.5 ± 5.2	8026.2 ± 43.8 (100%)	1918.5 ± 17.3 (100%)	4.18 ± 0.01
	A	374.5 ± 11.8	458.7 ± 7.4	171.6 ± 3.0	464.8 ± 7.5	1566.0 ± 16.7	357.0 ± 7.8	577.6 ± 6.1	195.2 ± 5.2	4165.5 ± 38.5 (51.8%)	196.7 ± 7.6 (11.4%)	21.18 ± 0.60
40	B	609.2 ± 17.3	2217.5 ± 40.1	296.3 ± 7.1	1009.2 ± 17.6	4324.7 ± 32.3	767.6 ± 12.2	1108.4 ± 13.2	358.2 ± 10.4	10691.1 ± 43.6 (100%)	2553.9 ± 17.1 (100%)	4.19 ± 0.01
	A	365.5 ± 12.3	896.8 ± 10.7	225.7 ± 7.1	559.0 ± 10.9	2468.2 ± 16.2	525.3 ± 8.5	829.5 ± 11.2	259.7 ± 8.7	6129.7 ± 36.2 (57.3%)	490.6 ± 9.3 (19.2%)	12.49 ± 0.16
50	B	775.7 ± 22.3	2765.2 ± 41.2	367.7 ± 8.5	1262.4 ± 23.5	5405.2 ± 39.6	982.7 ± 13.7	1416.9 ± 16.2	450.3 ± 10.1	13426.1 ± 125.7 (100%)	3195.8 ± 20.1 (100%)	4.20 ± 0.01
	A	486.3 ± 14.6	1266.1 ± 11.3	281.2 ± 11.6	762.9 ± 14.3	2970.6 ± 17.4	715.2 ± 12.2	1116.3 ± 9.2	324.6 ± 8.0	7923.2 ± 45.6 (59.0%)	870.9 ± 7.9 (27.3%)	9.10 ± 0.03
60	B	923.6 ± 22.2	3329.4 ± 30.2	430.2 ± 19.6	1506.4 ± 28.8	6472.6 ± 38.3	1178.1 ± 16.5	1700.2 ± 15.3	541.5 ± 12.6	16082.0 ± 111.3 (100%)	3828.2 ± 21.2 (100%)	4.20 ± 0.01
	A	615.5 ± 19.4	1767.8 ± 13.6	332.4 ± 7.0	974.3 ± 15.8	3787.0 ± 23.1	904.1 ± 18.3	1348.2 ± 10.3	388.5 ± 7.1	10117.9 ± 36.6 (62.9%)	1379.5 ± 12.2 (36.0%)	7.33 ± 0.04

[a] B, before AC treatment; A, after AC treatment. [b] Data in the blankets were percentages of total catechins or caffeine that remained in the solutions after AC treatment, and those before AC treatment were set as 100%. [c] TC/C, ratio of total catechins to caffeine.

Effect of Ethanol Concentration on Levels of Caffeine and Catechins. The effect of ethanol concentration on levels of caffeine and catechins is shown in **Table 1**. It showed that the levels of caffeine and catechins were low when ethanol was 0−15% (v/v) and then increased with the increase in ethanol concentration. The change in ratio of TC/C reversed to the levels of caffeine and catechins. In general, the polarity of solvents may affect the molecule attraction between adsorbate and adsorbent. In the case of high levels of ethanol, the polarity of solvents might be weakened with the increase in ethanol concentration, resulting in the decrease in the attraction between adsorbate and AC. That might be why less caffeine and catechins were adsorbed by AC at high ethanol concentration than at low ethanol concentration.

The concentration of total catechins in treatment water [0% (v/v) ethanol] was 3698.2 mg L^{-1}, which was 8% higher over the level of 5−15% (v/v) ethanol treatments (**Table 1**). To control an excessive loss of tea catechins, ethanol solution is not necessary during the decaffeination by AC.

Effect of Tea Extract Concentration on Removal of Caffeine and Catechins. Table 2 shows that the percentages of removed caffeine and catechins decreased with an increase in tea extract concentration, accompanying the decline in ratio of TC/C. It suggests that the removal of caffeine depends on the tea extract concentration. On the basis of the ratio of TC/C, the optimum concentration of 15−30 g L^{-1} is recommended for the tested material. However, the optimum concentration might be varied with initial caffeine levels of the tea materials used for processing decaffeinated instant tea. If partially decaffeinated tea is used, the concentration should be higher than 30 g L^{-1} so as to control excessive loss in tea catechins and to increase the processing efficiency.

Effect of pH on Removal of Caffeine and Catechins. The level of total catechins decreased with an increase in tea extract pH. However, there was no significant difference in caffeine levels between pH 3−6 and the ratio of TC/C showed nearly the same tendency (**Table 3**). This was consistent with the result of lignocellulose column separation (*11*). The natural tea solutions are at pH 5−6, and tea catechins are easily oxidized under alkaline conditions (*15*, *16*). That explains why the total catechins level declined quickly above pH 7, especially at pH 9 (**Table 3**). The results showed that it is not necessary to change the solution pH level during decaffeination by AC treatment.

Caffeine Level of Decaffeinated Instant Tea. Partially decaffeinated dry green tea with a caffeine level of 4.03 mg g^{-1} was used to prepare instant tea, during which further decaffeination was performed by AC. **Table 4** showed that the concentration of caffeine in the instant tea sample, which was further decaffeinated by AC, was 7.81 mg g^{-1}, which was 31% lower than that not treated by AC. The concentration of total catechins in the sample treated by AC was 319.15 mg g^{-1}, which was 16.2% lower than the control. The caffeine level of the

Table 3. Effect of pH on the Adsorption of Caffeine and Catechins by AC[a] (mg L^{-1})

pH	GC	EGC	C	EC	EGCg	GCg	ECg	Cg	total catechins	caffeine	TC/C[b]
control[c]	601.2 a	2212.6 a	289.9 a	1001.7 a	4317.8 a	758.7 a	1096.2 a	351.6 a	10629.7 a	2541.3 a	4.2 e
3	429.4 b	1037.3 b	242.8 b	628.9 b	2666.9 b	669.3 b	931.3 b	293.5 b	6899.4 b	510.9 bc	13.5 a
4	391.4 c	1010.5 b	238.2 b	593.1 c	2557.2 c	654.4 c	916.6 c	285.0 c	6646.4 c	500.7 c	13.3 ab
5	366.8 d	902.1 c	226.6 c	558.5 d	2471.7 d	529.7 d	826.8 d	259.8 d	6142.0 d	495.0 c	12.4 b
6	365.5 d	901.3 c	222.9 c	554.6 d	2469.3 d	528.8 d	822.6 d	256.8 d	6121.8 d	490.7 d	12.5 b
7	126.7 e	756.8 d	171.8 d	388.3 e	1907.8 e	402.9 e	767.8 e	202.4 e	4724.5 e	485.5 d	9.8 c
9	0.0 f	90.0 e	75.0 e	173.1 f	340.0 f	181.7 f	225.3 f	54.9 f	1139.9 f	739.5 b	6.2 d

[a] Data with different alphabetic letters in a same column were significantly different at $p < 0.05$. [b] TC/C, ratio of total catechins to caffeine. [c] Control: 4 g of the tea extract was dissolved in 100 mL of water.

Table 4. Chemical Compositions of Decaffeinated Tea Leaf and Instant Teas (mg g^{-1})

samples	GC	EGC	EC	EGCg	GCg	ECg	Cg	total catechins	caffeine
tea leaves[a]	17.52 ± 0.68	53.01 ± 0.95	7.91 ± 0.09	37.18 ± 0.12	8.26 ± 0.15	7.58 ± 0.07	1.31 ± 0.2	133.25 ± 1.19	4.03 ± 0.02
instant tea A[b]	51.13 ± 0.81	156.92 ± 1.98	22.92 ± 0.59	108.21 ± 2.13	23.52 ± 0.64	15.21 ± 0.41	3.11 ± 0.15	381.02 ± 1.51	11.32 ± 0.33
instant tea B[c]	38.06 ± 0.32	131.13 ± 1.77	19.48 ± 0.53	93.78 ± 1.56	20.40 ± 0.53	13.48 ± 0.32	2.82 ± 0.12	319.15 ± 2.79	7.81 ± 0.22

[a] Partially decaffeinated tea leaves were used for preparation of instant tea powder. [b] Instant tea that was not further decaffeinated by AC. [c] Instant tea that was further decaffeinated by AC.

instant tea by the further decaffeination of AC was below the maximum limit level 10 mg g^{-1}, whereas the control sample was 11.32 mg g^{-1}.

The above results suggest that partial removal of caffeine by AC from green tea extract solutions could be obtained and the removal of caffeine depended on treatment time and tea extract concentration while ethanol concentration and pH had little effect on the removal of caffeine. Because catechins were also partially removed during the decaffeination by AC, the treatment time and tea extract concentration should be chosen according to the initial caffeine level of the leaf tea materials used.

There have been many reports on the decaffeination of tea, including the decaffeination of tea leaves (*11*, *17*, *18*), separation of caffeine from active ingredient tea catechins (*11*, *12*, *19*−*21*), and extracted tea waste (*22*, *23*). The reported decaffeination methods included organic solvents separation, column treatments, supercritical CO_2 extraction, and microbial and enzymatic treatments. The results in this experiment showed that AC treatment could be used as a simple method of decaffeination of tea extract. It is also important that the AC can be easily reactivated and used several times. During our experiments, the used AC was reactivated as described in Pretreatment of AC of the present paper and was used four times, but its activity did not decrease. An organic solvent was not used in this method, and toxic organic solvent residues can be avoided. It does not need expensive equipment as compared with supercritical CO_2 extraction. The required treatment time (4 h) is shorter than those in microbial and enzymatic treatments (10−72 h) (*23*). These advantages make the technique promising for large-scale procedures. It was reported that the risk of aflatoxins contamination in coffee beans was increased by decaffeination (*24*). The effect of decaffeination on aflatoxins contamination in tea extract was not tested in the present paper, and it remains to be investigated.

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