JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Factors Affecting Transfer of Polycyclic Aromatic Hydrocarbons from Made Tea to Tea Infusion

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Factors affecting transfer percentages of 12 polycyclic aromatic hydrocarbons (PAHs) were investigated, including tea variety, tea/water ratio (TWR, g/mL), brewing times, washed tea or unwashed tea, and covered cup or uncovered cup. It was observed that %PAH transfer varied with tea variety and increased with the decrease of TWR. The mean %PAH transfer with TWR = 1/150 was 1.12 and 1.65 times higher than that with TWR = 1/100 and 1/50, respectively. %PAH transfer reduced greatly as the brewing times increased. The mean %PAH transfer in the first brewing time occupied 51.6% of the total three mean %PAH transfers in the three brewing times. The mean %PAH transfer decreased by 30.4% after the tea had been washed immediately before brewing. Brewing the tea within uncovered cup diminished %PAH transfer by a degree of 4.31−31.7% compared to brewing the tea within a covered cup.

KEYWORDS: **PAHs; tea; infusion; health risk**

INTRODUCTION

Tea is one of the most widely consumed beverages in the world. Scientific evidence has been mounting indicating that tea consumption might have health-promoting properties, including the effects of reduction of cholesterol, depression of hypertension, antioxidation, antimicrobial activity, and protection against cardiovascular disease and cancer (*1*−*5*). However, it was also demonstrated that certain comtaminants remaining in made tea might impose a health threat on tea drinkers. The main comtaminants investigated widely and intensively were some kinds of heavy metals, fluoride, and pesticides (*6*−*17*).

Polycyclic aromatic hydrocarbons (PAHs), a class of compounds that consist of two or more fused aromatic rings, are a well-known group of carcinogens found in various foods, and they have been intensively studied over a couple of years (*18*). Gaseous and particle-bound PAHs can be transported over long distances before deposition and may accumulate in vegetation (*19*−*22*). This phenomenon could indirectly cause human exposure to PAHs by food consumption and, therefore, might impose a health threat upon humans. Tea leaves possessing large surface areas may accumulate PAHs, especially from polluted air. The manufacturing process of tea leaves may also introduce PAHs into made tea (*23*). In fact, PAHs have been detected in varieties of made teas around the world (*24*−*27*).

It is important to evaluate the transfer percentages of pollutants from made tea to tea infusion, for tea is subjected to a brewing process before human consumption. The methods of brewing tea may significantly affect the transfer percentage, which has been studied for heavy metals, fluoride, and pesticides with the aim of assessing their health risk and selecting a brewing method that reduces the intake of pollutants (*7*, *9*, *12*−*17*). Our previous paper reported how the brewing duration affected the transfer of PAHs from a black tea to the tea infusion (*24*). However, specific investigation about how brewing methods affect the transfer of PAHs, even including other organic pollutants such as pesticides, is still lacking.

This research focused on the transfer percentages of PAHs from made tea to tea infusion with certain influencing factors, including tea variety, brewing times, tea/water ratio, washed or unwashed tea, and covered or uncovered cup.

MATERIALS AND METHODS

Materials. Standard solutions of PAHs were obtained from Supelco Co. All solvents [dichloromethane (DCM), acetonitrile, and hexane] used for sample preparation and analysis were of HPLC grade from TEDIA Co. Ultrapure water was acquired from a PALL system. Chromatography silica gel (200−300 mesh) used for sample purification was purchased from Huadong Medical Corp. (Hangzhou, China). Four brands of Chinese made teas—one green tea, one oolong tea, and two black teas—were bought from Hangzhou tea market in Zhejiang province, China. The green tea was produced in Zhejiang province. The oolong and black teas were produced in Fujian province.

Sample Preparation. The method for extraction of PAHs from tea samples was described in our previous papers (*23*, *24*). The tea samples were extracted by ultrasonication with DCM. After that, the extract

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10.1021/jf060189j CCC: $33.50
Published on Web 05/23/2006

Table 1. PAH Contents in Tea and Transfer Percentages to Infusion[a]

PAH	PAH contents in tea (μg/kg)				% PAH transfer to infusion			
	green tea	oolong tea	black tea 1	black tea 2	green tea	oolong tea	black tea 1	black tea 2
NA	51.4 ± 1.9	81.9 ± 4.0	114 ± 3	1240 ± 56	44.5 ± 4.6	17.1 ± 1.0	27.3 ± 1.6	32.2 ± 2.9
ACE	9.02 ± 0.09	11.7 ± 1.4	80.0 ± 13	195 ± 26	26.6 ± 5.1	19.9 ± 3.0	5.85 ± 1.12	11.5 ± 0.3
FL	15.2 ± 1.6	15.7 ± 1.9	70.0 ± 3.8	139 ± 8	22.6 ± 2.3	12.9 ± 1.6	32.5 ± 1.6	26.7 ± 1.9
PHEN	168 ± 13	115 ± 10	970 ± 64	3720 ± 160	13.0 ± 2.2	2.17 ± 0.47	16.9 ± 0.7	11.7 ± 0.2
AN	4.95 ± 0.95	5.51 ± 0.35	187 ± 13	400 ± 13	16.4 ± 3.1	1.37 ± 0.01	11.9 ± 0.8	10.2 ± 1.7
FLUR	60.0 ± 6.6	3.77 ± 0.65	352 ± 34	579 ± 34	6.31 ± 0.50	ND	7.17 ± 0.07	10.4 ± 1.9
PY	4.31 ± 0.45	5.47 ± 0.18	403 ± 27	924 ± 123	ND	ND	5.78 ± 0.10	7.38 ± 0.64
BkF	7.92 ± 0.62	2.85 ± 0.35	9.96 ± 0.58	133 ± 4	ND	ND	0.641 ± 0.121	2.11 ± 0.22
BaP	6.79 ± 0.86	4.00 ± 0.61	20.1 ± 1.2	246 ± 7	ND	ND	ND	0.392 ± 0.044
dBAn	0.771 ± 0.080	0.362 ± 0.030	1.55 ± 0.01	156 ± 4	ND	ND	ND	0.914 ± 0.081
BPe	3.69 ± 0.19	5.2 ± 0.91	8.09 ± 0.52	217 ± 6	ND	ND	ND	3.41 ± 0.18
IcdP	9.53 ± 1.27	5.79 ± 1.21	4.98 ± 0.72	24.2 ± 2.1	ND	ND	ND	3.03 ± 0.15
∑PAHs	342 ± 27	257 ± 22	2370 ± 160	7970 ± 450	10.8 ± 1.5*	4.45 ± 0.51*	9.02 ± 0.52*	10.0 ± 0.9*

[a] ND, not detected. An asterisk (*) indicates the mean percent transfer of the 12 PAHs with ND = 0.

was evaporated to dryness by a rotary evaporator and then was dissolved in hexane. The solution was filtered through a silica gel column with the elution of hexane and DCM (1:1, v/v) and then was evaporated to dryness. The residue was dissolved in HPLC grade acetonitrile for further analysis.

With respect to tea infusion preparation, the tea samples were brewed with boiling water under different infusion conditions according to the different customs of brewing tea in China. The effects of these conditions on the %PAH transfer from tea to tea infusion were examined as follows.

Effect of Tea Variety. One gram of each of the four tea varieties was infused in 100 mL of boiling ultrapure water, which was enclosed in a 100 mL conical flask, remaining at room temperature for 30 min.

Effect of Tea/Water Ratio (TWR). Tea samples of 0.67, 1.0, and 2.0 g were infused individually in 100 mL of boiling ultrapure water, which was enclosed in a 100 mL conical flask, remaining at room temperature for 30 min. The TWRs were 1/150, 1/100, and 1/50 (w/v tea infusion), respectively.

Effect of Brewing Times. One gram of a tea sample was infused in 100 mL of boiling ultrapure water, which was enclosed in a 100 mL conical flask, remaining at room temperature for 30 min. Afterward, the liquid portion was decanted and collected in another enclosed conical flask, regarded as the first infusion. Then, another 100 mL of boiling ultrapure water was poured into the original conical flask to brew the residual tea and to obtain the second infusion in the same way as the first brew. A third infusion was also obtained by the same processes.

Effect of Washed Tea. One gram of a tea sample was infused in 100 mL of boiling ultrapure water, which was enclosed in a 100 mL conical flask, remaining at room temperature for 30 min. To get the washed tea, 20 mL of boiling ultrapure water was first used to infuse 1.0 g of tea sample for 10 s according to the custom of washing tea in China. Afterward, the liquid portion was decanted, and then 100 mL of boiling ultrapure water was added into the flask to brew the residual tea at room temperature for 30 min, which is the same infusion procedure used with the unwashed tea.

Effect of Uncovered Cup. One gram of a tea sample was infused in 100 mL of boiling ultrapure water which was enclosed in a 100 mL covered conical flask, remaining at room temperature for 30 min. At the same time, another 1.0 g of the tea sample was infused in 100 mL of boiling ultrapure water in a 100 mL uncovered conical flask, remaining at room temperature for 30 min.

After 30 min of brewing in the conditions just discussed above, all of the tea liquor samples were decanted into other covered flasks and cooled to ≈30 °C before the extraction of PAHs. All of the above experiments were performed in triplicates.

The extraction of PAHs from the cooled tea liquor samples was carried out by a solid-phase extraction (SPE) cartridge system from Supelco according to the method of Zhou et al. (*28*). Before extraction, the C_{18}-bonded phase containing 500 mg of reversed phase octadecyl was first washed with 5 mL of DCM and then was washed with 5 mL of ultrapure water. Then the cooled samples of tea infusion were percolated through the cartridges with a flow rate of 5 mL min^{-1} under vacuum pump. After extraction, the PAHs trapped within the cartridges were eluted by passing 5 mL of DCM. Water was eliminated by Na_2SO_4 from the extracts before the solutions were evaporated to dryness by a gentle stream of nitrogen. The residues were dissolved in 2 mL of HPLC grade acetonitrile for further analysis.

PAH Analysis. The analysis of PAHs was described elsewhere (*29*, *30*). All extracts from the samples were filtered with a 0.22 μm minisart filter in a vial sealed with a PTFE-lined cap. Then 15 μL extracts were injected by autosampler to be analyzed by the HPLC system (Agilent 1100), which consists of a quatpump, a PAH column (Agilent, ∅ 4.6 × 250 mm), a fluorescence detector, a data processor, and a system controller.

Twelve kinds of PAHs were determined: naphthalene (NA), acenaphthelene (ACE), fluorene (FL), phenanthrene (PHEN), anthracene (AN), fluoranthene (FLUR), pyrene (PY), benzo[*k*]fluoranthene (BkF), benzo[*a*]pyrene (BaP), dibenz[*a*,*h*]anthraxcene (dBAn), benzo[*ghi*]perylene (BPe), and indeno[1,2,3,*cd*]pyrene (IcdP).

Quality Control. A strict regimen of quality control was performed in the experiment according to the method described in our previous papers (*23*, *24*). Recoveries for the 12 PAHs from tea samples and tea infusions ranged from 71.6 to 103% and from 86.3 to 112%, respectively, with all of their relative standard deviations (RSD) being <20.0%. The limit of determination for the 12 PAHs ranged from 0.23 to 4.1 pg. Each experiment was accompanied by two blank experiments to ensure that there were no PAHs in the reagents.

Calculation of Transfer Percentage of PAHs. The transfer percentage of PAHs from made tea to tea infusion was calculated by using formula

$$\text{transfer (\%)} = \frac{C_{tl} \times V_{tl}}{C_{mt} \times W_{mt}} \times 100$$

where C_{tl} represents the concentration (ng/mL) of PAHs in the tea liquor, V_{tl} is the volume (mL) of the tea liquor, C_{mt} is the content (ng/g) of PAHs in made tea, and W_{mt} is the weight (g) of made tea prepared for making infusion.

RESULTS AND DISCUSSION

Effect of Tea Variety. Table 1 shows the contents of PAHs in the four varieties of Chinese made teas. The total contents of the 12 PAHs (∑PAHs) in the tea samples were in the extent of 257 and 7970 μg/kg, very similar to the documented data (*23*−*26*). The concentrations of PAHs in tea liquor rose with the increase of PAH contents in made tea. The highest concentrations of the 12 PAHs, ranging from 0.007 to 4.345 μg/L, were observed in the infusion of black tea 2. The transfer percentages of PAHs are also listed in **Table 1**. In this study, %PAH transfer from the oolong tea (4.45%) was much lower

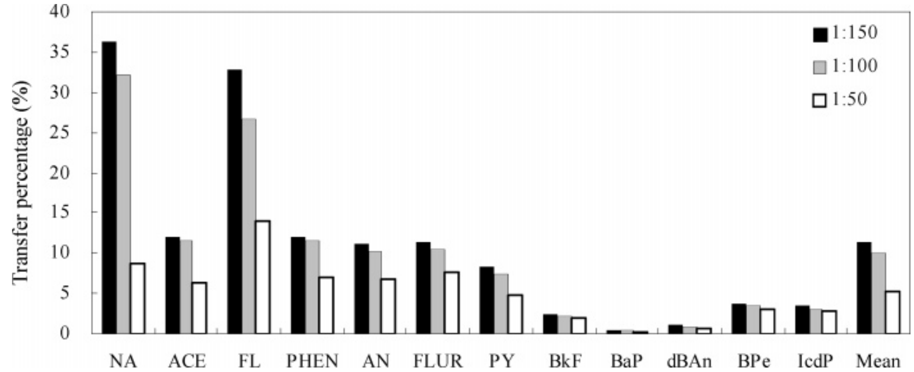

Figure 1. Effect of tea/water ratio on transfer percentages of PAHs from tea to infusion.

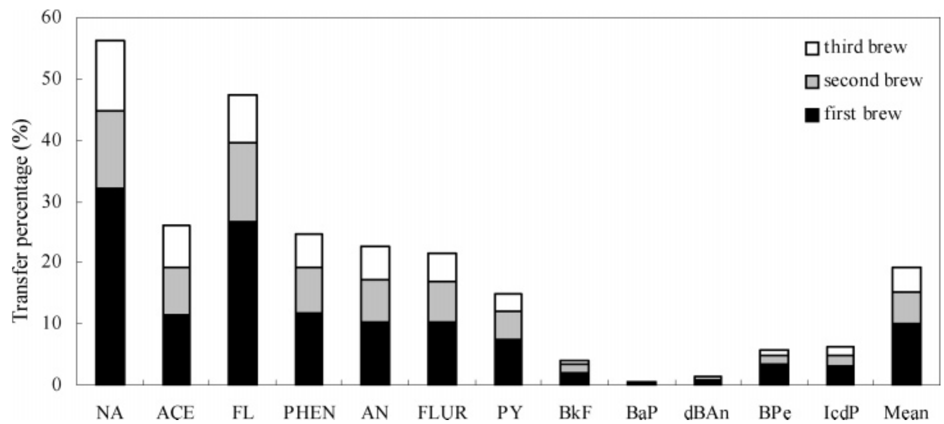

Figure 2. Effect of brewing times on transfer percentages of PAHs from tea to infusion.

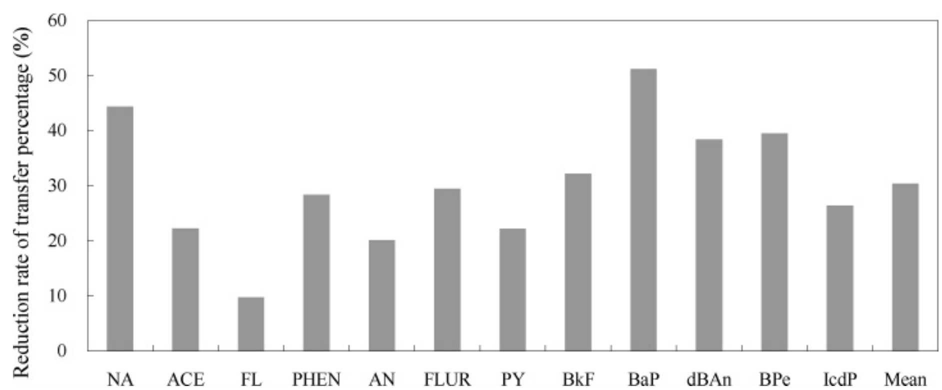

Figure 3. Reduction rate of %PAH transfer with washing tea before brewing.

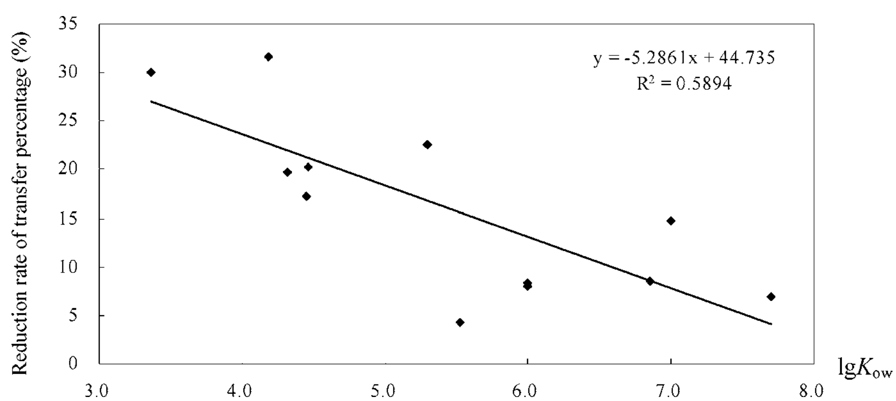

Figure 4. Relationship between log K_{ow} of PAHs and reduction rate of %PAH transfer with uncovered cup when brewed.

than that from the other three kinds of teas, for which the mean %PAH transfer was ≈10%. The reasons for the %PAH transfer variations with tea variety need further investigation and might be relative to the difference existing in natural properties of the tea variety (such as lipid content and fatty acid composition), manufacturing process (which might affect the PAH contents in made teas), and PAH pollution history (the longer polluted, the less transfer percentage). All %PAH transfers of each tea brand are closely relevant to the log K_{ow} of PAHs, which demonstrates that the lower the value of the log K_{ow}, the less transfer percentage, in accordance with our previous study (*24*) and other studies on organic pesticide residues (*13*, *14*).

Black tea 2 with the highest concentrations of PAHs in the infusion was chosen for further study on the effects of other factors.

Effect of Tea/Water Ratio. The effect of TWR on %PAH transfer is shown in **Figure 1**. It can be observed that %PAH transfer increased with the decrease of TWR, although the concentrations of PAHs in the infusion declined with the decrease of TWR, probably because the dispersion degree of made tea in water rose with the decrease of TWR. The mean %PAH transfer with TWR = 1/150 was 1.12 times and 1.65 times higher than that with TWR = 1/100 and 1/50, respectively. The %PAH transfer with TWR = 1/150 and 1/100 did not change much, but both were obviously higher than that with TWR = 1/50, implying that the effect of TWR on %PAH transfer was indistinguishable when TWR was lower than 1/100. It was easy to conclude that the effect of TWR on transfer percentages of the PAHs with two to four aromatic rings was obviously more remarkable than that with five or six aromatic rings (BkF, BaP, dBAn, BPe, and IcdP), and the reason might be the lower water solubility of the PAHs with five or six aromatic rings.

Effect of Brewing Times. The effect of brewing times on %PAH transfer from made tea to tea infusion is shown in **Figure 2**. It was observed that %PAH transfer decreased greatly with the increase of brewing times. The mean %PAH transfers of the second and third brewing times accounted for only 55.1 and 38.8% of that corresponding to the first brewing time, respectively. The mean %PAH transfer in the first brewing time occupied 51.6% of the total three mean %PAH transfers in the three brewing times. Considering that the highest transfer rate appeared in the first brewing time, the first infusion of polluted tea should be discarded or the polluted tea needs to be washed immediately before brewing.

Effect of Washed Tea. There is a custom of washing tea immediately before brewing, especially for black and oolong teas in China. Actually, black tea 2 was dried with the heat and smoke from the combustion of pine wood or coke (*23*). The small smoke particle, which may bind a certain amount of PAHs, can be deposited onto the leaf surface of the tea and then can be released into the infusion when it is washed. As shown in **Figure 3**, briefly washing the tea before brewing reduced the %PAH transfer with a mean reduction rate of 30.4% for the 12 PAHs. The reduced PAHs should mainly exist as particle bound, which could be poured out with the washing water, because most of the PAHs absorbed by tea leaves might not be released into the water within a short washing. This deduction was certified by the fact that the reduction rate increased with the increasing number of aromatic rings except for NA.

Effect of Uncovered Cup. There are two methods for brewing tea, covered cup and uncovered cup. PAHs, a group of semivolatile organic compounds, can volatilize from the hot tea liquor in the uncovered cup when it is brewed. Therefore, PAH concentrations of the liquor in the covered cup were higher than in the uncovered cup. Meanwhile, PAHs with lower values of log K_{ow} normally have greater volatility (*31*) and can more easily escape from the hot water. Consequently, the effect of the uncovered cup on the transfer percentages of PAHs with lower values of log K_{ow} was greater than that with a higher value of log K_{ow}. **Figure 4** shows the relationship between the reduction rate of %PAH transfer within the uncovered cup and the log K_{ow} of PAHs. It can be observed that the reduction rate of %PAH transfer decreased with the increase of log K_{ow}, in a scope of 4.31 and 31.7%. Therefore, it is better to leave the cup uncovered during the brewing of tea to reduce the PAH concentrations in the tea infusion and human health risk.

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Received for review January 21, 2006. Revised manuscript received April 7, 2006. Accepted April 11, 2006. This project was jointly supported by the National Natural Science Foundation of China (No. 20337010, 20507015), the National Basic Research Program of China (2003CB415004), and the Zhejiang Provincial Natural Science Foundation of China (No. M403041).

JF060189J