

## Polycyclic Aromatic Hydrocarbons: Pollution and Source Analysis of a Black Tea

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Investigations into the manufacturing process of one kind of black tea revealed that it included five steps: withering, rolling, fermentation, drying, and drying and sorting. A total of 16 polycyclic aromatic hydrocarbons (PAHs) were simultaneously measured in fresh leaves, withered leaves, rolled leaves, fermented leaves, crude black tea, and black tea sampled after each manufacturing stage and in the indoor and outdoor air of the drying house. It was observed that the total contents of the 16 PAHs ( $\Sigma$ PAHs) in the crude black tea and the black tea were obviously higher than those in the tea leaves sampled after each manufacturing step before the drying stage; the air  $\Sigma$ PAHs in the drying house were about 100 times higher than those outside the drying house. It can be concluded that quantities of PAHs were released into the drying house from the combustion of pine firewood during the drying stage, and then were absorbed by the tea leaves, thus resulting in the high PAH contents in the black tea.

**KEYWORDS:** PAHs; black tea; manufacturing process; pine firewood; air

### 1. INTRODUCTION

Next to water, tea is the most widely consumed beverage in the world. Global tea production in 2000 was 2.89 million tons, nearly 80% of which is black tea and about 20% of which is green tea, and some others are oolong tea and brick tea (1). Black tea is made by fermenting the slightly wilted leaves for many hours before being either smoke fired, flame fired, or steamed. In contrast, green tea is not fermented, but the leaves are treated by steaming or pan firing to inactivate the polyphenol oxidase, thus avoiding oxidation. Oolong tea is made by wilting the fresh leaves in the sun, then bruising them slightly, and partially fermenting them. The color of oolong tea is intermediate between that of green and black tea. Brick tea is a kind of black tea which is made of old leaves, fallen leaves, branches, fruits, and sometimes even roots. To make brick tea, the black tea is further pressed into bricks (where the name comes from) under high pressure, after full fermentation and other procedures normally used for making black tea.

The health effects of tea leaves have been studied by scientists for a long time (2, 3). There is a lot of scientific evidence indicating that tea consumption might have health-promoting properties, including the effects of reduction of cholesterol, depression of hypertension, antioxidation and antimicrobial effects, and protection against cardiovascular disease and cancer (4, 5). But residues of certain chemical contaminants in tea leaves, which may pose a health threat to tea drinkers, are occasionally detected and need to be monitored to avoid unacceptable levels of exposure. The main contaminants that

have been investigated widely and intensively are heavy metals, fluoride, and pesticides (1, 6–11).

Polycyclic aromatic hydrocarbons (PAHs), a class of compounds that consist of two or more fused aromatic rings, are a well-known class of carcinogens found in some foods, and they have been intensively studied over the past few years (12). Gaseous and particle-bound PAHs can be transported over long distances before deposition, and may accumulate in vegetation (13–16). This could indirectly cause human exposure to PAHs through food consumption, and thus might pose a human health threat.

Tea leaves possess a high surface area, so they may accumulate PAHs, especially from air. The manufacturing process of tea leaves may also lead to PAHs in the product, because many of them, especially black tea, are dried using combustion gases from burning wood, oil, or coal. PAHs are invariably present in the combustion gases, and can be absorbed by the tea product when they come into contact. The PAH contents in some tea products have been determined, with ranges from 497  $\mu\text{g}/\text{kg}$  (green tea) to 1162  $\mu\text{g}/\text{kg}$  (black tea) ( $n = 4$ ) (17) and from 13.41  $\mu\text{g}/\text{kg}$  (fennel tea) to 7536.33  $\mu\text{g}/\text{kg}$  (mate tea) ( $n = 11$ ) (18). It can be seen that PAH contents vary greatly with different kinds of tea product. This implies that the manufacturing process of tea leaves might be the main source of PAHs in the tea product with high PAH contents. However, little information is available on the changes of PAHs and other organic contaminant contents in tea leaves during the tea manufacturing process.

The main objectives of the present study were to determine the changes of PAH contents in tea leaves during the black tea

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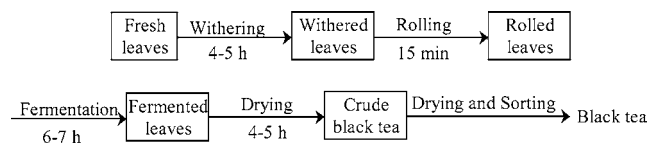


Figure 1. Typical flowchart of the manufacturing process of black tea.

manufacturing process, and then analyze the source of PAHs in the black tea.

## 2. MATERIALS AND METHODS

**2.1. Reagents and Reference Samples.** The mixed standard solution of 16 PAHs was obtained from Supelco Co. All solvents (dichloromethane, acetonitrile, hexane) used for sample preparation and analysis were HPLC grade from TEDIA Co. Chromatography silica gel (200–300 mesh) used for sample purification was purchased from Huadong Medical Corp., China.

**2.2. Materials and the Manufacturing Process for the Black Tea.** This study took the black tea, which was only produced in the Fujian province of China, as an example to investigate the changes of PAH contents in tea leaves during the tea manufacturing process. This black tea is characterized by its specific smokelike scent, which is directly absorbed from the smoke originated from the combustion of pine firewood during the drying stage.

There are five steps for the black tea manufacturing process, as shown in **Figure 1**. (1) “Withering”, in which the fresh leaves are air-dried for up to 4–5 h to reduce the initial moisture content to approximately 60%. (2) “Rolling”, in which the withered leaves are crushed and shaped by a rolling machine for about 15 min, thus partially destroying cell structures, to allow enzymatic oxidation of the flavonols in the presence of atmospheric oxygen. (3) “Fermentation”, in which the rolled leaves are compressed into a crate for about 6–7 h, thus resulting in significant compositional changes leading to the characteristic color and flavor of common black tea. (4) “Drying”, in which the fermented leaves are spread open in a big round shallow basket which was then laid on the beam of the drying house and then dried for about 4–5 h with the hot air and smoke originated from the combustion of pine firewood. After drying, the fermented leaves become the crude tea product with a moisture content of 2–3%, which sometimes is brewed to drink by some local people. The crude black tea possesses a heavy smokelike scent. (5) “Drying and sorting”, in which the crude product is smoke fired again to increase the specific smokelike scent, and then is sorted into the appropriate grade. The first four steps can be finished in a family workshop and tea factory. But, usually, most of the crude tea product is purchased by a tea company, and is made into black tea with step 5 only in the tea factory. To protect the manufacturing technology, the tea factory did not let us investigate the process. We only know that turpentine is fired as fuel to redry the crude black tea in the final stage.

**2.3. Sampling.** The fresh leaves, withered leaves, rolled leaves, fermented leaves, crude black tea, and black tea were sampled after each manufacturing stage. Each tea sample consisted of three subsamples obtained after the same stage, about 100 g per subsample. Air samples were collected from three sites, two sites of which were located in the drying house, while the other site was located outdoors about 50 m away from the house. Air sampling programs were carried out for 5 h with low-noise small samplers (MP-15CF minipumps, Shibita, Japan) located 2.0 m above the ground. The samplers equipped with Whatman glass fiber filters (GFFs; 25 mm, Whatman, England) collecting particle-bound PAHs and XAD-2 (2.5 g) retaining PAHs in the gas phase, respectively, were operated at flow rates of 1.0 L/min (19, 20). PAH concentrations in the air were shown with the sum concentrations in two-phase systems. These samples were transported to the laboratory immediately in an ice bag and stored in a –60 °C refrigerator until they were prepared for analysis.

**2.4. Sample Preparation.** A certain amount of ground tea samples was extracted by ultrasonication at 30 °C for 30 min with 20 mL of dichloromethane (DCM) and acetone (1:1, v/v). The extracts were then decanted and collected. This process was repeated in triplicate. All extracts were combined and passed through a column packed with

anhydrous Na<sub>2</sub>SO<sub>4</sub> using 10 mL of a 1:1 (v/v) mixture of DCM and acetone. The extracts were evaporated to dryness by a rotary evaporator and then dissolved in 2 mL of hexane. A 1 mL sample of the solution was filtered through a silica gel column, eluted with 10 mL of hexane and DCM (1:1, v/v), and then evaporated to dryness. The residue was dissolved in 2 mL of HPLC-grade acetonitrile for analysis. There were three replicates, with each sample undergoing the same procedures.

The air samples were extracted by ultrasonication at 30 °C for 30 min with a 20 mL mixture of DCM and acetonitrile (3:2, v/v). A 30 μL sample of dimethyl sulfoxide (DMSO) was added to 10 mL extract aliquots and then evaporated to dryness under a gentle flow of nitrogen gas at room temperature before addition of 1 mL of HPLC-grade acetonitrile (19, 20).

**2.5. Analysis of PAHs.** The analysis of PAHs was described elsewhere (16). All extracts of 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 an autosampler to be analyzed by the HPLC system (Agilent 1100) consisting of a quaternary pump, a PAH column (Agilent, C-18, 4.6 mm Ø × 250 mm), a UV detector, a data processor, and a system controller.

The following 16 PAHs were determined: naphthalene (NA), acenaphthene (ACE), acenaphthylene (ACY), fluorene (FL), phenanthrene (PHEN), anthracene (AN), fluoranthene (FLUR), pyrene (PY), benzo[*a*]anthracene (BaA), chrysene (CHRY), benzo[*b*]fluoranthene (BbF), benzo[*k*]fluoranthene (BkF), benzo[*a*]pyrene (BaP), dibenz[*a,h*]anthracene (dBAn), benzo[*ghi*]perylene (BPe), indeno[1,2,3-*cd*]pyrene (IcdP). The detection limits for the 16 PAHs ranged from 3.7 to 28.5 pg on-column, and from 0.16 to 1.27 μg/kg on a sample dry mass basis.

**2.6. Quality Control.** A strict regime of quality control was respected at all times during the experiment. Before the onset of the extraction and analysis program, PAH recovery studies were undertaken to demonstrate the availability of the method.

The tea samples, XAD-2, and filters were spiked with the mixed PAH standard solution, then extracted, purified, and analyzed in the same way as the samples. The six parallel experiments indicated that recoveries for the 16 PAHs were over 70% from the tea samples and over 90% from the XAD-2 and filters (19), and the relative standard deviations (RSDs) of the 16 PAHs were all below 20.0% for the tea samples and below 2.64% for the XAD-2 and filters (19).

Each series of experiments was accompanied by two blank experiments to make sure that there were no PAHs in the reagents.

## 3. RESULTS AND DISCUSSION

**3.1. Changes of PAH Contents in Tea Leaves during the Black Tea Manufacturing Process.** The 16 PAHs that were identified and their contents in the samples from each manufacturing stage are summarized in **Table 1**. It can be seen that the withered leaves and the rolled leaves contained more PAHs than the fresh leaves. This might be because that the withering and rolling processes were performed near the drying house and the leaves could absorb the PAHs from the environment during the manufacturing stages. While the result that the PAH contents in the fermented leaves are less than those in the withered and rolled leaves might be because of volatilization or degradation of the PAHs during the fermentation stage. The smokelike scent of the black tea is obviously heavier than that of the crude black tea, because the turpentine was fired as a fuel to redry the crude black tea. And this might also be the reason the black tea contained more PAHs than the crude black tea.

The 16 PAH contents (ΣPAHs) in the tea leaves sampled before the drying stage, with a range from 132 to 509 μg/kg comparable to that in some reported vegetation leaves (21, 22), are far less than those in the tea leaves sampled after the drying stage. This indicated that the PAHs in the black tea were mostly originated from the drying process. On the basis of the comparison of the PAH contents between the crude black tea and the fermented leaves, and between the black tea and the

Table 1. PAH Concentrations in Tea Leaves and Air<sup>a</sup>

PAH	tea PAH concn ( $\mu\text{g}/\text{kg}$ of dry mass)						air PAH concn ( $\mu\text{g}/\text{m}^3$ )		
	fresh leaves	withered leaves	rolled leaves	fermented leaves	crude black tea	black tea	in-1	in-2	out
NA	1.98 ± 0.39	14.6 ± 2.8	16.3 ± 3.1	11.7 ± 2.2	165 ± 25	231 ± 26	118	133	0.040
ACE	38.6 ± 4.1	56.8 ± 11	36.7 ± 6.9	7.19 ± 1.3	133 ± 10	219 ± 19	25.9	28.6	0.030
ACY	8.18 ± 1.6	174 ± 26	49.5 ± 8.1	26.0 ± 4.9	77.2 ± 11	143 ± 12	8.92	8.18	0.318
FL	9.30 ± 1.2	16.5 ± 3.2	38.6 ± 7.5	9.15 ± 1.0	184 ± 28	428 ± 45	4.06	12.0	0.700
PHEN	10.9 ± 2.1	53.5 ± 10	174 ± 30	20.5 ± 2.8	1120 ± 150	3930 ± 410	33.1	37.8	0.060
AN	0.420 ± 0.08	8.59 ± 1.7	43.7 ± 7.0	8.44 ± 3.6	286 ± 39	869 ± 110	10.6	11.7	ND
FLUR	1.59 ± 0.29	2.14 ± 0.32	9.88 ± 1.5	1.23 ± 0.20	312 ± 60	1640 ± 290	16.3	18.0	0.180
PY	54.1 ± 10	83.4 ± 16	72.7 ± 13	45.1 ± 8.5	388 ± 72	1340 ± 190	22.7	25.8	0.310
BaA	0.490 ± 0.03	0.850 ± 0.15	1.94 ± 0.31	1.54 ± 0.10	37.0 ± 4.4	198 ± 23	3.73	4.08	ND
CHRY	0.450 ± 0.05	1.63 ± 0.32	3.09 ± 0.54	1.63 ± 0.23	32.0 ± 1.6	200 ± 19	3.75	3.97	0.220
BbF	ND	ND	ND	ND	61.3 ± 6.5	100 ± 9.5	4.09	5.39	ND
BkF	ND	8.73 ± 1.7	36.2 ± 5.3	30.4 ± 5.8	107 ± 19	63.8 ± 12	4.12	4.22	0.930
BaP	ND	ND	ND	ND	8.37 ± 1.6	61.0 ± 9.8	3.26	3.76	0.130
dBAn	ND	ND	ND	ND	2.35 ± 0.35	8.42 ± 1.3	0.280	0.320	ND
BPe	4.57 ± 0.91	ND	ND	ND	30.3 ± 5.1	63.7 ± 9.9	2.07	2.13	ND
lcdP	2.06 ± 0.41	13.2 ± 2.6	27.2 ± 4.5	22.8 ± 4.1	28.7 ± 4.9	156 ± 16.7	2.33	2.63	ND
$\Sigma$ PAHs	132 ± 22	434 ± 77	509 ± 88	186 ± 35	2970 ± 431	9650 ± 1200	263	302	2.92

<sup>a</sup> NA = naphthalene, ACE = acenaphthene, ACY = acenaphthylene, FL = fluorene, PHEN = phenanthrene, AN = anthracene, FLUR = fluoranthene, PY = pyrene, BaA = benzo[a]anthracene, CHRY = chrysene, BbF = benzo[b]fluoranthene, BkF = benzo[k]fluoranthene, BaP = benzo[a]pyrene, dBAn = dibenz[a,h]anthracene, BPe = benzo[ghi]perylene, lcdP = indeno[1,2,3-cd]pyrene. ND = not detected. "In-1" and "in-2" are the air samples obtained in the drying house, while "out" is obtained outside the drying house.

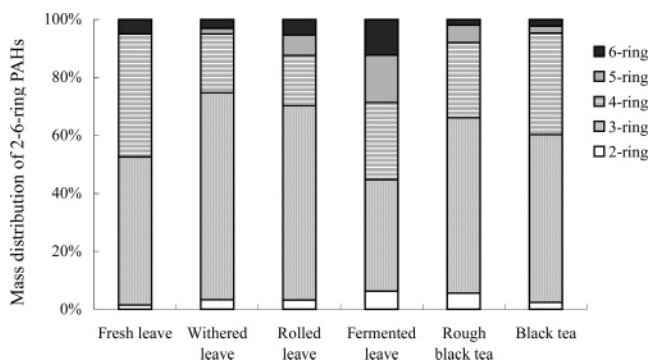


Figure 2. Mass distribution of two- to six-ring PAHs in the tea leaves as a percentage of the total PAHs in the tea leaves at the different stages of manufacture.

fermented leaves, it can be calculated that about 94% and 98% of  $\Sigma$ PAHs in the crude black tea and the black tea came from the drying process, respectively.

Figure 2 shows the mass distribution of two- to six-ring PAHs in the tea leaves as a percentage of the total PAHs in the tea leaves at the different stages of manufacture. Three-ring (ACE, ACY, FL, PHEN, AN) and four-ring (FLUR, PY, BaA, CHRY) PAHs were dominant in all tea samples. But the contribution of the three- to four-ring PAHs to the  $\Sigma$ PAHs decreased gradually from 93.6% in the fresh leaves to 64.9% in the fermented leaves during the four manufacturing steps before the drying stage, and the contribution of the five- to six-ring PAHs to the  $\Sigma$ PAHs increased gradually from 5.02% in the fresh leaves to 28.6% in the fermented leaves. After the drying stage, the contribution of the three- to four-ring PAHs to the  $\Sigma$ PAHs increased to 92.9% and the contribution of the five- to six-ring PAHs decreased to 4.7% in the black tea. This result implied that the volatilization or degradation of the three- to four-ring PAHs in the tea leaves happened more easily than that of the five- to six-ring PAHs during the first four tea manufacturing stages, while the tea leaves absorbed more three- to four-ring PAHs than five- to six-ring PAHs during the drying stage.

**3.2. Source Analysis of PAHs in the Black Tea.** The  $\Sigma$ PAHs in the black tea in this study are higher than those in other tea products previously reported (17, 18). It is known that the

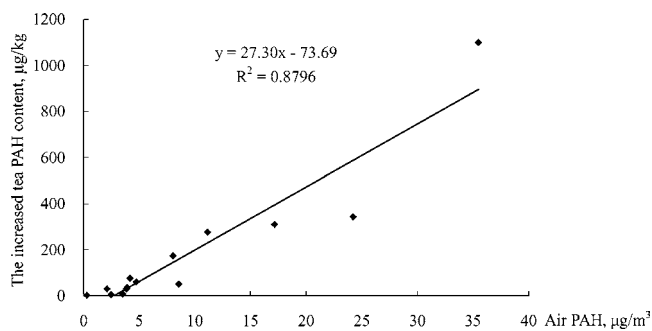


Figure 3. Correlation between the mean air PAH concentration and the increased tea PAH content during the drying stage.

combustion of firewood will release PAHs (23). So when the tea leaves absorb the scent of the smoke, they will also absorb the PAHs. This might be the main reason for the high content of PAHs in this black tea. To confirm this hypothesis, the air PAH contents in the drying house and outside the house were determined. The results are listed in Table 1. It was shown that the mean air  $\Sigma$ PAHs in the drying house is about 100 times higher than that outside the house. This indicated that quantities of PAHs were released into the drying house from the combustion of pine firewood.

The amount of PAHs absorbed by tea leaves is a function of the air concentration and physicochemical properties (e.g., hydrophobicity, vapor pressure) of the compound as well as ambient temperature (13). In this study, the increased PAH (except for NA and ACE) contents in the crude black tea compared to that in the fermented leaves were correlated to the mean concentrations of the air PAHs in the drying house with  $R^2 = 0.8796$  (Figure 3).

Investigations into the source of PAHs have used the molecular ratios of specific hydrocarbons (20, 24). The concentration ratios of FLUR/PY, PHEN/AN, and BaA/CHRY in the crude black tea, black tea, and drying house air, which are listed in Table 2, are similar, but obviously differ from those in the other samples. This implies that the PAHs in the crude black tea, black tea, and drying house air came from the same source.



**Table 2.** Concentration Ratios of Specific PAHs in the Tea Leaves and Drying House Air

sample	fresh leaves	withered leaves	rolled leaves	fermented leaves	crude black tea	black tea	drying house air
FLUR/PY	0.03	0.03	0.14	0.03	0.80	1.22	0.71
PHEN/AN	25.95	6.23	3.98	2.43	3.92	4.52	3.19
BaA/CHRY	0.76	0.52	0.63	0.94	1.16	0.99	1.01

These results confirmed that the PAHs in the black tea mainly came from the combustion of the pine firewood.

**3.3. Conclusion.** The manufacturing process of black tea consists of five steps: withering, rolling, fermentation, drying, and drying and sorting.  $\Sigma$ PAHs in the crude black tea and black tea are much higher than those in the tea leaves sampled before the drying stage. The air  $\Sigma$ PAHs in the drying house were about 100 times higher than those outside the drying house. It is obvious that the combustion of pine firewood during the drying stage released PAHs into the drying house, which were then absorbed by tea leaves, thus resulting in the high PAH contents in the black tea.

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