original article

Determination of polycyclic aromatic hydrocarbons concentration in eight brands of black tea which are used more in Iran

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ABSTRACT

Aims: The objective of this study was investigation of the polycyclic aromatic hydrocarbons (PAHs) concentration in eight brands of black tea which is used more in Iran.

Materials and Methods: In the present study, PAHs content of eight brands of black tea were extracted and analyzed with gas chromatography-mass spectrometry (GC-MS) equipped with Selected Ion Monitoring (SIM) technique.

Results: The average of total PAHs compounds in analyzed teas was in the range of 139 to 2082 μg kg^{-1}. PAHs with 5 to 6 rings were not found in the teas samples. Four rings PAHs compounds composed 46% of the total PAHs compounds and were the most dominant compounds. The same compounds were also dominant in tea bags.

Conclusion: In the tea liquor, after brewing times of 10 and 120 minutes, the maximum and minimum releasing percentage of PAHs was observed, respectively. The result of this study confirmed the presence of PAHs in the tea leaves and tea liquor. Assuming that every person use 10 g of tea leaves every day and 10 minutes take for brewing time, the daily uptake of 16 PAHs will be 1.2 μg.

Key words: Black tea, gas-chromatography, poly aromatic hydrocarbons

INTRODUCTION

After water, tea is the most consumed beverage in the world.[1,2] Tea is consumed by approximately two-thirds of the world's population.[3,4] All types of teas are made from the plant Camellia Sinensis which are grown in tropical and subtropical regions. China, India, Srilanka, Kenya, Indonesia, Vietnam, Turkey and Japan are the greatest producers of tea in the world. Production tonnage of tea in the world was 3.6 million tons in 2007, which 30.6 percent of this amount is produced in China.[4] There are different types of tea like white tea, green, oolong, black and pyorrhea, however about 78% of produced tea in the world is black tea.[3,5] A few data has been published on the health effects of tea in the last years. Tea contains many compounds including polyphenols, caffeine, and tein. Some of tea properties for health are decreasing effect on cholesterol and blood pressure; antibacterial and antioxidant effects; and protection against heart-cardiovascular disease.[4]

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However, certain contaminants may exist in tea leaves which threaten the health of consumers. The major pollutants that are widely present in tea are heavy metals, fluoride and pesticides.[2] PAHs are one group of the pollutants those are found in various foods including dairy products, vegetables, fruits, oils, smoked meat and black tea. Presence of PAHs in the food is caused by processing and cooking of food (smoking, grilling, baking and frying).[6]

PAHs are included over 200 chemical compounds with at least two aromatic rings which are composed of carbon and hydrogen atoms. Most of PAHs compounds in the environment have between two and seven rings.[7] These compounds are derived from both natural and artificial sources.[8-10] PAHs are ubiquitous environmental pollutants which are found in abundance in the environment.[10-13] They are thermodynamically stable due to negative resonance energy, having a high melting and boiling point, low water solubility and low vapor pressure.[14,15] US environmental protection agency selected 16 PAHs as pollutants with high priority due to their high toxicity and carcinogen properties.[12,16-18] PAHs can be transferred in gas form or combined with other particles and may accumulate on the plants. Therefore, PAHs are considered as potentially toxic compounds to human health.[2] Exposure to these compounds may result into various cancers such as kidney, bowel, pancreas, and skin.[15] Several studies have been conducted on the PAHs concentration in black tea.[2,19] Lin *et al*. (2005) showed that total values of 16 PAHs in samples of black, pyorrhea, oolong and green teas were 8800, 685, 769 and 323 μg kg^{-1}, respectively.[2] Schlemitx and Pfannhauser (1997) reported that the amounts of total 16 compounds of PAHs in black and green teas were 776 and 549 μg kg^{-1}, respectively.[19] The aim of this study was to investigate the concentrations of 16 types of PAHs in eight brands of black tea which are used more in Iran.

MATERIALS AND METHODS

Standards and reagents

All chemicals including PAHs standard solution (containing 16 types PAHs), acetonitrile, anhydrous disodium sulfate, silica gel, hexane and dichloromethane were of analytical grade and provided from Merck Co (Germany).

Tea samples

The 8 brands of black tea were purchased from tea stores in Isfahan (Iran) in 2011. Seven brands of the tea are produced and processed outside of Iran and one of them is produced inside the country. The experiments were conducted in duplicates and the average values were considered.

PAHs extraction from tea leaves

Tea sample (10 g) was mixed with 20 ml dichloromethane under ultrasonication in 30°C for 30 min (this process is repeated three times), the extract was then filtered and collected. The obtained extracts were mixed and evaporated using a rotary evaporator equipped with water bath to dryness. The dried residual was then dissolved in 2 mL of hexane. This solution was filtered through silicagel column, washed with 10 mL hexane/dichloromethane (1:1 v/v), evaporated by rotary evaporator and the residuals were dissolved in 2 mL acetonitrile HPLC-grade.

PAHs extraction from tea liquor

To extract the PAHs from tea liquor, 20 g of tea sample was placed into the funnel Erlenmeyer with 500 mL ultra pure water and the sample was boiled. Then, it was located into a water bath with temperature of 90-92°C for the period of 10, 30, 60, 120 minutes, respectively. Liquid phase was filtered and poured into another funnel Erlenmeyer and cooled to 30°C. The liquid was extracted with 50 mL of dichloromethane for 30 min through ultrasonication in 30°C. The dichloromethane which placed under water phase was separated and transferred to another Erlenmeyer (These steps were repeated three times). All the extracts obtained were mixed with each other and filtered through anhydrous Na_2SO_4 column following by 10 mL mixture of hexane/dichloromethane (1:1 v/v). The solvents achieved from the previous steps were evaporated and dried by a rotary evaporator. The dried residuals were dissolved in 2 mL of hexane, passed through a silicagel column and washed with hexane/dichloromethane (1:1 v/v). The obtained solution was again dried using a rotary evaporator and the residuals were dissolved in 2 mL of HPLC-grade acetonitrile.

Sample analysis

Samples analysis was conducted on the basis of EPA-8270 using an Agilent gas chromatography- mass spectrometry (GC-MS, 6890 N) with the following details; the instrument was equipped with a mass detector (MS) including Selecting Ion Monitoring (SIM) mode. The injection port was held at 320°C and used in split less mode. Sample volume was 3 μL and the carrier gas was helium at flow rate of 1.0 mL min^{-1}. The column was a 30 m × 0.2 mm capillary with a 1 μm stationary phase thickness.

Quality control method

The experiments quality control was carried out based on Lin *et al*. method.[2] To confirm the extraction method, the PAHs standard solution (containing 16 PAHs compounds) was spiked into 10 and 20 g of tea. The extraction method and analysis of spiked samples were similar to the real samples as explained above. However, 30 minutes was considered for brewing time to calculate the percentage of extraction recovery. Percentage of extraction recovery in leaves and liquid tea were 25 and 21% for naphthalene, 93.6 and 45.2% for three rings cyclic compounds, 39.9-66.0 and 35- 61% for four to six ring cyclic compounds, respectively.

RESULTS

Table 1 shows the physical and chemical properties of 16 PAHs which are investigated in this study. The results are presented in Tables 2 and 3 and Figures 1 and 2. Table 2 and Figure 1 indicate the obtained results for 16 PAHs in the tea leaves. As seen in Table 2, two PAHs with most concentrations in all of analyzed brands of the tea leaves were phenanthrene (182 μg kg^{-1}) and naphthalene (172 μg kg^{-1}), respectively. The 5 and 6 rings PAHs including Benzo[b] fluoranthene (Bbf), Benzo[k] fluoranthene (Bkf), Benzo[a] pyrene (BaP), Dibenzo[ah] anthracene (dBAn), Indeno[123-cd] pyrene (IcdP) and Benzo[ghi] perylene (Bpe) were not found in all tea samples [Tables 2 and 3]. Figure 1 shows that the most PAHs produced in tea leaves have 2-4 rings. Figure 2 also presents that 3-4 rings PAHs are the most conventional compounds that released in the tea liquor.

DISCUSSION

The average concentrations of 16 PAHs in 8 brands of tested teas were in the range of 139 to 2082μg kg^{-1} [Table 2]. As seen in Table 2, the maximum level of PAHs was found in the tea brand 5 (2082 μg kg^{-1}) and after that in the tea brand 8 (tea bag: 1192 μg kg^{-1}). The minimum concentration of PAHs was also assigned to brand 2 of tea (139 μg kg^{-1}). Among 8 tested samples, 2-4 rings PAHs composed 23, 31 and 46% of overall PAHs [Table 2 and Figure 1] There were not any kinds of 5 or 6 rings PAHs compounds in both of the samples (tea leaves and tea liquor). Lin *et al.* (2005) reported that the maximum concentrations of 16 types of PAHs in black tea was 8800 μg kg^{-1}, that 161.63 μg kg^{-1} of this contents were PAHs with 5 or 6 rings.[2] In another study by Singh and Vishal (2011) which was conducted on five brands of tea, the amount of 26 PAHs compounds was found between 101.9-399.9 μg kg^{-1} which 6.9-75 μg kg^{-1} were related to

Table 1: Physical and chemical properties of 16 types of PAHs in the present study[2,20]

PAHs compounds	Abbreviations	Number of benzene rings	Solubility in water (mg L^{-1})	Octanol-water partition coefficient (log K_{ow})	Boiling point (°C)
Naphthalene	NA	2	32	3.36	217.9
Acenaphthylene	ACY	3	3.93	4.08	280
Acenaphthene	ACE	3	3.4	4.32	297
Fluorene	FL	3	1.9	4.18	295
Phenanthrene	PHEN	3	1-1.3	4.46	340
Anthracene	AN	3	0.05-0.07	4.45	339.9
Fluoranthene	FLUR	4	0.26	5.53	384
Pyrene	PY	4	0.14	5.3	404
Benzo[a] anthracene	BaA	4	0.01	5.6	437.6
Chrysene	CHRY	4	0.002	5.6	448
Benzo[b] fluoranthene	BbF	5	0.0014	6.6	No data
Benzo[k] fluoranthene	BkF	5	0.0008	6.85	480
Benzo[a] pyrene	BaP	5	0.0038	6	311
Dibenzo[ah] anthracene	dBAn	5	0.0005	6	524
Indeno[123-cd] pyrene	IcdP	6	0.00053	7.7	536
Benzo[ghi] perylene	BPe	6	0.00026	7	>500

PAHs: Polycyclic aromatic hydrocarbons

Table 2: Concentration of poly aromatic hydrocarbons in 8 brands of the tea leaves (μg kg^{-1})

PAHs compounds	Different brands of investigated tea in this study								Average
	1	2	3	4	5	6	7	8	
NA	99.20	81.60	178.40	202.16	32	168.00	112.00	217.60	172.37
ACY	6.19	4.87	11.95	5.57	13.98	6.64	8.91	7.57	8.21
ACE	ND	ND	ND	ND	ND	ND	ND	ND	ND
FL	9.83	13.23	49.07	14.16	31.15	7.94	37.04	96.14	32.32
PHEN	146.72	19.30	146.72	43.49	551.38	ND	192.07	356.10	181.97
AN	3.74	9.98	6.55	3.12	34.07	9.43	6.43	13.90	10.90
FLUR	171.50	4.93	171.50	8.10	409.98	8.04	95.04	175.74	130.60
PY	155.58	4.87	155.58	8.28	379.90	7.59	98.28	162.15	121.53
BaA	45.38	ND	45.38	ND	222.66	ND	37.49	93.92	55.60
CHRY	55.27	ND	55.27	ND	119.05	ND	39.90	68.96	42.30
BbF	ND	ND	ND	ND	ND	ND	ND	ND	ND
BkF	ND	ND	ND	ND	ND	ND	ND	ND	ND
BaP	ND	ND	ND	ND	ND	ND	ND	ND	ND
dBAn	ND	ND	ND	ND	ND	ND	ND	ND	ND
IcdP	ND	ND	ND	ND	ND	ND	ND	ND	ND
BPe	ND	ND	ND	ND	ND	ND	ND	ND	ND
∑PAHs	693.41	138.79	820.41	284.87	2082.18	207.63	627.15	1192.07	755.81

PAHs: Polycyclic aromatic hydrocarbons, ND: Non detectable

Table 3: Concentration of PAHs in tea liquor and release percentage of the 16 compounds in various times

PAHs compounds	Concentration of PAHs in liquid tea (µg mL^{-1}) in various times (min)				Release percentage of PAHs			
	10	30	60	120	10	30	60	120
NA	ND	ND	ND	ND	-	-	-	-
ACY	0.03	0.05	0.07	ND	45.21	60.81	93.52	-
ACE	ND	ND	ND	ND	-	-	-	-
FL	0.86	0.13	0.13	ND	89.80	13.44	13.00	-
PHEN	2.64	0.87	1.11	0.16	74.05	24.38	31.11	4.61
AN	ND	ND	0.05	ND	-	-	32.49	-
FLUR	0.23	0.29	0.28	0.06	12.96	16.66	15.72	3.41
PY	0.21	0.28	0.21	0.06	13.15	17.48	13.26	3.84
BaA	ND	0.05	0.03	ND	-	5.46	3.64	-
CHRY	ND	0.06	0.04	ND	-	8.18	5.45	-
BbF	ND	ND	ND	ND	-	-	-	-
BkF	ND	ND	ND	ND	-	-	-	-
BaP	ND	ND	ND	ND	-	-	-	-
dBAn	ND	ND	ND	ND	-	-	-	-
IcdP	ND	ND	ND	ND	-	-	-	-
BPe	ND	ND	ND	ND	-	-	-	-
∑PAHs	4.0	1.7	1.9	0.29	16.45	11.62	13.83	0.74

Release has not achieved, ND: Non detectable, PAHs: Polycyclic aromatic hydrocarbons

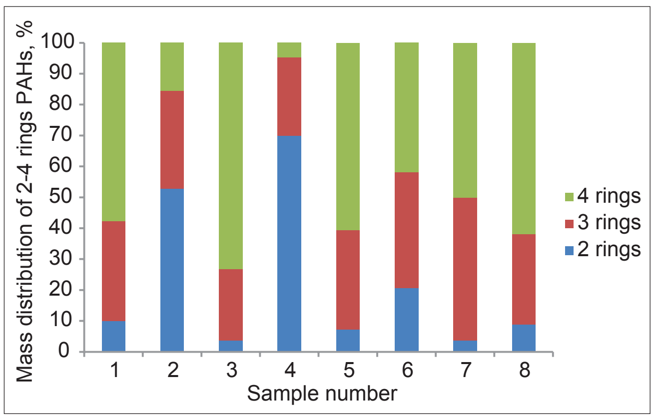

Figure 1: Mass distribution of 2-4 rings PAHs in 8 kinds of black tea

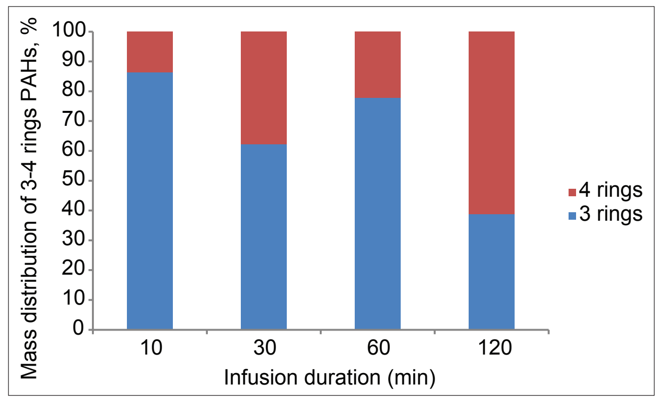

Figure 2: Mass distribution of 3-4 rings PAHs in tea liquor after various times

5-6 rings PAHs compounds.[21] In order to assess the release of PAHs compounds in the tea liquor, tea bag (No. 8) has been studied and analyzed. Unlike the study conducted by Lin *et al.*,[2] PAHs compounds with 2 and 4 rings has not been found in all the brewing times [Table 3]. It is evident that all the released PAHs compounds in infusion included 2 and 3 rings of PAHs. As can be seen in Table 3 and Figure 2, the maximum and minimum released amounts of PAHs in infusion were occurred within 10 minutes and 120 minutes of brewing, respectively. However, Lin *et al.* (2005) reported that maximum and minimum percentages of released PAHs were happened in the brewing times of 30 and 60 minutes, respectively.[2] In all the brewing times, except for the time of 120 minutes, the release rate of 3 rings PAHs compounds was more than of 4 rings PAHs, which it can be due to the octanol-water partition coefficient (log k_{ow}). As can be observed in Table 1, by increasing the number of rings of these compounds, their log k_{ow} also increase. Daily uptake of 16 PAHs per capita is nearly equals to1.2 µg (in average) while 10 g of tea leaves be used per day and the brewing time of tea to be10 minutes.

CONCLUSION

This study was conducted to investigate the 16 types of PAHs in eight brands of black tea which are used more in Iran. The amount of 16 types of PAHs in all brands of tea was between 139 to 2082 µg kg^{-1}. Among 8 tested samples, 2-4 rings PAHs composed 23, 31, and 46% of overall PAHs and the 5 to 6 rings PAHs compounds were not found in all tested tea samples (tea liquor and tea leaves). In the tea liquor, the brewing of 10 and 120 minutes has released the maximum and minimum percentage of PAHs compounds, respectively.

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