Priority PAHs in Some Coffee Brands

A Thesis Submitted

In partial fulfilment for the award of the

Degree of

Master of Science in Chemistry

Submitted by
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CERTIFICATE

This is to certify that the thesis entitled **"Priority PAHs in different coffee brands"** being submitted by Rashmi in partial fulfilment of the requirements for the award of the degree of Master of Science in Chemistry and submitted to Thapar University, Patiala is a bonafied work carried out under my supervision and that no part of this thesis has been submitted for the award of any other degree.

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CANDIDATE'S DECLARATION

I do hereby declare that the work being presented in the thesis entitled "**Priority PAHs in different coffee brands"** in partial fulfilment of the requirements for the award of the degree of **MASTER OF SCIENCE IN CHEMISTRY** and submitted to School of Chemistry and Biochemistry, Thapar University, Patiala is an authentic record of my own work which I completed during a period of six months from January 2012 to June 2012 under the supervision of **Dr. Satnam Singh,** ***Associate Professor, School of Chemistry and Biochemistry, Thapar University, Patiala.*** To the best of my knowledge the matter being presented has not been presented elsewhere for the award of any other degree or diploma.

Place: Patiala

Rashmi

RASHMI

Date: 16 July, 2012

This is to certify that the above statement made by the candidate is correct and true to the best of my knowledge.

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All these thanks are, however, only fraction of what is due to almighty for granting me an opportunity and strength to successfully accomplish this assignment.

Rashmi

RASHMI

Place: Patiala

Date: 16 July, 2012

My Parents and Guide

God, my guide, my parents, my brother Rahul Sharma who taught me the basics of life.

Thank you all for being with me in all odd and pleasing times.

ABSTRACT

Sixteen Polycyclic Aromatic Hydrocarbons described as priority pollutants by United State Environmental Protection Agency (USEPA) are of special concern due to their carcinogenic and mutagenic properties. Owing to these properties their determination in most drinkable beverage deserves further investigation. In this respect, four coffee samples viz., Nescafe Classic, Nescafe Premium, Bru & Suncafe and Coffee Beans (roasted) were studied. Soxhlet extraction was used for the extraction of PAHs. Three to thirteen PAHs from list of 16 PAHs (USEPA) were found to be present. Concentrations of total PAHs in different brands of coffee samples varied from 831.7-1589.7 μg/kg. Benzo[a]pyrene (2A: probable human carcinogen) was found in Nescafe Premium while naphthalene (2B: possible human carcinogen) was found in all coffee samples except in roasted coffee beans with their average amount ranging from 18.3 μg/kg and 36.1-194.4 μg/kg, respectively.

1. INTRODUCTION

1.1 PAHs SOURCES

PAHs are two to eight-ring fused ring system without any heteroatoms and formed by incomplete combustion of organic materials during industrial and other human activities, such as processing of coal and crude oil, combustion of natural gas, including heating, vehicle traffic, cooking and tobacco smoking, as well as in natural processes such as carbonization [1-6]. In any incomplete organic combustion, PAHs formation and emission mechanisms are classified into two processes viz., pyrolysis [1] and pyrosynthesis [2-6]. Organic compounds are partially cracked to smaller and unstable fragments upon heating (pyrolysis). These fragments are mainly reactive free radicals which might leads to more stable PAHs formation through recombination reactions (pyrosynthesis). Formation and sequential growth of PAHs take place by reactions with stable and radical species, lower molecular weight PAHs and acetylene, followed by the nucleation or inception of small soot particles, soot growth by coagulation and mass addition from gas phase species, and carbonization of the particulate material. Additionally, number of heterocyclic aromatic compounds (e.g. carbazole and acridine) as well as Nitro-PAHs could also be generated by incomplete combustion. However, the present contributions are from the different important sources, such as residential heating (coal, wood, and oil), vehicle exhausts, industrial power generation, incinerators, production of coal tar, coke, petroleum catalytic cracking etc. [7]. These sources may also vary considerably from one place to another. Beside this, stationary sources may also contribute to total percentage of PAHs emissions. The stationary category comprises a wide variety of combustion processes including residential heating, industrial activities (e.g. aluminium production, coke manufacture etc.), incineration of waste and power generation which result in high concentrations of atmospheric PAHs in the vicinity of major sources. However, in urban or suburban areas, the mobile sources such as vehicular petrol and diesel engines are additionally the major contributors to PAHs release in atmosphere.

1.2 Toxicity of PAHs and its exposure to humans

Emissions of PAHs by incomplete combustion of organic mass are of special environmental concern because of their possibility of interacting with biological nucleophiles, such as proteins, inhibitors for the regular metabolic functions of the cells thus possess carcinogenic nature [8, 9]. The chronic exposure to carcinogenic PAHs concomitant with exposures to multiple chemicals or biological agents in indoor-air (respireable suspended particulate matter) could be an attributable risk factor, acute/chronic pulmonary illnesses, asthma, pulmonary tuberculosis and lung cancer [10]. The differences in carcinogenic activity of many of these compounds are related to the structure of compound [11]. Out of number of PAHs, United State Environmental Protection Agency (USEPA), the Joint Food and Agricultural Organization / World Health Organization, Expert Committee on Food Additives (JECFA) and Scientific Committee on Food (SCF) have classified priority compounds in food, which are found to be abundant and toxic in nature viz., benzo[a]anthracene (BaA), benzo[b]fluoranthene (BbF), indeno[1,2,3-*cd*]pyrene (IND), benzo[*k*]fluoranthene (BkF), benzo[*a*]pyrene (BaP), dibenzo-[*a,h*]anthracene (DBA), naphthalene (Nap), acenaphthene (Acp), acenaphthylene (AcPy), fluorene (Flu), phenanthrene (PA), anthracene (Ant), fluoranthene (FL), pyrene (Pyr), chrysene (CHR) and benzo (g,h,i)perylene (BghiP). BaP, DBA, and BaA have been classified [12] as probable human carcinogens (2A) while BbF, BkF, IND and Nap have been classified as possible human carcinogens (2B) by International Agency on research on cancer (IARC).

Since, some PAHs are carcinogenic as well as mutagenic so their identification and minimization are imperative in the combustion process [13]. These PAHs may be transformed to even more toxic compounds by chemical reactions such as sulfonation, nitration or photo oxidation. Traces of nitric acid are known to transform some PAHs into nitro-PAHs [14]. Organic compounds released from their sources in gas phase or can be associated with particles by nucleation and condensation, forming particulate matter. The particulate form of PAHs are initially in the gaseous phase at high combustion temperature, however when the temperature decreases, gaseous phase PAHs adsorb or deposit on fly ash particles / vegetation thus results in PAHs contaminated food [15]. Traces of PAHs have been found in oils, vegetables, tea and coffee intake of carcinogenic PAHs through foods in Italy was higher (~1.4 µg/day) than estimated (0.13 ng/day) intake through respiration [16].

In total, results from PAHs analyses in 33 food categories/subcategories were evaluated [17] and 30% of the samples found to be contaminated with 16 priority PAHs, however BaP has not been detected. Beside this, individual compounds were grouped and summed in order to check whether their sums would better reflect the occurrence of carcinogenic PAHs in different food categories. The selection of the individual PAHs was based on the frequency of their results above the limit of detection (LOD).

Besides this, the sum of four PAHs (PAH4) viz., BaP, CHR, BaA and BbF, as well as the sum of two PAHs (PAH2) viz., BaP and CHR were calculated. The correlation between PAH2 and PAH4 was 0.92. The samples which were found to be negative for PAH2, contains at least one PAH (other than 16 priority PAHs) having concentrations 26% above the LOD. The frequency varied between 2% and 9% for the individual PAHs. Of samples negative for PAH4, 14% and 6% identified concentrations above the LOD for at least one other PAH for samples tested for all 16 PAHs, respectively. The frequency varied between 1% and 6% for the individual PAHs or PAH combinations. Overall, it was concluded that PAH4 was better indicators of the occurrence of PAHs than PAH2 [17].

Coffee is one of the most drinkable beverages used worldwide [18]. Scientific evidence has been indicating that coffee consumption might have health promoting effects, including improvement of digestion and sense of sensation, antioxidation, as stimulant, reduction of chronic diseases, effective against diabetes mellitus, as better glucose tolerance etc. [19-32]. However, it has also been demonstrated that certain contaminants such as of pesticides, parrafins, mycotoxins, nitrosamines, heterocyclic amines and polycyclic aromatic hydrocarbons might impose a health threat on their drinkers [33, 34]. Roasting is a crucial step for the production of coffee, as it enables the development of color, aroma, and flavor, which are essential for the characterization of the coffee quality. At the same time, roasting may lead to the formation of non-desirable compounds, such as polycyclic aromatic hydrocarbons (PAHs) which are very toxic and impose threat on human health as listed by USEPA (Table 1.1).

Table 1.1: Sixteen priority PAHs listed by USEPA and IARC

S. No	Name	Classification*	
		** PP (US EPA, 1997)	(IARC, 1987, 2002)
1	Acp	√	--
2	AcPy	√	--
3	Ant	√	--
4	BghiP	√	--
5	BaA	√	2A
6	BaP	√	2A
7	BbF	√	2B
8	BkF	√	2B
9	CHR	√	--
10	DBA	√	2A
11	FL	√	--
12	Flu	√	--
13	IND	√	2B
14	Nap	√	2B
15	PA	√	--
16	Pyr	√	--

*2A= Probable Human carcinogenic
*2B= Possible Human Carcinogenic
** Priority Pollutant

Table 1.2: Abbreviation of 16 PAHs as priority Pollutants (USEPA, 1997)

S.No.	PAHs	Abbreviation
1	Acenaphthene	Acp
2	Acenaphthylene	AcPy
3	Anthracene	Ant
4	Benzo[g,h,i]perylene	BghiP
5	Benzo[a]anthracene	BaA
6	Benzo[a]pyrene	BaP
7	Benzo[b]fluoranthene	BbF
8	Benzo[k]fluoranthene	BkF
9	Chrysene	CHR
10	Dibenzo[a,h]anthracene	DBA
11	Fluoranthene	FL
12	Fluorene	Flu
13	Indeno[1,2,3]pyrene	IND
14	Naphthalene	Nap
15	Phenanthrene	PA
16	Pyrene	Pyr

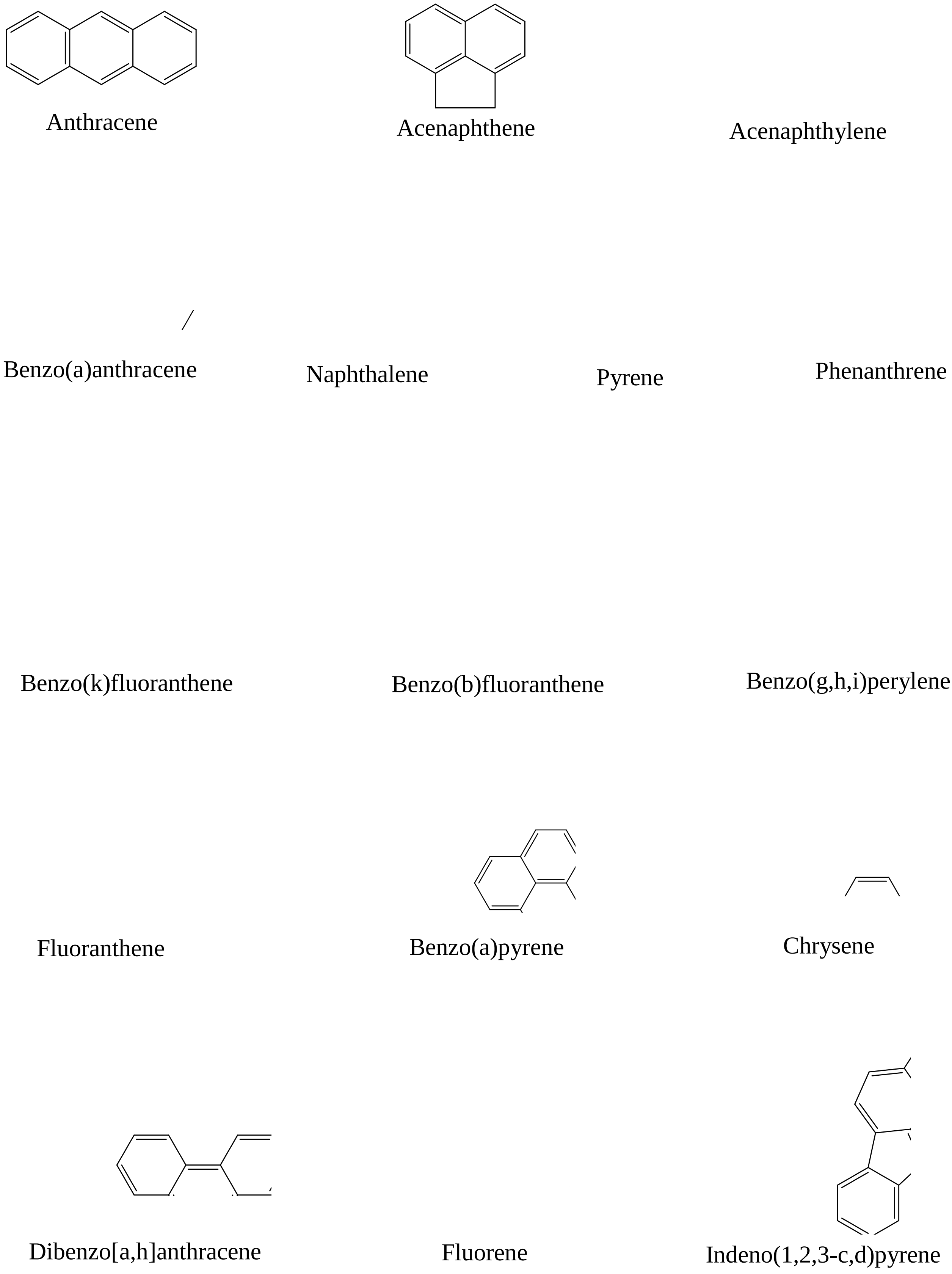

Fig.1: Structural formulas of 16 PAHs as priority pollutants (USEPA)

2. LITERATURE REVIEW

Potentially toxic 16 priority PAHs has been determined in food stuff such as edible oils [35-37], vegetables [38-43], smoked meat [44-50], tea [51,52], coffee [53-60] etc. Edible oils found to be contaminated with different levels of PAHs. Studies conducted by Hossian et al. [35], Purcaro et al. [36] and Formberg et al. [37] showed that the contamination level in oils with PAHs is in a range from 0.01-0.61 μg/kg , 0.2-1.4 μg/kg and 0.2-0.8 μg/kg, respectively. Beside this, vegetables have also been found to be contaminated with PAHs. Studies conducted by Zohair [38] and Monica [39] clearly reveals that vegetables form Egyption and Brazilan markets, respectively are contaminated with PAHs ranging from 1.22 to 12.63 ppb and 3.77 to 13.57 μg/kg, respectively. In addition to this, studies conducted by Shen, Tuteja, Waqur and Tao et al. also revealed the contamination of vegetables i.e. tomato, spinach, potato, carrot and spinach by different of levels of PAHs ranging form 7.1 - 231.2 ng/g, 22.89 - 85.55 μg/kg , 2.1 - 8.35 μg/kg and 2.25 and 7.82 μg/g respectively [40-43].

Since, it is reported that PAHs are formed by incomplete combustion of organic mass so their determination in meat products has been carried out by various techniques such as GC-MS, GC-HRMS [44, 45] etc. e.g. results obtained by Mottier showed the average values of PAHs ranging from 0.5-1.0 μg/kg [46]. Jira et al. [47] has reported PAHs presence of benzo[a]pyrene (2A: most probable carcinogenic) in meat sugasse (0.43 μg/kg) whereas the sum content of benzo[a]pyrene, benzo[a]anthracene, chrysene and benzo[b]fluoranthene was 0.28 μg/kg. Jasna et al. [48] studied PAHs content in different types of smoked meat products by using GC-HRMS method and found that none of the sample had amount more than acceptable limit (5 μg/kg) for benzo[*a*]pyrene. However, a report by Reinik [49] revels that 3.4% of total samples were contaminated by BaP, more than its acceptable limit and the total PAHs concentrations detected were 16 $\mu g\ kg^{-1}$ in smoked meat, 19 $\mu g\ kg^{-1}$ in smoked sausage and 6.5 $\mu g\ kg^{-1}$ in smoked chicken samples. Farhadian et al. [50] analyzed nine types of grilled meat PAHs, i.e. fluoranthene, benzo[b]fluoranthene and benzo[a]pyrene using HPLC with fluorescence detector. The differences in PAH concentrations among (charcoal, gas and oven grilling) were found to be significant, ranging from 3.51-106 ng/g. Fluoranthene was found in all samples; the highest concentration of total PAHs was 132 ng/g found in beef satay and the lowest was 3.51 ng/g in oven grilled chicken.

Contamination of PAHs in tea was found to be between 3 and 20 µg/kg for 3 Russian mixtures, 5 brands of Chinese tea, 1 Russian Smoke tea, 1 Chinese smoke tea, 497 - 1,162 µg/kg for two green teas and two brick teas. 13-7,536 µg/kg for 11 different brands, 48 - 1,703 µg/kg for 34 different brands, 9,650±1,200 µg/kg for black tea samples of the same producing area and 323-8,800 µg/kg for 8 brands of Chinese tea [51]. Additionally, PAHs in 5 Indian black tea brands has also been determined and found to be 101.9 to 464.0 µg/kg [52]. The PAHs content in tea infusion was reported to highlight that a maximum of 11% of the PAHs present in the tea leaves are transferred to infusion [53]. Different kinds of tea products have different PAHs content. Thereby indicating that the manufacturing process of tea leaves might be the main source of PAHs in the tea product with high PAHs contents. It was also pointed out that little information is available on the changes of PAHs and other organic contaminant contents in tea leaves during the tea manufacturing process.

Coffee is one of the most commonly consumed beverages worldwide [18]. Over 90% of coffee production takes place in developing countries, while consumption happens mainly in the industrialized economies [54]. During the coffee production, roasting is a crucial step, as it enables the development of color, aroma, and flavor, which are essential for the characterization of its quality. At the same time, roasting may lead to the formation of not desirable compounds, such as polycyclic aromatic hydrocarbons (PAHs). Justin et al. [55] studied the PAHs content in roasted coffee beans and the effect of roasting temperature on the PAHs. It has been found that at temperature > 220^{o}C formation of phenanthrene, anthracene, and benzo[a]anthracene occurs, however at temperature > 260^{o}C pyrene and chrysene were formed. Beside this, traces of benzo[g,h,i]perylene were also determined indicating transformation of low molecular PAHs to high molecular PAHs. Additionally, reports by Justin et al. [56, 57] indicates the presence of 3 and 11 PAHs and their amount ranging from 0.16-0.87 μg Kg^{-1} and 0-100 ng L^{-1} for grounded coffee and coffee brew, respectively. Furthermore, presence of 7 and 8 PAHs in different coffee brands and coffee brew has been determined by GC-MS and GC-ECD by Santino [58] and Gabriela et al. [59], and total PAHs amount was reported to be 0.52-1.8 μg/l and 0.001 μg/Kg - 90.732 μg/Kg, respectively. Beside this, HPLC has also been used for the determination of PAHs in coffee brew and grounded coffee [60, 61]. A report by Sayadi et al. [60] describes a HPLC method with fluorimetric detection for PAHs determination in coffee brew and its quantity was determined to be 1.65-2.87 ng L^{-1}. However, Bishnoi et al. [61], who have also used the HPLC for the same, reports the PAHs content 16.47-18.24 μg/L in different coffee brands, collected from Mumbai city (India). However, there is a need to study PAHs by Gas Chromatography with Flame Ionization Detector (FID) for commonly available coffee brands in Indian market. The present study comprises identification and quantification of 16 priority PAHs in different grounded coffee samples available in local market.

OBJECTIVE

1. To study 16 Polycyclic Aromatic Hydrocarbons (priority pollutants) in coffee samples.

3. MATERIALS AND METHODS

3.1 Reagents and Chemicals

Different brands of coffee viz., Nescafe Classic, Nescafe Premium, Bru and Suncafe were purchased from local market of Patiala, Punjab (India). However, roasted coffee beans were collected from Coffee Board of India, New Delhi (India). Dichloromethane, acetone, sodium sulphate (anhydrous) and hexane were obtained from SD fine chemicals. Silica gel (mesh size 60-120) and acetonitrile (HPLC grade) was purchased from Loba Chemicals Pvt. Ltd. Standard 16 PAHs solution for Gas chromatography (GC) was obtained from Sigma Aldrich. All chemical were used without further purification.

3.2 Sample preparation for different brands of coffee

In a typical sample preparation, 50 g of grounded coffee was extracted with mixture of dichloromethane (DCM) and acetone (1:1 *v*/*v*) using soxhlet apparatus. The extract thus obtained was passed through anhydrous sodium sulphate and then evaporated to dryness. Residue thus acquired was dissolved in 2 ml of hexane. The contents were passed through silica gel column and eluted with hexane and DCM (1:1 *v*/*v*), evaporated to dryness. The residue was dissolved in 1ml of acetonitrile and kept in refrigerator till analysis by GC.

3.3 PAHs analysis GC – FID parameters

The identification and quantification of PAHs was carried out by using a gas chromatography (NUCON-5765) with ZB-5 MS capillary column (30 m X 0.25 mm, 0.25 μm film thickness) and flame ionization detector. The column oven temperature was held isothermally at 60^oC for 5 min, temperature programmed at 6^oC /min to 320^oC and then to 321^oC @1^oC/min. The injection port was maintained at 250^oC, and the detector was maintained at 275^oC. All injections were done using 1μl of injection volume with split ratio of 1:5 having nitrogen as a carrier gas at a constant flow rate of 1mL/min. PAHs in samples were determined on the basis of comparison of retention times with standard solution and quantified by response factor method [62, 63].

4. RESULTS AND DISCUSSION

Five coffee samples viz. Nescafe Classic, Nescafe Premium, Bru, Suncafe and Roasted Coffee Beans have been analyzed for PAHs content by gas chromatograph. The Gas chromatogram of 16 PAHs (Sigma-Aldrich) under optimized conditions is given in Fig. 4.1 and their corresponding retention times are given in Table 4.1. PAHs were quantified by response factor method (Equ. 1 & 2) and results obtained are given in Tables 4.2 to 4.6.

$$\text{Response factor} = \frac{\text{Peak area of standard analyte}}{\text{Concentration of analyte amount}} \tag{1}$$

$$\text{Amount of analyte} = \frac{\text{Peak area of analyte}}{\text{Response factor}} \tag{2}$$

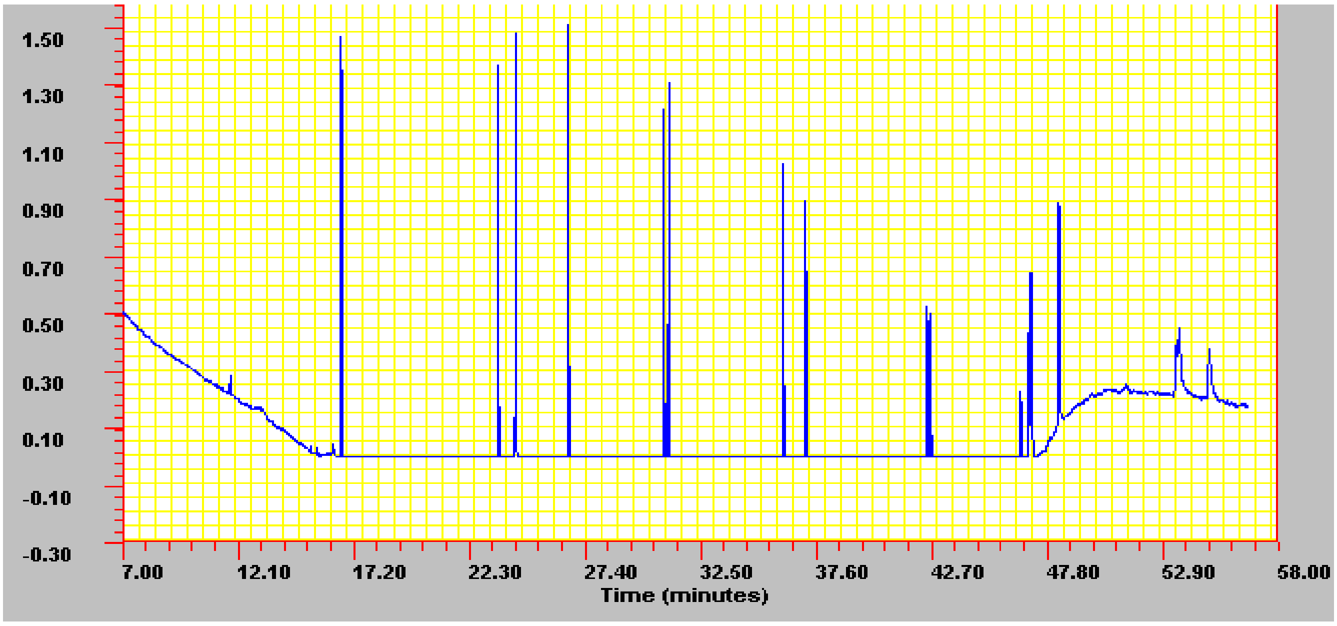

Fig. 4.1: Gas Chromatogram of standard solution of 16 PAHs (Sigma-Aldrich)

Table 4.1: Retention time of 16 PAHs

S.No.	Polycyclic Aromatic Hydrocarbons (PAHs)	Retention Time(min)
1	Naphthalene (Nap)	16.6
2	Acenaphthene (Acp)	23.3
3	Acenaphthylene (AcPy)	24.2
4	Fluorene (Flu)	26.4
5	Phenanthrene (PA)	30.5
6	Anthracene (Ant)	31.0
7	Fluoranthene (FL)	36.1
8	Pyrene (Pyr)	37.0
9	Benzo[a]anthracene (BaA)	42.3
10	Chrysene (CHR)	42.4
11	Benzo[b]fluoranthene (BbF)	46.4
12	Benzo[k]fluoranthene (BkF)	47.0
13	Benzo[a]pyrene (BaP)	47.0
14	Indeno[1,2,3]pyrene (IND)	53.3
15	Dibenzo[a,h]anthracene (DBA)	53.4
16	Benzo[g,h,i]perylene (BghiP)	54.6

Four brands of grounded coffee viz., Nescafe Classic, Nescafe Premium, Bru & Suncafe and Roasted Coffee Beans were studied for the presence of 16 priority PAHs (table 4.2-4.6). Nescafe Classic coffee was found to possess 8 PAHs viz., Nap, Acp, AcPy, Flu, PA, Ant, FL and Pyr. The sum total concentration of these PAHs was 1589.7 µg/Kg. Nap (possible human carcinogenic: 2B) was determined and found to be 121.0 µg/Kg. However, other possible human carcinogenic: 2B viz., BkF, BbF and IND were not detected. Beside this, 8 PAHs including three probable human carcinogenic: 2A viz., BaA, BaP and DBA were remained undetected in Nescafe Classic coffee sample.

Nescafe Premium showed the presence of 13 PAHs and their total concentration was found to be 1117.8 µg/Kg (table 4.3). Two PAHs viz., DBA and BghiP (six-member ring) were not detected though four possible human carcinogenic: 2B PAHs namely Nap, BbF, BkF and IND were determined and found to be 36.1, 17.7, 39.7 and 243.0 µg/Kg, respectively. Beside this, 2 PAHs viz., BaA and BaP (out of three probable human carcinogen: 2A) were determined and found to be 106.6 and 18.3 µg/kg, respectively.

Table 4.2: Concentration of 16 PAHs in sample of Nescafe Classic Coffee

S.No	PAHs	Retention Time (min)	Concentration	
			µg/ml	µg/Kg
1	Nap	16.6	6±2.1	121.1
2	Acp	23.3	12.2 ±4.4	206.8
3	AcPy	24.2	2.6±1.8	92.0
4	Flu	26.4	7.2±4.8	143.2
5	PA	30.5	2.8±1.0	60.0
6	Ant	31.0	3.9±2.9	84.4
7	FL	36.1	32.8±3.5	648.0
8	Pyr	37.0	12.7±6.5	234.2
9	BaA	--	--	--
10	CHR	--	--	--
11	BbF	--	--	--
12	BkF	--	--	--
13	BaP	--	--	--
14	IND	--	--	--
15	DBA	--	--	--
16	BghiP	--	--	--
	Total			1589.7

-- not detected

Bru coffee sample was also found to be contain 9 PAHs viz., Nap, AcP, AcPy, Flu, PA, Ant, FL, Pyr and BaA with total amount of 831.7 µg/Kg (table 4.4). However, out of four possible human carcinogenic: 2B (Nap, BbF, BkF and IND) only Nap (194.4 µg/Kg) has been determined while that of three probable human carcinogenic: 2A (BaA, BaP and DBA) were remained undetected

Table 4.3: Concentration of 16 PAHs in sample of Nescafe Premium Coffee

S.No	PAHs	Retention Time (min)	Concentration	
			µg/ml	µg/Kg
1	NaP	16.6	1.8±9.0	36.1
2	Acp	23.3	--	--
3	AcPy	24.2	3.9±3.7	79.2
4	Flu	26.4	4.7±9.3	95.7
5	PA	30.5	3.4±2.5	69.5
6	Ant	31.0	7.0±3.1	141.6
7	FL	36.1	2.7±1.9	54.2
8	Pyr	37.0	2.1±1.2	41.2
9	BaA	42.3	5.3±3.2	106.6
10	CHR	42.4	4.7±2.7	95.0
11	BbF	46.4	0.9±4.5	17.7
12	BkF	47.0	1.9±7.1	39.7
13	BaP	47.0	4.9±1.2	18.3
14	IND	53.3	12.1±2.1	243.0
15	DBA	53.4	--	--
16	BghiP	54.6	--	--
	Total			1117.8

-- not detected

Hence, Nescafe Premium, Nescafe Classic and Bru grounded coffee samples showed the presence of thirteen (table 4.2), eight (table 4.3) and nine (table 4.4) PAHs. However, Suncafe was found to contain four PAHs (table 4.5) viz., Nap, Flu, PA and Ant found to be 136.2, 755.8, 387.2 and 56.2 µg/Kg, respectively. Therefore, out of four PAHs (possible human carcinogenic:

2B) only NaP has been detected while other three PAHs of same category were absent. Beside this, three PAHs (probable human carcinogenic: 2A) were remained undetected.

Table 4.4: Concentration of 16 PAHs in sample of Bru Coffee

S.No.	PAHs	Retention Time (min)	Concentration µg/ml	Concentration µg/Kg
1	NaP	16.6	9.7±2.9	194.4
2	Acp	23.3	0.4±1.0	7.7
3	AcPy	24.2	2.0±1.3	40.9
4	Flu	26.4	7.8±3.5	157.5
5	PA	30.5	4.2±1.8	85.7
6	Ant	31.0	5.2±3.5	103.6
7	FL	36.1	6.9±3.8	139.5
8	Pyr	37.0	0.4±1.2	9.7
9	BaA	42.3	4.6±2.7	92.8
10	CHR	42.4	--	--
11	BbF	46.4	--	--
12	BkF	47.0	--	--
13	BaP	47.0	--	--
14	IND	53.3	--	--
15	DBA	53.4	--	--
16	BghiP	54.6	--	--
	Total			831.7

--not detected

On contrary to the four grounded coffee sample viz., Nescafe Premium, Nescafe Classic, Bru and Suncafe only three PAHs viz., Flu, PA and Ant has been found in Roasted Coffee Beans (table 4.6). Total amount of these three PAHs have been found to 1249.7 µg/Kg. However, possible human carcinogenic: 2A and probable human carcinogenic: 2B were not detected. Analysis of these five coffee samples has revealed the impact of manufacturing process on the PAHs content. Similar findings have been found in literature [15].

Furthermore, variation in mass percentage for 2 to 6 ring PAHs in various coffee brands is shown in figure 2. Nescafe Classic and Bru coffee samples showed the presence of 2, 3 and 4 ring PAHs. However, Nescafe Premium showed the presence of 2 to 6 ring PAHs whereas Suncafe showed presence of only 2 and 3 ring PAHs.

Table 4.5: Concentration of 16 PAHs in sample of Suncafe Coffee

S.No	PAHs	Retention Time (min)	Concentration	
			µg/ml	µg/Kg
1	NaP	16.6	6.81±2.2	136.2
2	Acp	23.3	--	--
3	AcPy	24.2	--	--
4	Flu	26.4	37.8±5.2	755.8
5	PA	30.5	78.3±3.2	387.2
6	Ant	31.0	2.81±1	56.2
7	FL	36.1	--	--
8	Pyr	37.0	--	--
9	BaA	42.3	--	--
10	CHR	42.4	--	--
11	BbF	46.4	--	--
12	BkF	47.0	--	--
13	BaP	47.0	--	--
14	IND	53.3	--	--
15	DBA	53.4	--	--
16	BghiP	54.6	--	--
	Total			1335.4

--not detected

The mass percentage of 2, 3 and 4 ring PAHs for Nescafe Classic was 7.61, 36.88 and 55.49%, respectively, while that for Bru coffee sample is 23.37, 47.54 and 29.08%, respectively. Nescafe Premium showed the mass percentage of 2, 3, 4, 5 and 6 rings PAH as 3.21, 34.49, 26.53, 13.91 and 21.71%, respectively. Furthermore, mass percentage of 2 and 3 rings PAH in Suncafe was found to be 10.1 and 89.9%, respectively. However, mass percentage of 3 ring PAHs in roasted coffee beans was 100%. The variation in mass percentage of 2-6 ring PAHs for different coffee samples is due to difference in manufacturing process that plays a key role in formation of PAHs.

Table 4.6: Concentration of 16 PAHs in sample of Roasted Coffee Beans

S.No	PAHs	Retention Time (min)	Concentration	
			µg/ml	µg/Kg
1	NaP	16.6	--	--
2	Acp	23.3	--	--
3	AcPy	24.2	--	--
4	Flu	26.4	18.8±1.3	361.4
5	PA	30.5	35.5±8.3	710.0
6	Ant	31.0	8.9±4.7	178.3
7	FL	36.1	--	--
8	Pyr	37.0	--	--
9	BaA	42.3	--	--
10	CHR	42.4	--	--
11	BbF	46.4	--	--
12	BkF	47.0	--	--
13	BaP	47.0	--	--
14	IND	53.3	--	--
15	DBA	53.4	--	--
16	BghiP	54.6	--	--
	Total			1249.7

-- not detected

Investigation into the source of PAHs has been proposed on the basis of molecular ratios of some specific hydrocarbons. For instance if the ratio of FL to Pyr is greater than 1.0 then it suggests pyrolytic origin, whereas if ratio is less than 1.0 then it is characteristic of petroleum hydrocarbons. Moreover, if ratio of PA/Ant is less than 10 then it suggests combustion sources, while if it is greater than 10 it implies petrogenic sources [38]. In case of coffee samples during our study, the ratio of FL/Pyr was found to be 2.8, 1.3 and 14.4 for Nescafe Classic, Nescafe Premium and Bru, respectively. However, in case of Roasted Coffee Beans and Suncafe, FL and Pyr were undetectable. The ratio of PA/Ant was in range from 0.7 to 6.8 for all the coffee samples. The ratio of FL/Pyr > 1.0 and those of PA/Ant < 10 indicated that PAHs contamination was due to incomplete combustion products via pyrolytic process. These results are in agreement to those reported in literature [38].

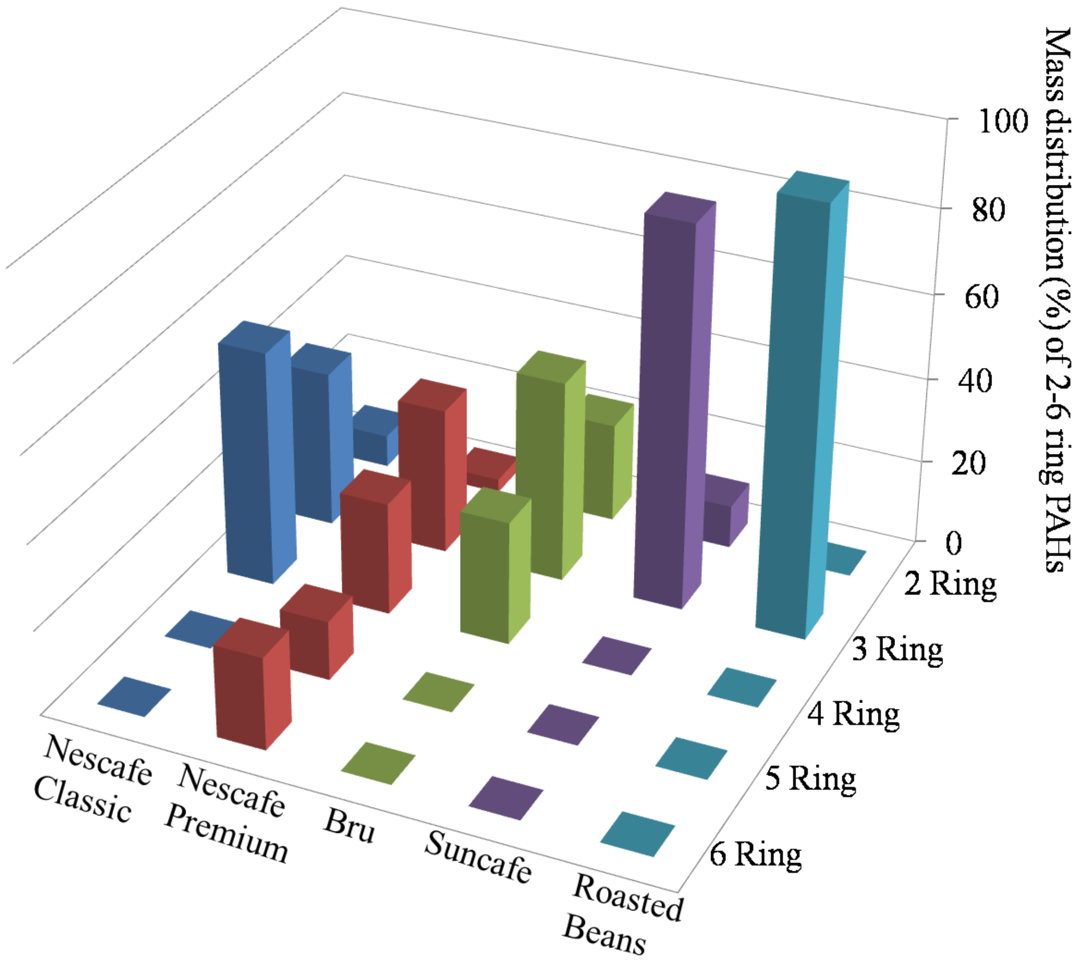

Fig. 2 Mass distribution of two to six ring PAHs in the different coffee brands (% age of ∑PAHs)

CONCLUSION

Indian grounded coffee brands and Roasted Coffee Beans were found to be contaminated by different levels of PAHs. Three PAHs viz., Flu, Ant and PA were present in all coffee brands. Decreasing order of Nap (possible human carcinogen: 2B) is: Bru, Suncafe, Nescafe Classic and Nescafe Premium. Sum total of 16 PAHs in Nescafe Classic is about 2 times than that found in Bru coffee. Two PAHs viz., BghiP and DBA were not detected in all the coffee samples. Variation in mass percentage of 2-6 ring PAHs and its amount (831.7-1589.7 μg/Kg) clearly showed that difference in the manufacturing process has created an impact. The molecular ratios of PA/Ant and FL/Pyr suggested the origin of PAHs to be incomplete combustion of organic mass via pyrolytic process.

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