Polycyclic Aromatic Hydrocarbons formed during Roasting Process in Arabica Coffee Beans

A Thesis Submitted

In partial fulfilment for the award of the degree of

MASTER OF SCIENCE

IN

CHEMISTRY

Submitted by
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Under the Supervision of
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CERTIFICATE

This is to certify that the project entitled **"Polycyclic Aromatic Hydrocarbons formed during Roasting process in Arabica Coffee Beans"** being submitted by Sanchita, Roll No. 301102011 in partial fulfillment of the requirements for the award of degree of **Master of Science**, in School of Chemistry and Biochemistry, Thapar University , Patiala, is a bonifide work carried out under my supervision and guidance. The report has not been submitted for the award of any otherdegree or certificate in this or any other university.

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CANDIDATE'S DECLARATION

I hereby declare that the work which is being presented in the dissertation entitled "**Polycyclic Aromatic Hydrocarbons formed during Roasting process in Arabica Coffee Beans**" in partial fulfillment of the requirements for the award of the degree of **Master of Science** in Chemistry, School of Chemistry and Biochemistry, Thapar University, Patiala is an authentic record of my own work during a period of six months from January 2013 to July 2013, under the supervision of Dr. Satnam Singh, Associate Professor and Head, School of Chemistry and Biochemistry, Thapar University, Patiala. The report has not been submitted for the award of any other degree or certificate in this or any other university.

Sanchita

Place : Patiala

Date : 9-07-13

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

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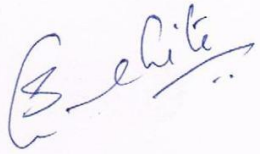
Words fail me to express my thanks to my family for their selfless sacrifice, encouragement and heart full blessings that continue to enlighten my life.

Above all I thank almighty God for blessing me with strength and wisdom to complete this project successfully.



SANCHITA

Date : 9-07-13

Place: Patiala

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ABSTRACT

Roasting is a critical process in coffee production, as it enables the development of flavor, colour and aroma, and may lead to the formation of non-desirable compounds, such as polycyclic aromatic hydrocarbons (PAHs). Among the number of PAHs, sixteen of them have been classified as priority pollutants by United State Environmental Protection Agency (USEPA) are of special concern due to their carcinogenic and mutagenic properties. In this regard, Arabica coffee beans were roasted under controlled conditions to monitor the formation of PAH during the roasting process. Soxhlet extraction method was used for the extraction of PAHs. Five to eleven PAHs from list of 16 PAHs (USEPA) were found to be present. Concentrations of total PAHs in green and roasted coffee beans was found to be vary from 30.0-955.8 μg/kg. Benzo[a]pyrene (2A: probable human carcinogen) was not detected in any roasted coffee sample. However, 2-ring PAH viz., naphthalene (2B: possible human carcinogen) was found to convert into higher ring PAHs, as a result its concentration gradually decreases and becomes undetectable in coffee beans after 100^{o}C of roasting temperature.

1. INTRODUCTION

1.1 PAHs SOURCES

Polycyclic Aromatic Hydrocarbons (PAHs) are two to eight-ring fused ring system without any heteroatom, 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 (respirable 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 that serve as the source for human exposure by these pollutants. For example, the intake of carcinogenic PAHs through foods in Italy was found to be (~1.4 μg/day) which is higher than estimated (0.13 ng/day) intake through respiration [16]. 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 Limit of Detection (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 [17]. 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. [18-31]. 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 colour, aroma, and flavor, which are essential for the characterization

of the coffee quality. For instance, the temperature and time during the roasting process varies from 150 to 300^{o}C and 5-30 min, respectively, depending upon the requirement for colour, aroma and taste of coffee [33b and 33c] e.g., Nescafe Cap Colombie is a light roast with a smooth and fruity coffee whereas, Nescafe Espresso, is fully roasted coffee, having its characteristic golden-brown layer in its brew [33c]. However, at the same time roasting may lead to the formation of non desirable compounds, such as polycyclic aromatic hydrocarbons (PAHs) that are of concern and impose threat on human health. Priority PAHs listed by USEPA and IARC, respectively are listed in Table 1.1.

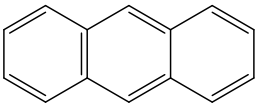
Table 1.1: Sixteen priority PAHs listed by USEPA and IARC

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	√	--

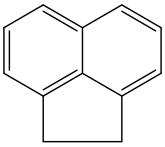
***2A= Probable Human Carcinogenic**
***2B= Possible Human Carcinogenic**
****Priority Pollutants**

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

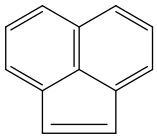
S.No.	PAHs	Abbreviation
1	Acenaphthene	Acp
2	Acenaphthylene	AcPy
3	Anthracene	Ant
4	Benzo[g,h,i]perylene	BghiP
5	Benzo[a]anthracene	BaA
6	Benzo[a]pyrene	BaP
7	Benzo[b]fluoranthene	BbF
8	Benzo[k]fluoranthene	BkF
9	Chrysene	CHR
10	Dibenzo[a,h]anthracene	DBA
11	Fluoranthene	FL
12	Fluorene	Flu
13	Indeno[1,2,3]pyrene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



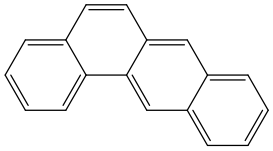
Anthracene



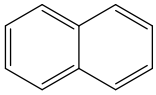
Acenaphthene



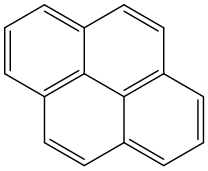
Acenaphthylene



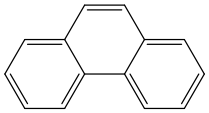
Benzo(a)anthracene



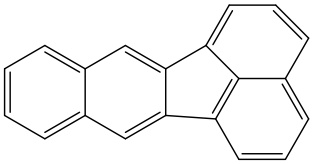
Naphthalene



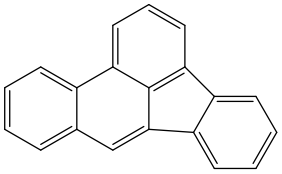
Pyrene



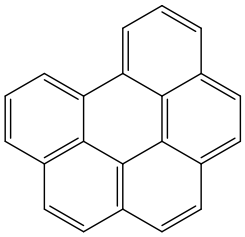
Phenanthrene



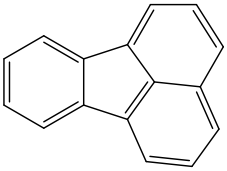
Benzo(k)fluoranthene



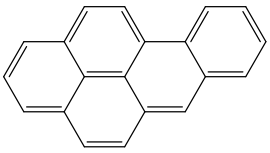
Benzo(b)fluoranthene



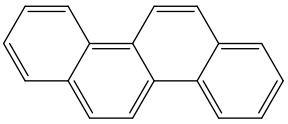
Benzo(g,h,i)perylene



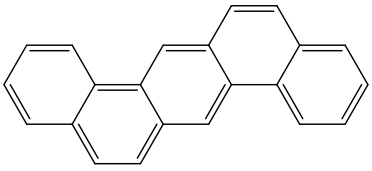
Fluoranthene



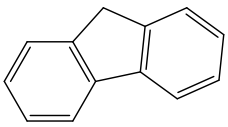
Benzo(a)pyrene



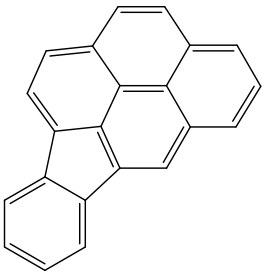
Chrysene



Dibenzo[a,h]anthracene



Fluorene



Indeno(1,2,3-c,d)pyrene

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 [34-36], vegetables [37-42], smoked meat [43-49], tea [50,51], coffee [53-59] etc. Edible oils found to be contaminated with different levels of PAHs. Studies conducted by Hossian et al. [34], Purcaro et al. [35] and Formberg et al. [36] showed that the contamination level oils with PAHs 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 [37] and Monica [38] clearly revealed that vegetables form Egyption and Brazilan market, 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. for also revealed the contamination of vegetables by different of levels of PAHs ranging form 7.1 - 231.2 ng/g, 22.89 - 85.55 μg kg^{-1} , 2.1 - 8.35 μg kg^{-1} and 2.25 and 7.82 μg/g respectively [39-42].

PAHs are formed by incomplete combustion of organic mass as a results of their determination in meat products has been carried out, using various techniques such as GC-MS, GC-HRMS etc. [43,44]. For example, observation obtained by Mottier showed the average values of PAHs ranging from 0.5-1.0 μg/kg [4] in barbecued meat sausages. Jira et al. [46] 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 [47] 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 [48] revels that 3.4% of total samples were contaminated by Bap, more than its acceptable limit and the total PAHs concentrations detected were 16 μg kg^{-1} in smoked meat and ham, 19 μg kg^{-1}

in smoked sausage and 6.5 μg kg^{-1} in smoked chicken samples. Farhadian et al. [49] 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 [50]. The PAHs contents in tea infusion were reported to highlighting that a maximum of 11% of the PAHs present in the tea leaves are transferred to infusion [51]. 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 contents in tea leaves during the tea manufacturing process [50].

Coffee is one of the most commonly consumed beverages worldwide [17]. Over 90% of coffee production takes place in developing countries, while consumption happens mainly in the industrialized economies [52]. During the coffee production, roasting is a crucial step, as it enables the development of colour, aroma, and flavor, which are essential for the characterization of its quality. At the same time, roasting may lead to the formation of undesirable compounds, such as polycyclic aromatic hydrocarbons (PAHs). Justin et al. [53] studied the PAHs content in roasted coffee beans and the effect of roasting temperature on the PAHs. It was found that at temperature > 220^{o}C phenanthrene, anthracene, and benzo[a]anthracene and 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. [54, 55] indicated 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 [56] and Gabriela et al. [57], 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 [58,59]. A report by Sayadi et al. [58] described a HPLC method with fluometric detetction for PAHs determination in coffee brew and its quantity was determined to be 1.65-2.87 ng L^{-1}. However, Bishnoi et al. [59], 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). On the other hand, there seems no report indicating a detailed investigation on the effect of temperature during roasting on the formation of PAHs by Gas Chromatography with Flame Ionization Detector (FID). A report by Houessou et al. [60] depicting the HPLC-FD (FD = fluorometric detector) method for the determination of these PAHs, signifies the role of temperature (180-260^{o}C) during the roasting process of grounded Arabica coffee. In order to address this issue present study is devoted to monitor the changes in quantity, number and mass of 16 priority PAHs listed by USEPA, as a function of temperature during the roasting process in Arabica coffee beans.

OBJECTIVES

1. To study the effect of temperature during roasting process on the number, quantity and mass of 16 Polycyclic Aromatic Hydrocarbons (priority pollutants) in Arabica coffee beans

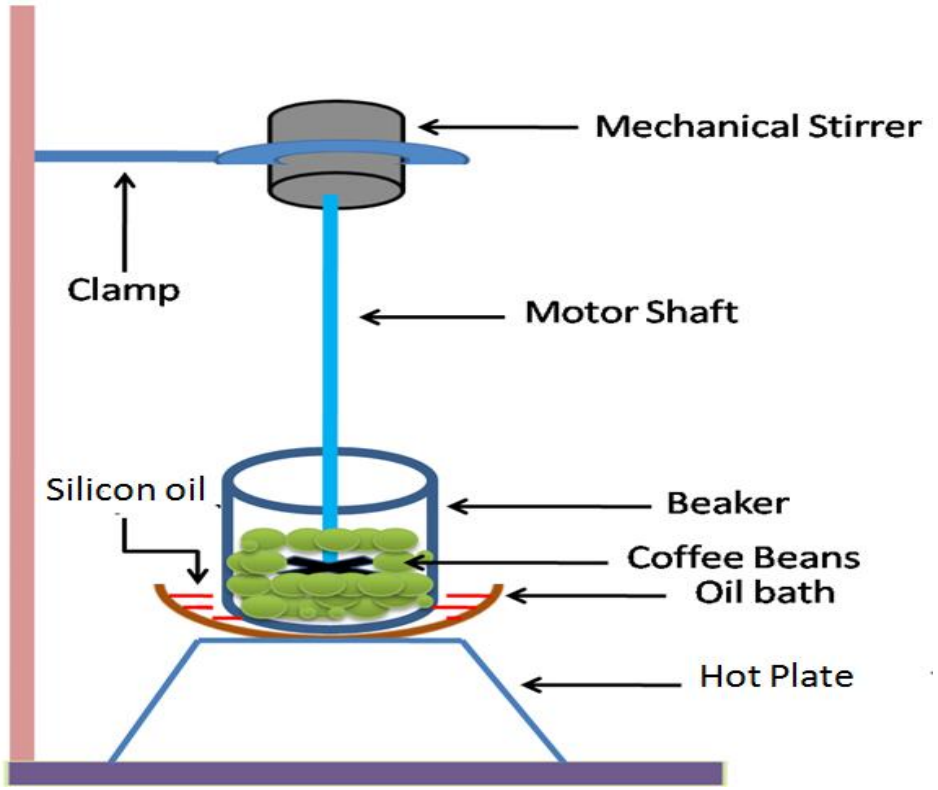
3.0 MATERIALS AND METHODS

3.1 Reagents and Chemicals

Arabica coffee beans were purchased from M.K.C Foods Pvt. Ltd., Bangalore, Karnataka (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 Roasting of Arabica coffee beans

Roasting of Arabica coffee beans was carried out by heating 100 g of green coffee beans contained in a beaker placed in a silicon oil bath at different temperatures ranging from 50-250^{o}C with stirring by mechanical stirrer (scheme-I) for 30 min.



Scheme-1 Roasting of green coffee beans

3.3 Sample preparation for different brands of coffee

In a typical sample preparation, 50 g of green/ roasted coffee beans were 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 chromatograph (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^{o}C for 5 min, temperature programmed at 6^{o}C /min to 320^{o}C. The injection port was maintained at 250^{o}C, and the detector was maintained at 275^{o}C. 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 [1,61,62].

4.0 RESULTS AND DISCUSSION

The determination of 16 priority PAHs (listed by USEPA) have been carried out by gas chromatography. The Gas chromatograph of 16 PAHs (Sigma-Aldrich) under operating conditions is given in Fig. 4.1 and their retention times are given in Table 4.1. PAHs were quantified by response factor method and results obtained are shown in table 4.2 a and 4.2 b, with their corresponding GC-chromatographs (Fig. 4.2- 4.10).

1. Response factor = (Peak area of standard analyte) / (Analyte amount)

2. Amount of analyte = (Peak area of analyte) / (Response factor)

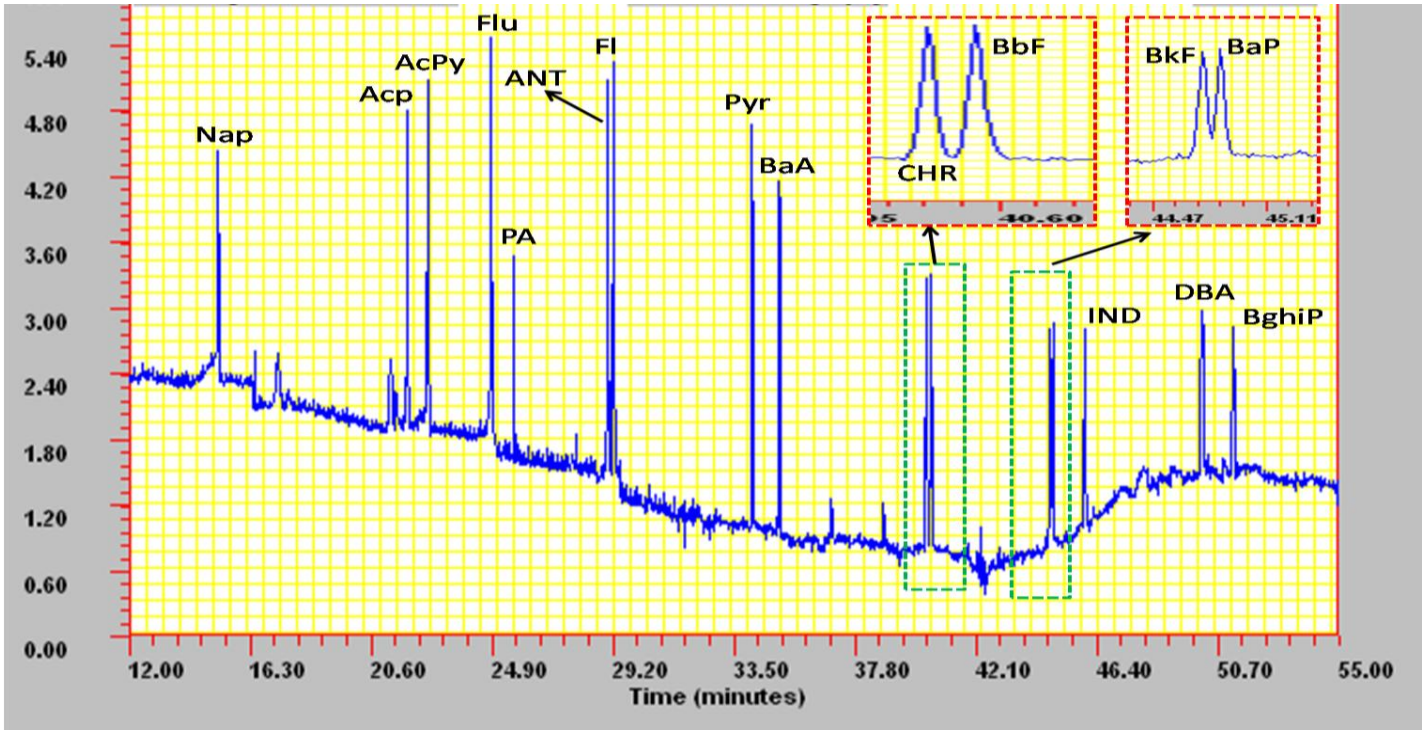


Fig. 4.1: Gas Chromatogram of standard solution of 16 PAHs (Sigma-Aldrich)

Table 4.1: Retention time of 16 PAHs

S.No.	Polycyclic Aromatic Hydrocarbons (PAHs)	Retention Time (min)
1	Nap	15.2
2	Acp	21.9
3	AcPy	22.6
4	Flu	24.9
5	PA	25.7
6	Ant	29.0
7	FL	29.3
8	Pyr	34.1
9	BaA	35.2
10	CHR	40.3
11	BbF	40.6
12	BkF	44.7
13	BaP	44.8
14	IND	46.0
15	DBA	50.2
16	BghiP	51.3

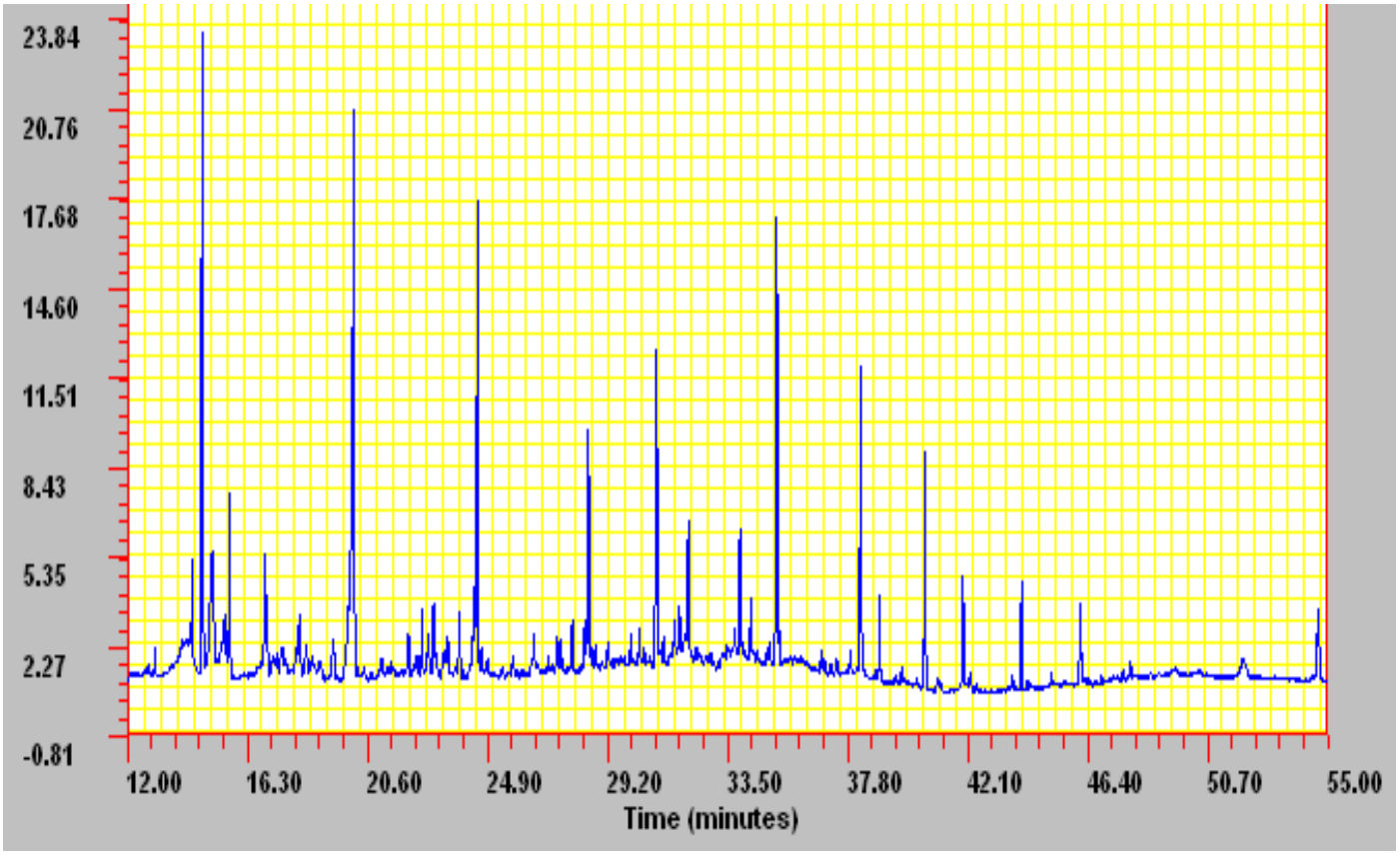


Fig. 4.2: Gas Chromatogram of sample obtained from coffee beans roasted at 50 ^{o}C for 30 min

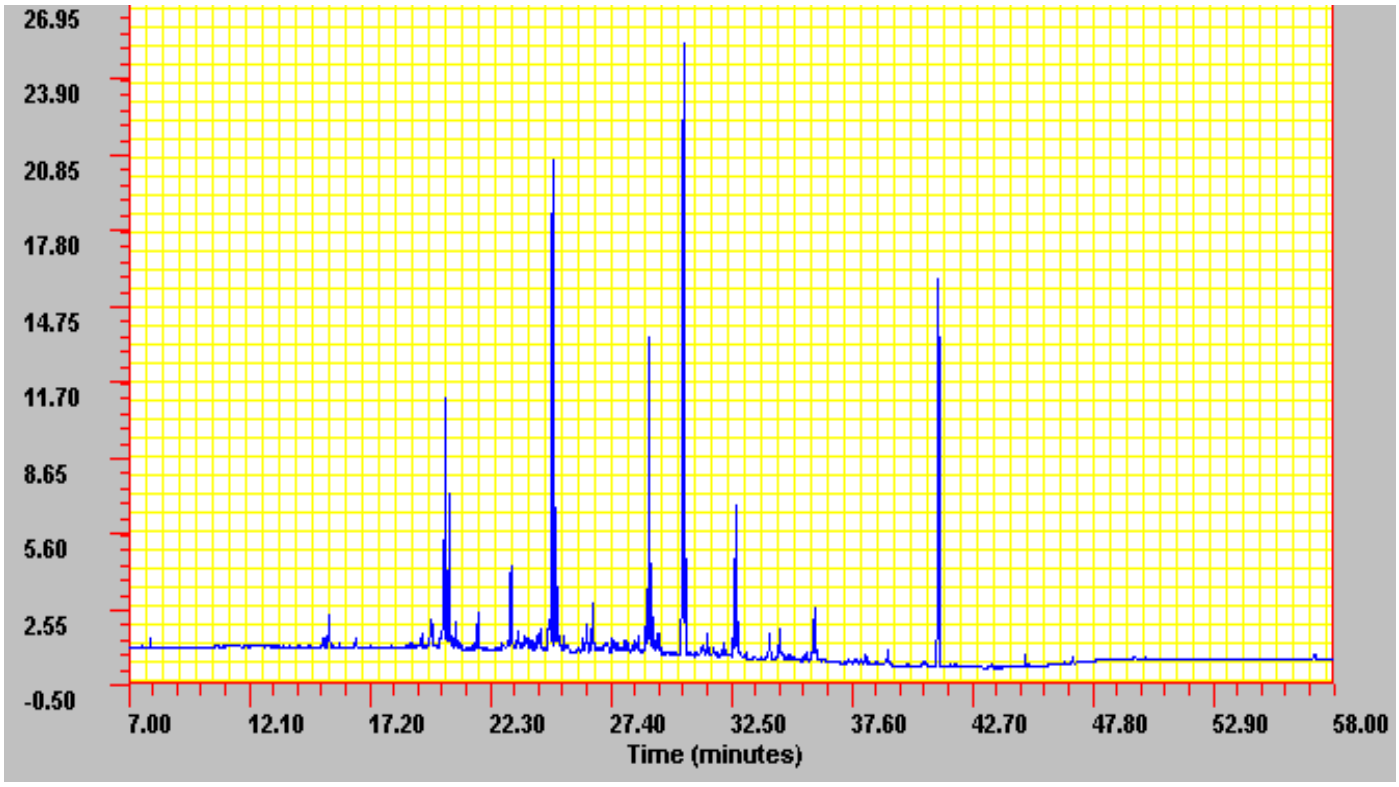


Fig. 4.3: Gas Chromatogram of sample obtained from coffee beans roasted at 75 ^{o}C for 30 min

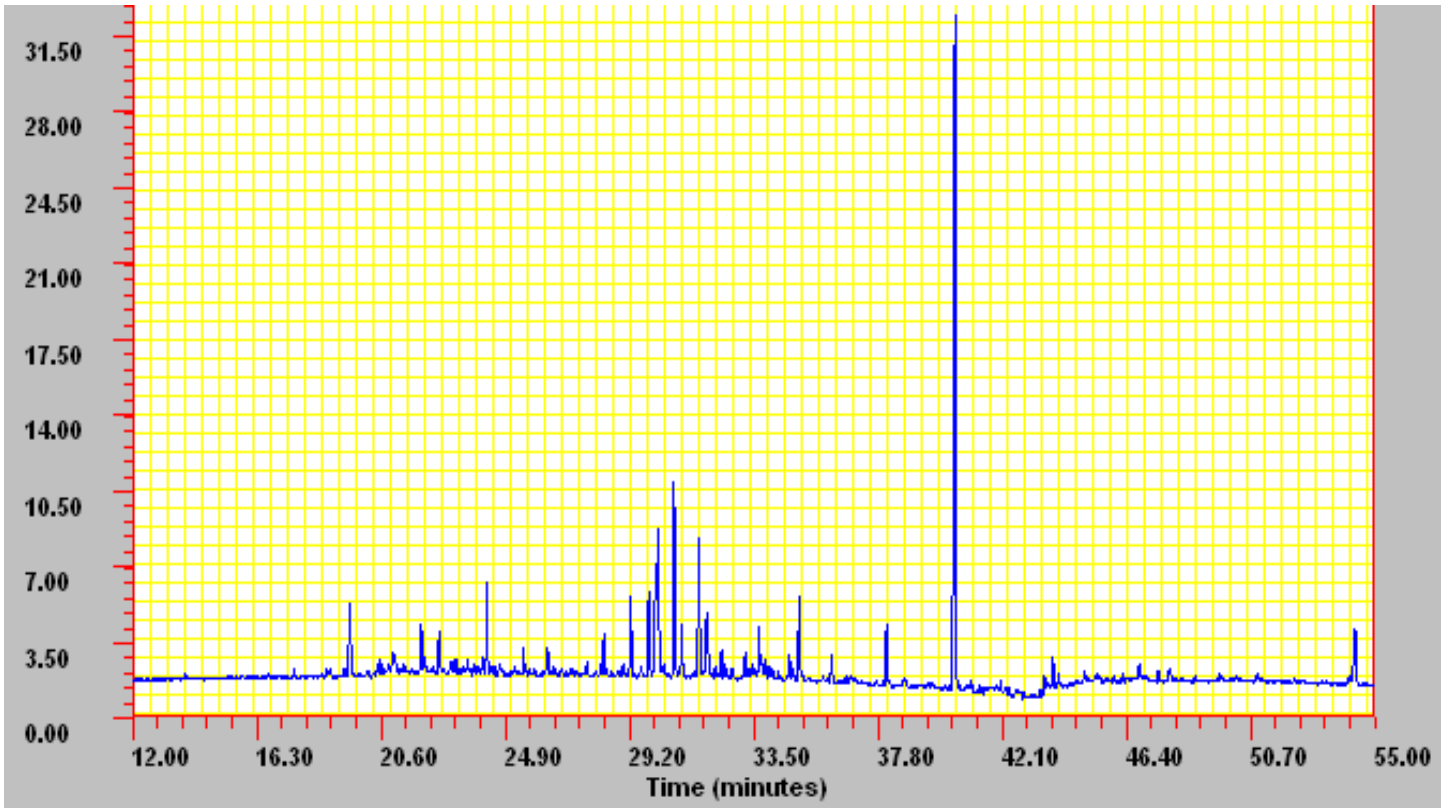


Fig. 4.4 : Gas Chromatogram of sample obtained from coffee beans roasted at 100 ^{o}C for 30 min

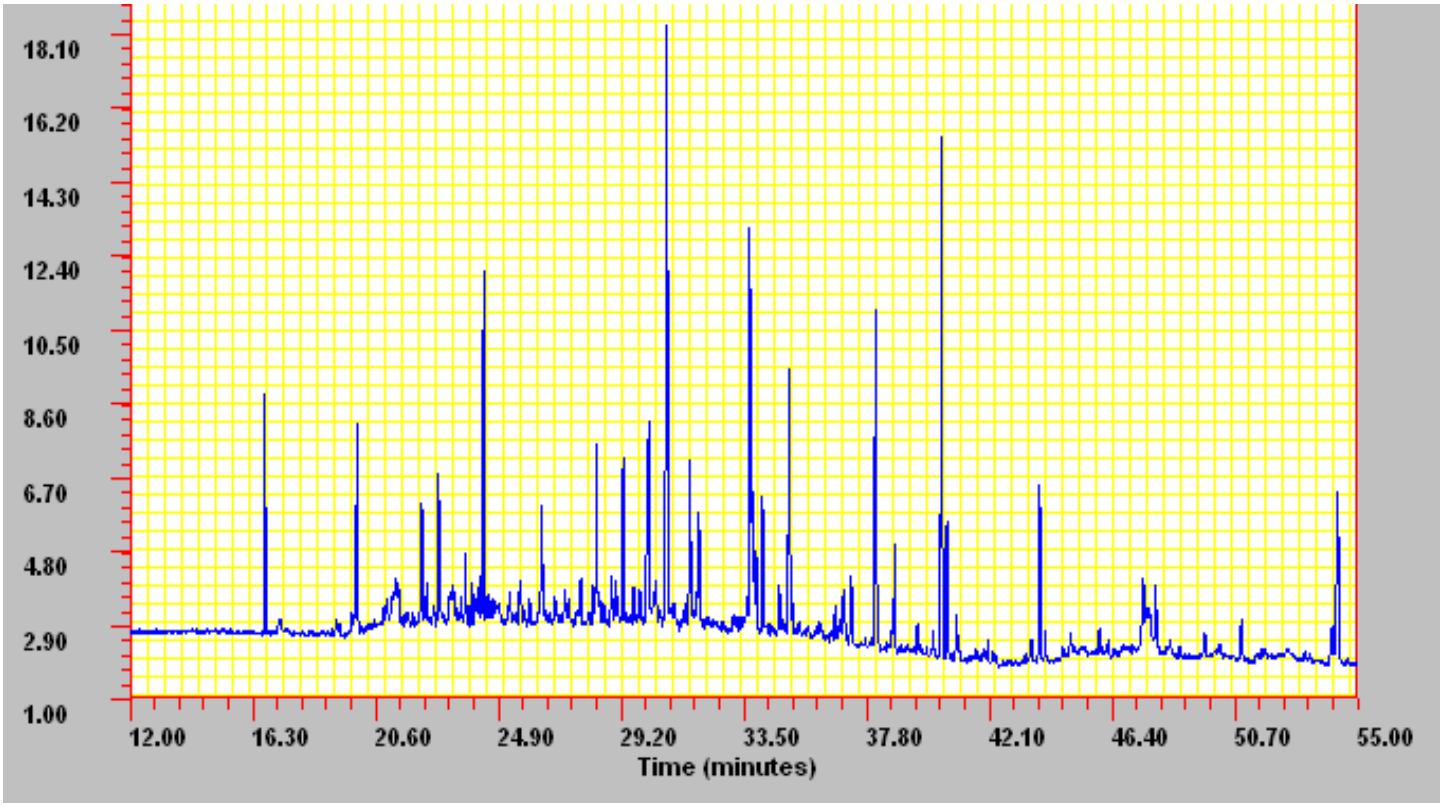


Fig. 4.5: Gas Chromatogram of sample obtained from coffee beans roasted at 125 ^{o}C for 30 min

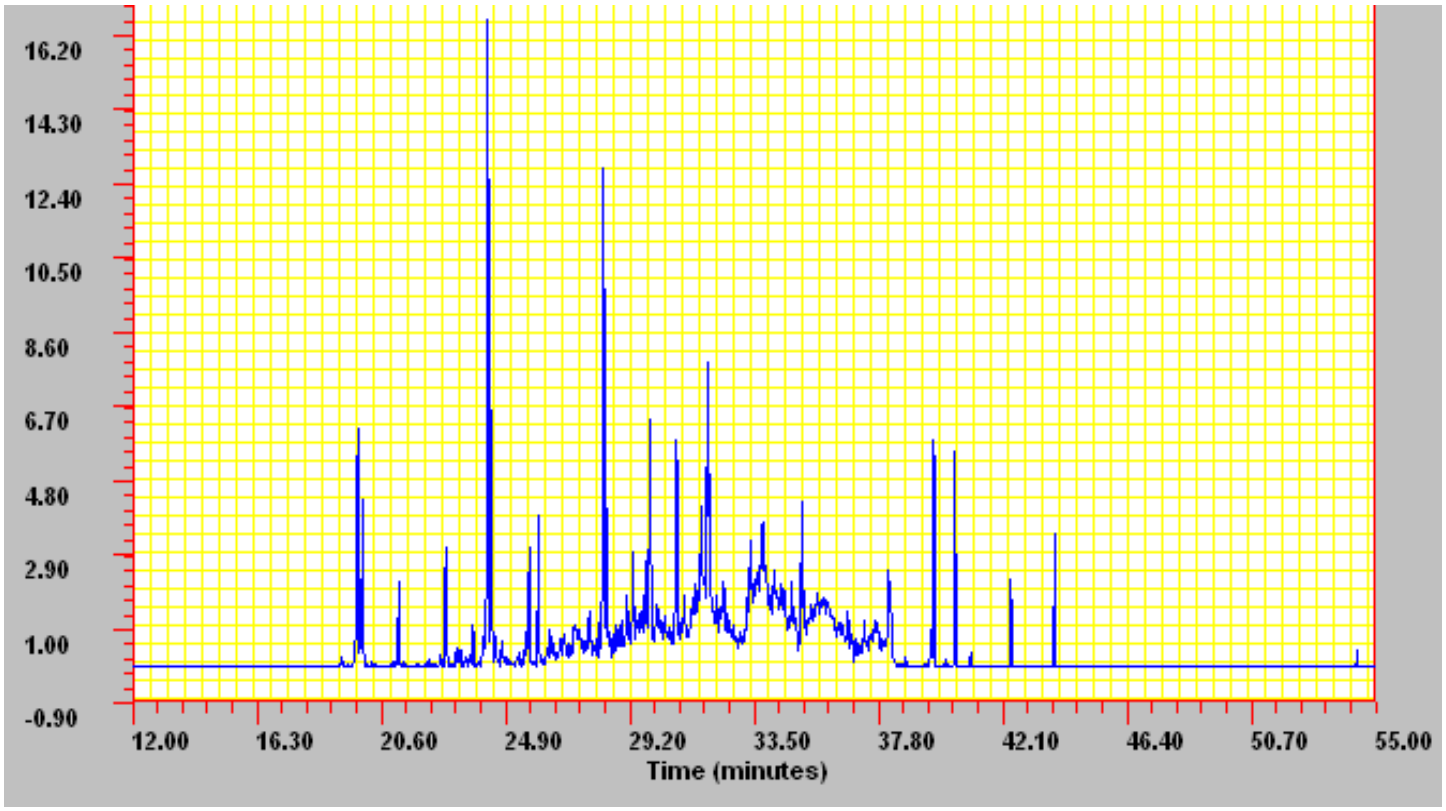


Fig. 4.6: Gas Chromatogram of sample obtained from coffee beans roasted at 150 ^{o}C for 30 min

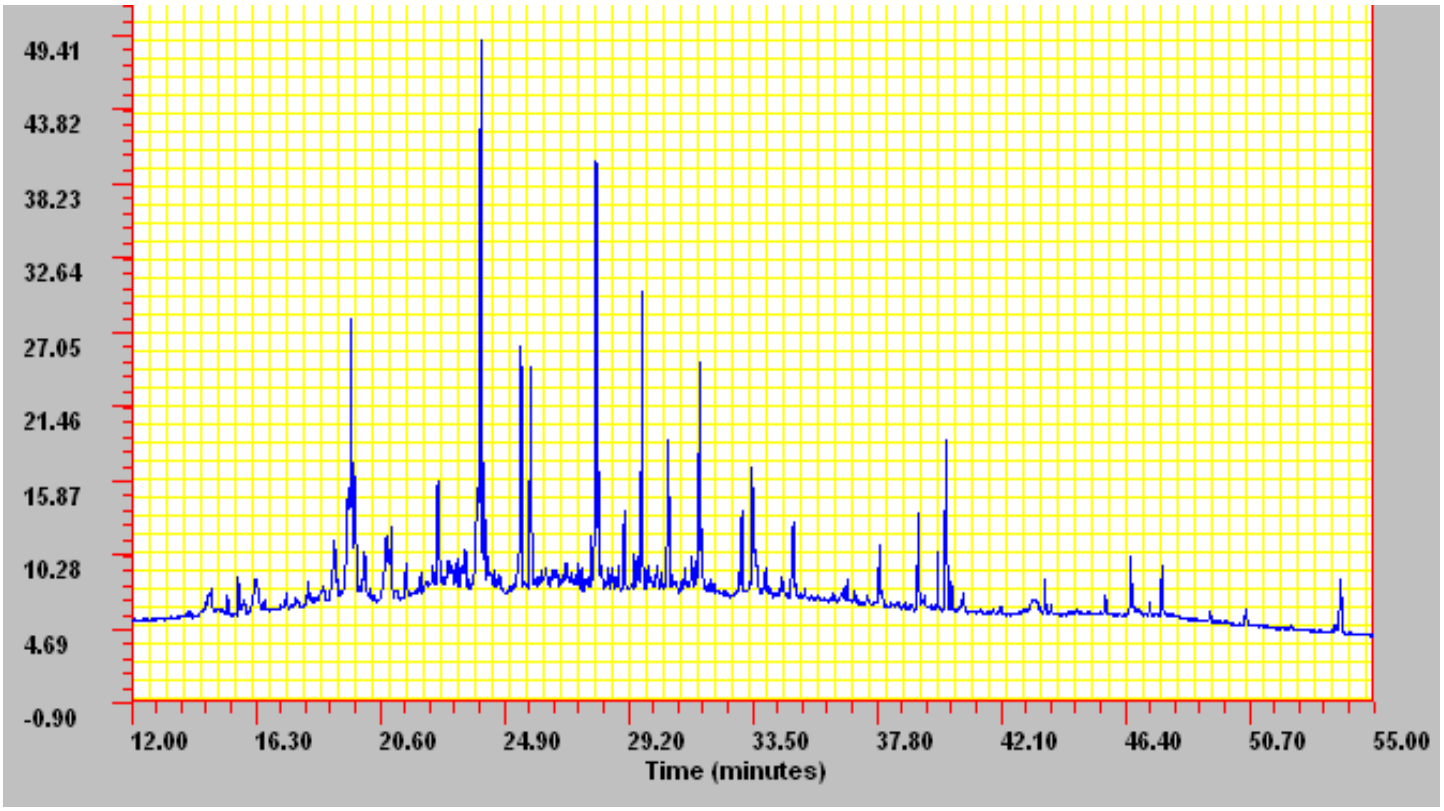


Fig. 4.7: Gas Chromatogram of sample obtained from coffee beans roasted at 175 ^{o}C for 30 min

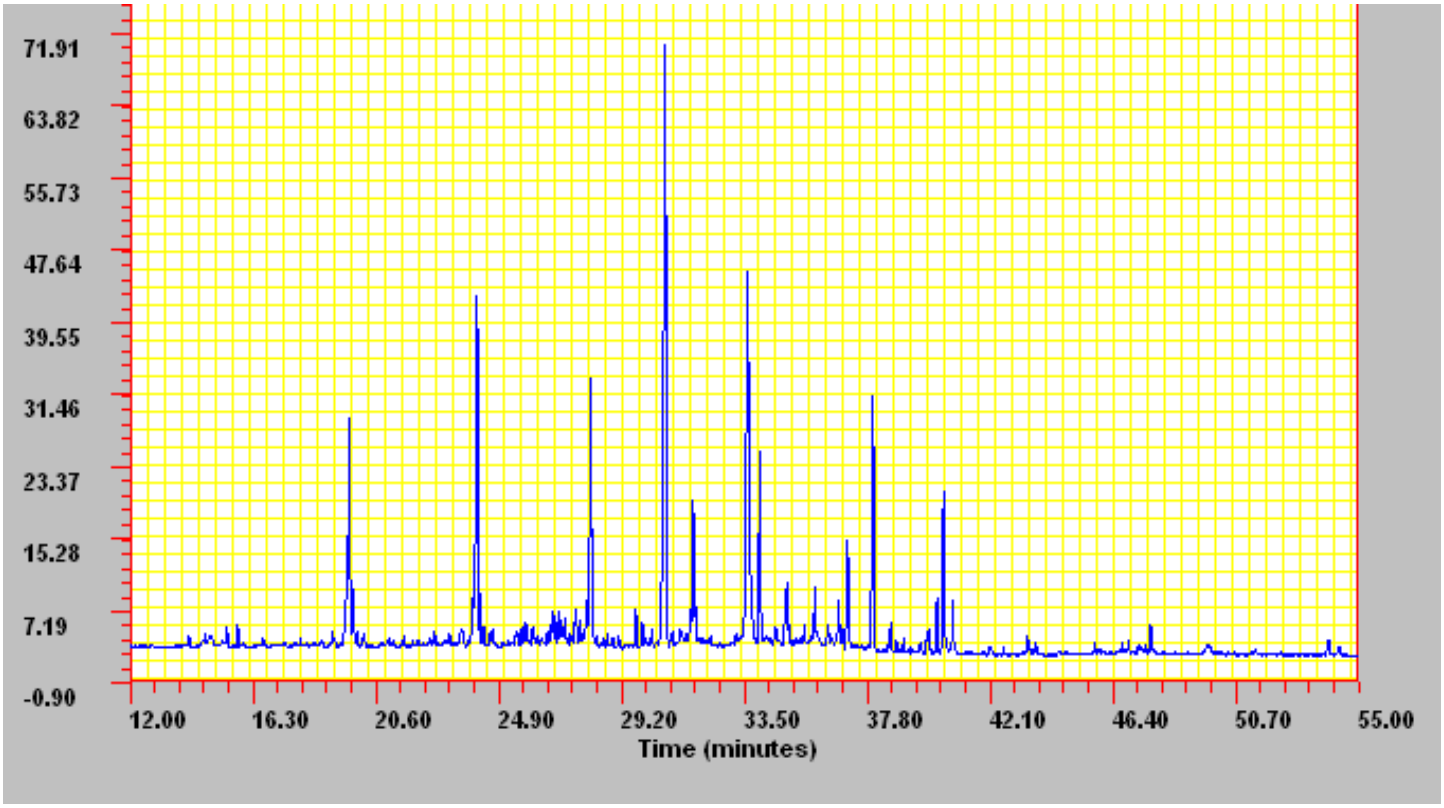


Fig. 4.8: Gas Chromatogram of sample obtained from coffee beans roasted at 200 ^{o}C for 30 min

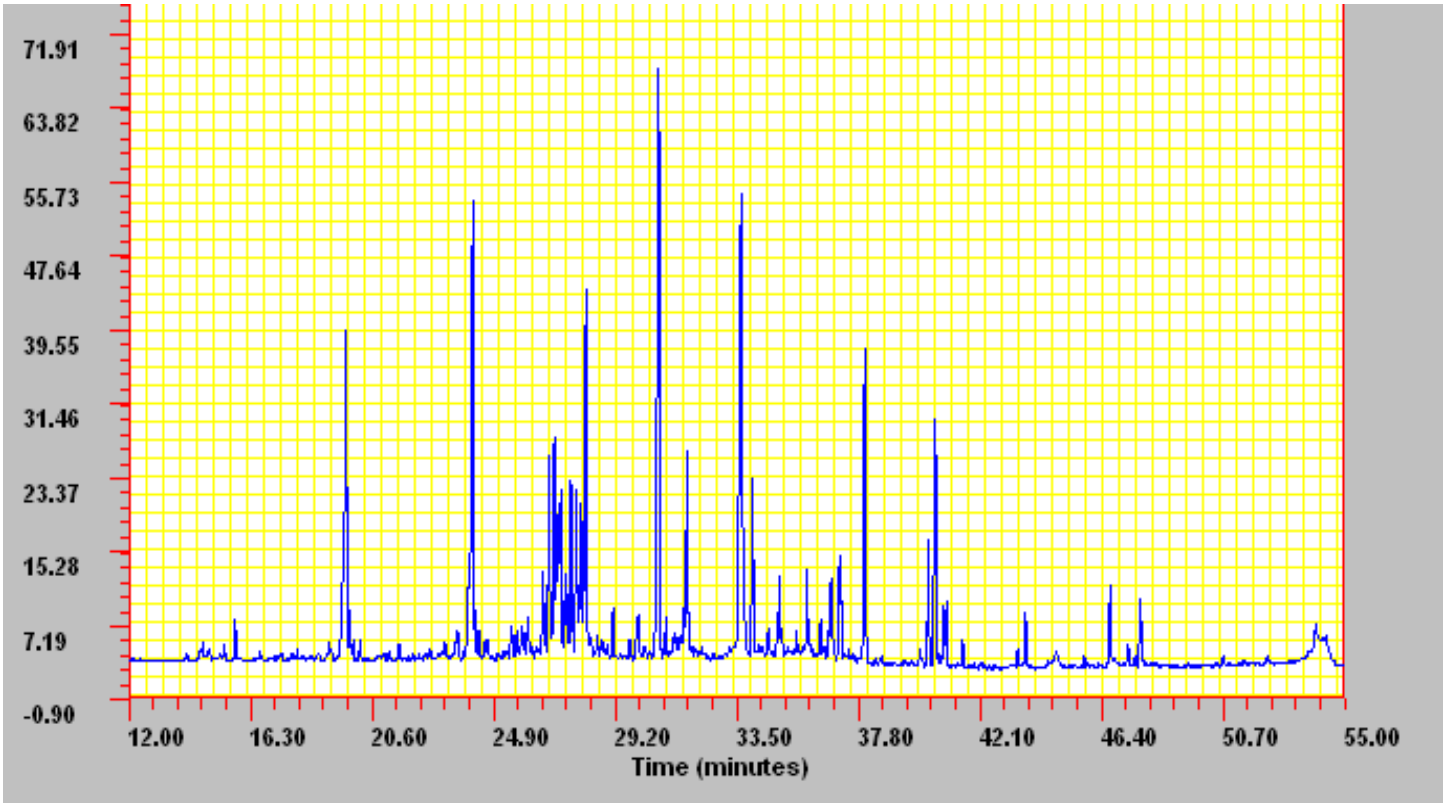


Fig. 4.9: Gas Chromatogram of sample obtained from coffee beans roasted at 225 ^{o}C for 30 min

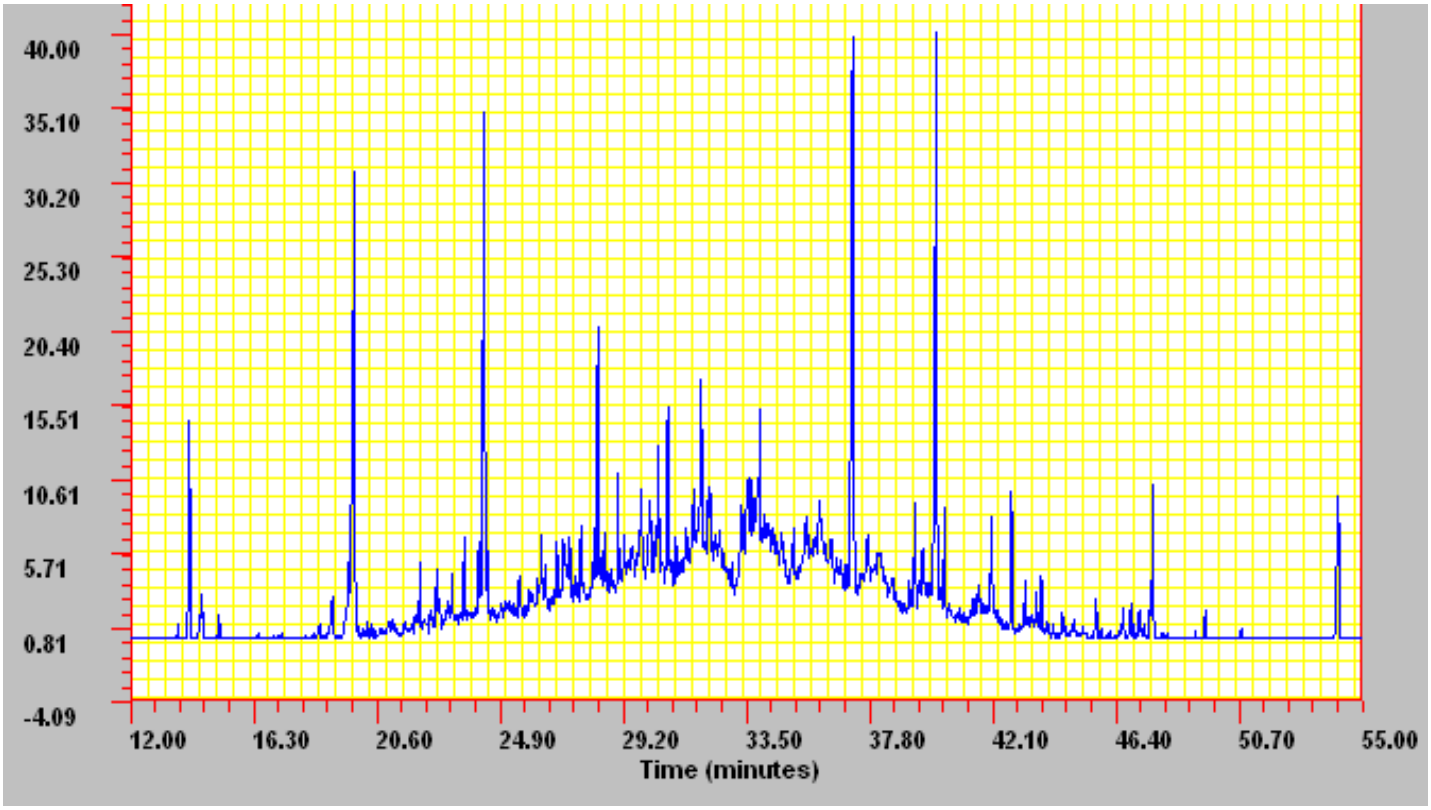


Fig. 4.10: Gas Chromatogram of sample obtained from coffee beans roasted at 250 ^{o}C for 30 min

Roasting is a critical step in coffee production and is responsible for the flavor and aroma apart from the development in colour of the coffee beans. Roasting of green coffee beans (for 30 min) at various temperatures (50-250^{o}C), resulted in gradual change in colour of coffee beans from light green to dark brown (Fig. 4.11). Certainly, such type of colour change in coffee beans is ascribed to the release of caffeol (coffee oil), which increases with increase in temperature during the roasting process, and gets enough burnt up, resulted into brown colour of coffee beans [33b].

Fig. 4.11 Colour of Arabica coffee beans with increase in temperature during roasting process

The effect of temperature (50-250^{o}C) during roasting process on concentration of 16 priority PAHs in Arabica coffee samples were investigated (Table 4.2a and 4.2 b). It has been found that all the coffee samples were contaminated with different levels of PAHs ranging from 100-955.3 µg/kg. Besides roasted coffee beans, green coffee beans were also analyzed for determination of the possible PAHs contamination of coffee beans and provide the actual amount of PAHs formation during roasting process.

Table 4.2a: Amount of PAHs formed during roasting of Arabica coffee beans

S.No	PAHs	Amount of PAHs at different roasting temperature							
		50°C		75°C		100°C		120°C	
		µg/ml	µg/kg	µg/ml	µg/kg	µg/ml	µg/kg	µg/ml	µg/kg
1	Nap	0.44±0.2	8.81	--	--	0.03±0.1	0.65	--	--
2	Acp	--	--	--	--	0.01±0.1	0.27	--	--
3	AcPy	0.02±0.1	0.11	1.15±0.4	23.05	0.59±0.3	11.96	--	--
4	FLU	--	--	--	--	0.64±0.9	12.87	--	--
5	PA	--	--	--	--	--	--	--	--
6	Ant	0.98±0.2	10.47	4.14±1.2	82.87	0.25±0.1	5.09	3.67±2.1	20.26
7	FL	0.26±0.1	1.02	0.82±0.5	16.52	0.08±0.3	1.73	0.32±0.1	3.31
8	Pyr	1.45±0.3	16.98	0.80±0.1	16.18	0.76±0.1	15.25	1.59±0.9	22.48
9	BaA	0.97±0.4	15.46	1.31±0.9	26.33	4.77±4.3	95.47	2.11±1.8	23.78
10	CHR	--	--	--	--	--	--	29.69±9.5	388.95
11	BbF	--	--	--	--	--	--	7.48±2.3	--
12	BkF	--	--	--	--	--	--	--	--
Total			52.8		164.8		142.3		458.8

-- not detected

Table 4.2b: Amount of PAHs formed during roasting of Arabica coffee beans

S.No	PAHs	Amount of PAHs at different temperature									
		150°C		175°C		200°C		225°C		250°C	
		µg/ml	µg/kg	µg/ml	µg/kg	µg/ml	µg/kg	µg/ml	µg/kg	µg/ml	µg/kg
1	Nap	--	--	1.72±0.8	34.46	0.16±0.3	3.31	0.89±0.6	17.83	1.23±1.1	24.6
2	Acp	--	--	0.26±0.4	5.23	0.03±0.1	0.68	--	--	--	--
3	AcPy	0.88±0.5	17.76	0.53±0.4	10.63	0.21±0.2	4.27	--	--	--	--
4	FLU	0.34±1.0	6.85	0.70±1.0	14.17	0.14±0.4	2.85	--	--	--	--
5	PA	0.21±0.2	4.24	0.05±0.1	1.05	0.05±0.0	1.13	--	--	--	--
6	Ant	2.37±1.9	47.52	0.25±0.3	5.01	1.39±1.2	27.88	3.67±2.1	73.43	5.92±2.6	118.4
7	FL	0.20±0.1	4.07	0.08±0.1	1.70	0.22±0.2	4.40	0.32±0.1	6.52	0.75±0.2	15
8	Pyr	2.62±1.5	52.40	1.20±0.9	24.05	0.42±0.3	8.45	1.59±0.9	31.84	1.78±0.6	35.6
9	BaA	1.04±0.8	20.9	12.80±15.3	256.02	0.85±1.1	17.05	2.11±1.8	42.24	2.72±1.5	54.4
10	CHR	16.24±5.0	324.96	10.10±6.0	202.10	34.90±18.1	698.16	29.69±9.5	593.98	25.87±7.8	517.4
11	BbF	1.43±1.2	28.78	1.57±4.7	31.58	0.10±0.2	2.17	7.48±2.3	149.70	8.38±3.0	167.6
12	BkF	--	--	--	--	--	--	0.89±0.6	17.83	1.14±0.5	22.8
Total			507.5		586		770.4		933.4		955.8

It has been found that the green coffee beans were contaminated with only one, two ring PAH viz., naphthalene (30 µg/kg) that could be due to the deposition of the PAH bounded particulate matter over the vegetation [16-19]. In comparison to the individual PAHs, four PAHs viz., Fl, Pyr, PA and Ant was found in all the roasted coffee samples with their combined concentration ranging from 30-40 µg /kg. Concentrations of the BaA (2A: probable human carcinogen) was found be 15.46-256.02 µg /kg in all the coffee samples, while for Nap (2B: possible human carcinogen) the minimum detected concentration ca. 0.65 µg /kg (at 100^{o}C) in comparison to its highest amount 34.46 µg/kg (at 175^{o}C). However, the most toxic PAHs [18,25] viz., BaP and DBA, remained undetected.

Considering the sum total of twelve detected PAHs (∑PAHs) in the roasted coffee samples, concentrations were found to increase (52.8-955.8 µg /kg) with rise in temperature (50-250^{o}C) during roasting process. A significant increase in ∑PAHs was observed at 75^{o}C, which was even more pronounced at 250^{o}C of roasting temperature, with respective ∑PAHs of 164.8 and 953.0 /kg and determined to be around 4 and 21 times more in comparison to the PAHs concentration at 50^{o}C (52.8 µg /kg). Moreover, with increase in temperature during roasting, increase in number of PAHs has been found upto 175^{o}C (11 PAHs) that decreases with further increase in temperature (200-250^{o}C), although the trend for increase in ∑PAHs with rise of roasting temperature still continues. Such type of variation in number of PAHs, signifies the conversion of the lower ring PAHs to corresponding higher ring PAHs. However, the increase in amount of ∑PAHs was due to the conversion of organic mass containing compounds viz., 6-chlorogenic in Arabica coffee that reported to serve as precursor for PAHs [63]. Similar results for the effect of roasting temperature (210-260^{o}C) were reported by Houessou et al. [60], however the amount of PAHs determined are much less in relation to the present study, ascribed to the roasting time (5 min), roasting conditions and type of roaster used.

Variation in mass percentage for 2, 3, 4 and 5 ring PAHs in Arabica coffee beans after each roasting temperature ranging from 50-250^{o}C with step-up difference of 25^{o}C are shown in figure 4.12. Green coffee beans showed the presence of only Nap, two ring PAH (not shown here). However, after their roasting at 50^{o}C, 3 and 4 ring PAHs were predominant with their respective amounts of 31 and 67%. At 75^{o}C of roasting temperature the mass percentage of 3-ring PAHs (72.87%) are more than 4-ring PAHs (25.22%) while the converse is true for the 100 and 125^{o}C of roasting temperature, suggesting the initial conversion of the 2-ring PAH to the corresponding 3-ring PAHs at 75^{o}C, that converts into the 4-ring PAHs above this temperature. After roasting the coffee beans at 150^{o}C, the contribution of 2, 3 and 4 ring PAHs to the ∑PAHs decreases, with addition of 5-ring PAHs in roasted beans, revealing the conversion of lower ring to the higher ring PAHs, and this trend continues till 250^{o}C of temperature during roasting.

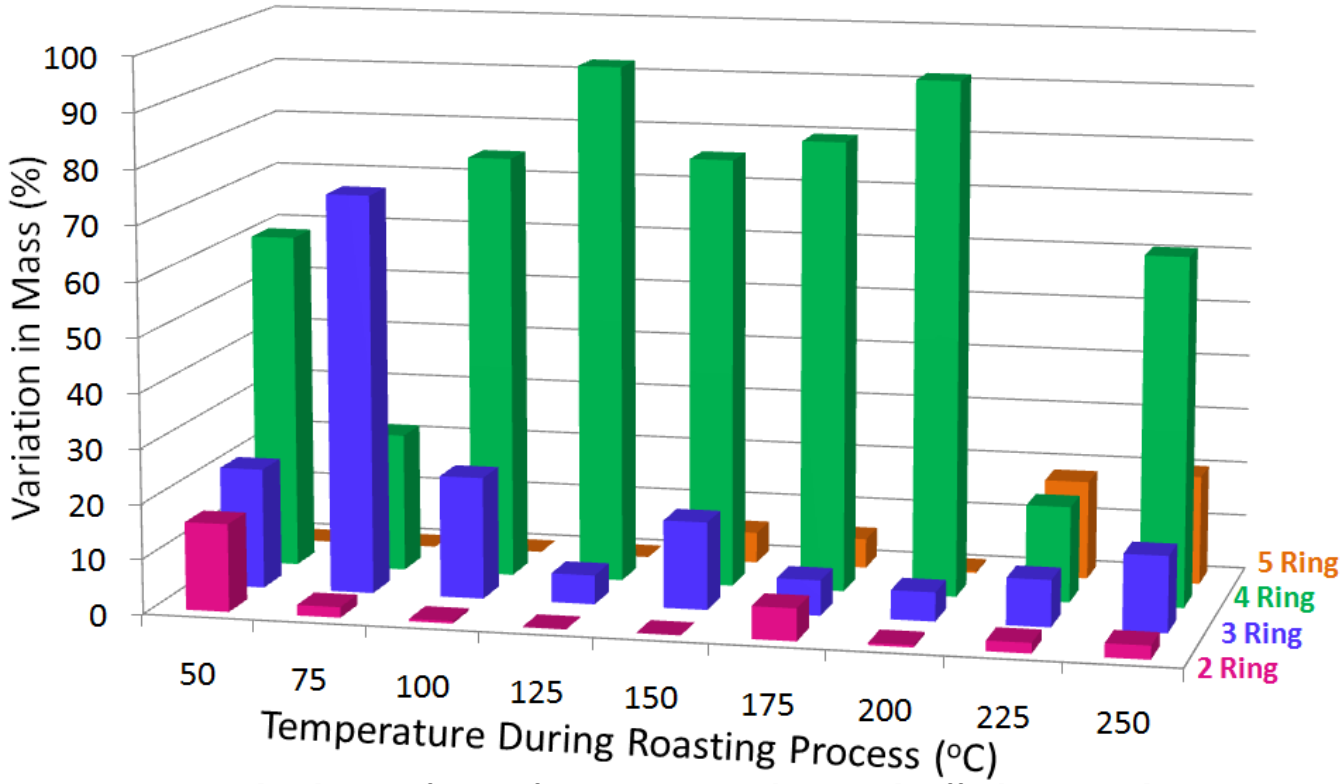


Fig. 4.12 Mass distribution of two to five ring PAHs in the roasted coffee bean samples (%age of ∑PAHs)

CONCLUSIONS

Green and Arabica roasted coffee beans are found to be contaminated by different levels of PAHs. Four PAHs viz., Fl, Pyr, PA and Ant were present in all coffee samples except in green beans. Sum total of 16 PAHs in roasted coffee beans at 250^{o}C is about 21 times than that found in green coffee beans. Four PAHs viz., BaP, IND, BghiP and DBA were not detected in all the coffee samples. Variation in mass percentage of 2-5 ring PAHs and its concentration (30.0-955.8 μg/kg) clearly showed that increase in temperature during roasting has created an impact.

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