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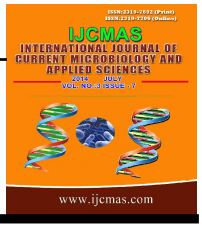

Original Research Article

Detection of polycyclic aromatic hydrocarbons levels in Egyptian meat and milk after heat treatment by Gas chromatography-mass spectrometry

Abou-Arab, A.A.K.[1]; Abou-Donia, M. A. M.[1]; El-Dars, F. M. S. E.[2]; Ali, O. I. M.[2] and Hossam A. Goda[3]

[1]Food Toxicology and Contaminants, National Research Center,
[2]Chemistry Department, Faculty of Science, Helwan University, Cairo Governorate, Egypt
[3]Quality Control Manager, Luna for Perfumes and Cosmetics (Lunapac)
**Corresponding author*

A B S T R A C T

The presence of polycyclic aromatic hydrocarbons (PAHs) in Egyptian beef meat and milk from different sources at Greater Cairo Urban Region were investigated. The PAHs' concentrations in non-boiled and boiled milk and meat samples were determined using gas chromatography- mass spectrometry. The present work indicated that the PAHs concentration in milk varied according to sample source, where the highest mean levels of PAHs was detected in raw milk from farm (1.011 µg/kg), followed by commercial raw milk (0.370 µg/kg). However, the lowest level was detected in pasteurized milk (0.198 µg/kg). Boiling had an important role for reducing PAHs concentration in milk and meat. The results showed that boiling decreased the levels of PAHs more efficiently with increasing boiling time. Regards to meat, the total PAHs was 2.611 µg/kg. Treatment of meat samples by boiling for 40 min reduced the total and carcinogenic PAHs by 14.52 and 2.69%, respectively. The reduction percentage of the total and carcinogenic PAHs by using food additives (salt, pepper, baharrat, cumin, rosemary and onion) as a pretreatment before boiling reached to 91.32% and 89.79%, respectively.

K e y w o r d s

PAHs,
Milk, Meat,
GC-MS,
Detoxification,
Boiling.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of chemicals composed of two or more fused rings that are mainly formed as a result of pyrolitic processes, especially the incomplete combustion of organic materials during industrial and other human activities such as processing of coal, vehicle traffic, cooking, and tobacco smoking (Wise et al., 1993; Mottier et al., 2000).

The quantity and composition of PAHs produced are closely related to the reaction conditions, temperature and amount of air, therefore, may vary considerably (Vaessen et al., 1988).

PAHs are classified as light or heavy based on the number of fused aromatic rings contained in their structure. The light PAHs

contain less than 4 member fused aromatic rings, while the heavy PAHs contain more than 4 member fused aromatic rings. The light PAHs are volatile, and volatility decreases with an increase in the number of aromatic rings in their structure. Heavy PAHs however, are more stable and are found in many environmental matrices. PAHs are generally lipophilic, with members of each class having similar character and subject to the same environmental fate. The stability and distribution of the PAHs in the natural environment are influenced by the chemical structure, chemical configuration and physic-chemical properties of them (Dabestani and Ivanov 1999).

The contaminated atmosphere by PAHs may lead to contamination of foods causing dangerous effects. Some PAHs are shown to have toxicological, carcinogenic and mutagenic effects on humans and animals. PAHs comprise the largest class of known chemical carcinogens. The relationship between cancer and the environment is largely conditioned by investigations involving PAHs exposures (Armstrong et al., 2004). PAHs can reach to the human body by different modes of exposure as direct inhalation of polluted air, ingestion of contaminated water, soil and ingestion of contaminated food. The movement of PAHs in the environment depends on properties such as how easily they dissolve in water, and how easily they evaporate into the air. In general, PAHs do not easily dissolve in water. They are present in air as vapors and are stuck to the surfaces of small solid particles. They can travel long distances before they return to earth with rainfall.

There have been investigations of PAHs contamination and transformation in milk and dairy products (Grova et al., 2000; Bulder et al., 2006). This transformation based on the often high levels in dried grass. Kan et al. (2003) performed an exploring study on the transfer of PAHs from feed to milk in lactating cows. They reported very low transfer of PAHs to milk. Acenaphthene, phenenthrene, fluoranthene, pyrene and chrysene were detected to some extent in the milk, but the more heavy PAHs were not present at the levels above the detection limit of 0.1 ng/g fat. PAHs have been also detected in different food types as meat (Yabiku et al., 1993; Moll et al., 1993).

The most commonly used techniques for PAHs determination are high-performance liquid chromatography with fluorescence detection (HPLC-FLD), gas chromatography with mass spectrometry (GC–MS), gas chromatography with flame ionization detector (GC-FID) and solid phase microextraction (SPME) followed by GC–MS (Kishikawa et al., 2003; Vigeas et al., 2012; Jira, 2004; Olatunji et al., 2014; Aguinaga et al., 2007).

The aim of the present study was to evaluate the levels of PAHs in some Egyptian beef meat and milk from different sources in Greater Cairo Urban Region and their influences by heat treatment. The effect of pretreatment of meat samples with different spices in the process of removing PAHs with boiling was also investigated.

Materials and Methods

Chemicals and Reagents

A mixture of 15 polyaromatic reference standards containing acenaphtene, acenaphthylene, anthracene, benzo(a)anthracene, benzo(a)pyrene, benzo(k)fluoranthene, benzo(ghi)perylene, chrysene, dibenzo(a,h) anthracene, fluoranthene, fluorene, indeno(1,2,3,-cd)pyrene, phenanthrene, pyrene and 2-bromonaphthalene was purchased from

Supleco Inc., USA., PAHs working solutions (10 µg/mL) was prepared from a stock solution of PAHs containing 200 µg/mL. Dichloromethane, n-hexane, cyclohexane, ethanol, acetonirile (Pestiscan Chromatography grade), anhydrous sodium sulphate, acetone (BDH chemicals), florisil (Magnesium silicate) were obtained from Merck, Germany.

Quality assurance procedures and precautions were carried out to ensure reliability of the results, all materials used for processing were screened by nitric acid, hot water, chromic acid, acetone and distilled water for possible removal of PAHs contamination according to the method of Tuteja et al., (2011).

Food samples

A total of 18 milk samples from different sources (raw milk from farm, commercial milk and pasteurized milk) were used in this study. Also, a total of 30 fattened beef meat slabs used in this study were collected from different regions in Greater Cairo Urban Region. All samples were collected during the period of January 2012 up to March 2012.

Food additives

Onion and spices (cumin, baharrat, black pepper and rosemary were obtained from the local market at Giza, Egypt. Salt (sodium chloride) was obtained from the Egyptian Salts and Minerals Co., Egypt. The spices were ground to a fine powder and the spices mixture was prepared by mixing equal weights from the former spices and then mixing again carefully. Mixture of sunflower oil and soya oil was purchased from Alexandria for oils Company, Egypt.

Samples preparation, extraction and clean-up of PAHs

The samples of milk and meat were quite representative since they collected from districts where foodstuffs were scattered throughout the different regions in Cairo. Sub-samples (1 kg for each) were taken randomly from the composite sample and used for analysis. The different weights of samples were homogenized separately for extraction and clean-up.

For milk, 10 g was mixed with 100 mL 0.4 M NaOH in ethanol: water (9:1 v/v) at 60 °C for 30 min and extracted twice with n-hexane according to the method of Kishikawa et al. (2003). The extract was evaporated to dryness and dissolved in 100 µL acetonitrile and filtered through a membrane filter (0.45 mm).

With respect to meat, the homogenized processed meat samples were allowed to stand and equilibrate to laboratory temperature. Then, 50 g was mixed with 2 M KOH in ethanol: water (9:1 v/v) and refluxed for 1.5 h. The saponificated material was rinsed with cyclohexane and water then concentrated according to the method of Chen et al. (1996).

The extracted milk and meat samples were cleaned up using activated florisil (60/100mesh) and anhydrous sodium sulphate and eluted by methylene chloride and n-hexane. The eluate was collected and evaporated to dryness by rotary evaporator at room temperature.

Instrumentation and analysis conditions

The dry eluate sample was dissolved in 1 mL n-hexane and injected into a Hewlett Packered Gas Chromatograph 5890 fitted with HP-5 fused silica capillary column (50

m x 0.2 mm i.d. x 0.3 µm film thickness) and connected to Hewlett Packered 5970 series mass selective detector. The carrier gas was helium at a flow rate of 1.0 mL/min. The injection port temperature was 275˚C with electron energy of 70 eV. The quadruple temperature was 280˚C. The instrument was tuned on perflorotributylamine (PFTBA). The oven programme was as follows; initial temperature 70 ˚C, continue for 5 min, followed by an increase to 290˚C at 3˚C/min, then finally hold for 30 min. Calibration was done using external standards (mixture of 15 compounds). The mass spectrometer was operated in selective ion monitoring mode using separate ions to identify and confirm compounds.

PAHs detoxification by boiling

From milk

Samples of farm milk were boiled (100°C) for 5, 10 and 15 min on open flame. The boiling procedure was carried out on naturally contaminated samples by high levels of PAHs.

From meat

Beef meat was divided into six equal portions (5.0 cm length x 4.0 cm width x 0.6 cm height, ≈ 50 g). The first portion treated by a mixture of equal quantities of salt, black pepper and baharrat. The second portion was treated by a mixture of equal quantities of salt, black pepper, baharrat and onion paste. The third portion was treated by a mixture of equal quantities of salt, black pepper, baharrat and cumin. The fourth portion was treated by a mixture of equal quantities of salt, black pepper, baharrat and rosemary. The fifth portion was treated by a mixture of equal quantities of salt, black pepper, baharrat, cumin, rosemary and onion paste. The sixth portion was control (boiling meat samples untreated by food additives). Each portion was boiled at 100 °C for 40 min (until well done cooking) after treating by the different spices for 1 h. The boiled samples were prepared as mentioned before to PAHs analysis.

Recovery

Recovery results of PAHs from milk and meat samples under investigation were studied according to the method of Chantara and Sangchan (2009). The results showed that the recovery average percentage was ranged between 95 to 97%.. The average of triplicate analysis was calculated for each PAH.

Statistical analysis

The data obtained from this study was statistically subjected to analysis of variance (ANOVA) and means separation was by Snedecor and Cochran (1980). The least significant difference (L.S.D) value was used to determine significant difference between means and to separate means at ($P \leq 0.05$) using SPSS package version 15.0.

Results and Discussion

PAHs in some Egyptian milk

PAHs levels in raw milk (farm and commercial) and pasteurized milk samples collected from local markets were determined (Table 1 and Fig. 1). Data indicated that PAHs concentrations in different samples are quite variable among the different type of the samples source. The results proved that, the highest mean levels of PAHs were detected in raw milk from farm (1.011 µg/kg) followed by commercial raw milk (0.370 µg/kg). On the other hand, the lowest level was detected in pasteurized

milk, which recorded 0.198 µg/kg. Data revealed that 2-bromonaphthalene, acenaphthylene, acenaphthene, fluorene, benzo(ghi)perylene and Indeno(1,2,3-cd)pyrene were not detected in any samples of milk under investigation. On the other hand, anthracene was detected only in commercial milk at mean level 0.029 µg/kg, while phenanthrene (0.296 µg/kg), chrysene (0.082 µg/kg) and benzo(k)fluorancene (0.045µg/kg) were detected only in the farm milk.

The present study indicated that pasteurized milk samples contained only fluoranthene at mean value 0.198 µg/kg. The obtained data proved that total carcinogenic PAHs were 0.291 and 0.465 µg/kg in raw farm and commercial milk, respectively but were not detected in pasteurized milk. The ratio of phenanthrene to pyrene shows that PAHs in farm milk result from pyrolytic process (Zohair, 2006). On the other hand, raw milk samples contained PAHs levels higher than that were detected in pasteurized milk, i.e. the raw milk that contain more triglyceride provide higher level of PAHs. These results are acceptable with those reported by Kishikawa et al. (2003). They showed that PAHs are incorporated in fats of milk owing to their lipophilic nature. Triglyceride is known to be main components of fats in commercial. In France, PAHs were found in milk at total levels of 37 and 27 ng/g fat (Grova et al., 2002)

Lutz et al. (2005) indicated that the parent compounds of PAHs did not detect, but found the hydroxy-metabolites from phenanthrene and pyrene in the milk from cows which were chronically exposed to PAHs through oral soil intake. They also concluded that it is likely that low molecular mass PAHs with less than 5 rings are transferred to the milk as native compound after oral exposure in addition; evidence from literature suggests that even more PAHs are transferred as metabolites, possibly including those of the high molecular mass PAHs.

PAHs in some Egyptian meat

PAHs levels in beef meat samples that collected from different locations in Greater Cairo Urban Region (GCUR) were determined. The data presented in Table 2, which indicated that total PAHs concentration was 2.611 µg/kg and total carcinogenic PAHs was 2.086 µg/kg. The results showed that the 3-4 rings PAHs are predominant in meat samples. While, 2-bromonaphthalene, acenaphthylene, acenaphthene, benzo(ghi)perylene and indeno(1,2,3-cd)pyrene were not detected in meat samples under investigation. Data also proved that the highest concentration of PAHs in the collected meat samples was chrysene (0.774 µg/kg). However, the lowest concentration of PAHs was anthracene (0.033 µg/kg). On the other hand, fluorene, phenanthrene, pyrene, fluoranthene, benzo(a)anthracene, benzo(k)fluorancene, benzo(a)pyrene and dibenzo(a,h)anthracene were detected in the collected meat samples.

The ratio of fluoranthene to pyrene was higher than 1, which means that PAHs from pyrolytic origin while the ratio of phenanthrene to anthracene was lower than 10, which means PAHs from combustion source. Similar results were obtained by Chung et al. (2011) who reported that the average PAHs levels in beef did not exceed 0.80 µg/kg. This indicates that PAHs levels in uncooked food largely depend on the origin of the food and can be subject to regional variations. El-Badry (2010) demonstrated that the total PAHs in chicken meat was of low level (0.209 µg/kg) in agreement with WHO, (1998) that mentioned raw foods are not associated with high level of PAHs.

Detoxification of PAHs from milk and meat by boiling

The role of boiling at 100°C for 5, 10 and 15 min on PAHs levels in milk was investigated and the results presented in Table 3 and illustrated in Fig. 2. The results demonstrated the efficient role of boiling process in reduction of PAHs from contaminated samples.

The role of boiling at 100 °C for 40 min on PAHs levels in meat was studied and data presented in Table 4. The results demonstrated that the efficient role of boiling process on the reduction of PAHs in contaminated meat samples without (control) or with food additives for 1 h before boiling like salt, pepper and baharrat, cumin, rose marry, onion was clear. It was found that the reduction percentages of total and carcinogenic PAHs after boiling meat samples without additives were 14.52 and 2.69%, respectively. The results showed that the treatment of meat samples with a mixture of salt, pepper, baharrat and a mixture of salt, pepper and baharrat, cumin, rose marry, onion had a similar effect on removing of the total carcinogenic PAHs after boiling; where reduction percentages reached to 89.52 and 89.79, respectively. Also, it can be concluded from the results represented in Table 4 that the most effective treatment of meat before boiling was by using all food additives (salt, pepper and baharrat, cumin, rosemary and onion) and this resulted in reduction of total PAHs and total carcinogenic PAHs by 91.32 and 89.79%, respectively.

In conclusion, the present investigation showed the efficient role of boiling in the reduction of PAHs from the investigated foodstuffs. For raw milk samples, the data indicated that boiling decreased the levels of PAHs more efficiently with increasing boiling time. These results proved that the levels of PAHs compounds in beef meat samples decreased during boiling process with or without additives. The maximum reduction was achieved in case of pretreatment of meat samples with all food additives (spices).

It could be recommended that heat treatment play an important role in decreasing some contaminants as PAHs and may be effective in the reduction of the daily intake of PAHs containing food.

Fig.1 Levels of PAHs in some Egyptian milk samples from different sources in Cairo

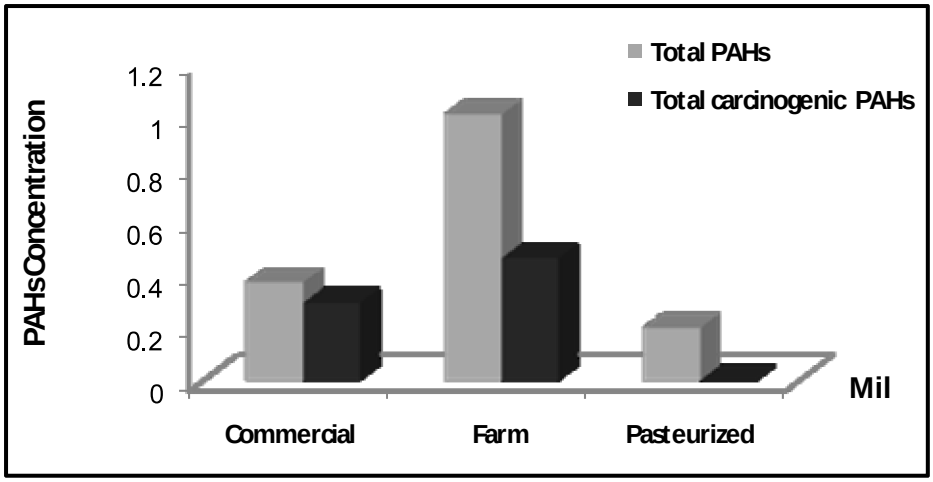

Table.1 Levels of PAHs in some Egyptian milk samples from different sources of Greater Cairo Urban Region (GCUR)

PAHs Compounds	Mean concentration (µg/kg) ± SD		
	Farm milk	Commercial milk	Pasteurized milk
2-Bromonaphthalene	ND	ND	ND
Acenaphthylene	ND	ND	ND
Acenaphthene	ND	ND	ND
Fluorene	ND	ND	ND
Anthracene	ND	0.029±0.01	ND
Phenanthrene	0.296±0.03	ND	ND
Pyrene	0.101±0.01	0.050±0.01	ND
Fluoranthene	0.149±0.02	ND	0.198±0.02
Chrysene**	0.082±0.01	ND	ND
Benzo(a)anthracene*	0.203±0.02	0.255±0.02	ND
Benzo(k)fluorancene**	0.045 ±0.01	ND	ND
Benzo(a)pyrene*	0.091±0.01	0.023±0.01	ND
Dibenzo(a,h)anthracene*	0.044±0.02	0.013±0.01	ND
Benzo(ghi)perylene	ND	ND	ND
Indeno(1,2,3,-cd)pyrene**	ND	ND	ND
Total PAHs	1.011	0.370	0.198
Total carcinogenic PAHs	0.465	0.291	ND

ND: Not detectable. SD: Standard Deviation

* IARC Group 2a: Probably carcinogenic to human according to IARC.

** IARC group 2b: Possibly carcinogenic to humans according to IARC.

* & ** classified as carcinogenic to human by US EPA and WHO/IPCS.

Table.2 Levels of PAHs in some Egyptian raw meat samples collected from Greater Cairo Urban Region (GCUR)

PAHs Compounds	Mean concentration (µg/kg) ± SD
2-Bromonaphthalene	ND
Acenaphthylene	ND
Acenaphthene	ND
Fluorene	0.081±0.01
Anthracene	0.033±0.01
Phenanthrene	0.190±0.02
Pyrene	0.108±0.01
Fluoranthene	0.113±0.01
Chrysene**	0.774±0.06
Benzo(a)anthracene*	0.586±0.06
Benzo(k)fluorancene**	0.110±0.01
Benzo(a)pyrene*	0.519±0.05
Dibenzo(a,h)anthracene*	0.097±0.01
Benzo(ghi)perylene	ND
Indeno(1,2,3,-cd)pyrene**	ND
Total PAHs	2.611
Total carcinogenic PAHs	2.086

ND: Not detectable. SD: Standard Deviation

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Fig.2 Mean of PAHs levels (µg/kg) in milk and reduction percentage after boiling

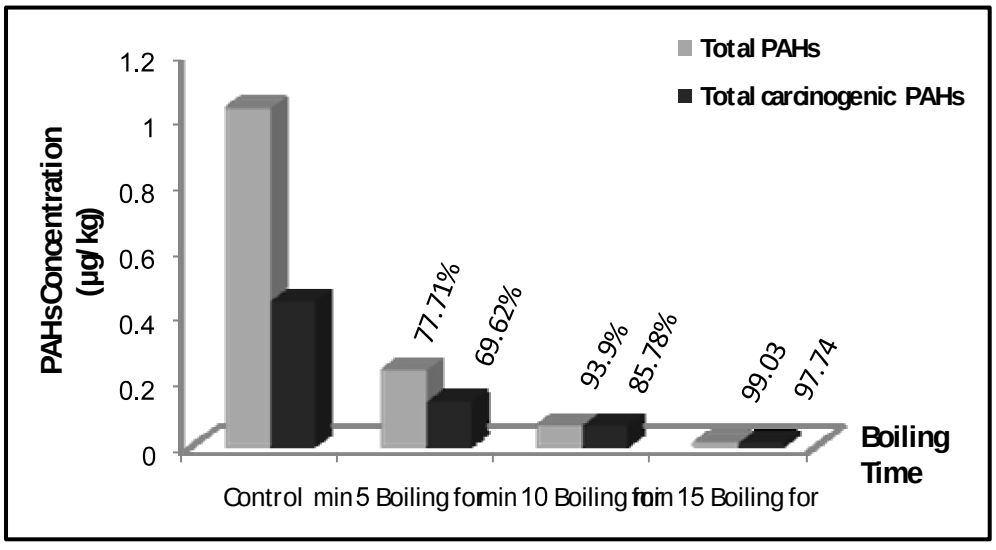

Table.3 Mean of PAHs levels (µg/kg) in milk and reduction percentage after boiling

PAHs compounds	Mean concentration (µg/kg)			
	Control	Boiling for 5 min	Boiling for 10 min	Boiling for 15 min
2-Bromonaphthalene	ND	ND	ND	ND
Acenaphthylene	ND	ND	ND	ND
Acenaphthene	ND	ND	ND	ND
Fluorene	ND	ND	ND	ND
Anthracene	0.063 ± 0.02	ND (100%)***	ND (100%)	ND (100%)
Phenanthrene	0.273 ± 0.03	ND (100%)	ND (100%)	ND (100%)
Pyrene	0.109 ± 0.02	ND (100%)	ND (100%)	ND (100%)
Fluoranthene	0.144 ± 0.02	0.095 ± 0.01 (33.75%)	ND (100%)	ND (100%)
Chrysene**	0.086 ± 0.01	ND (100%)	ND (100%)	ND (100%)
Benzo(a)anthracene*	0.196 ± 0.02	0.116 ± 0.02 (40.82%)	0.063 ± 0.01 (67.86%)	0.010 ± 0.01 (94.90%)
Benzo(k)fluorancene**	0.038 ± 0.01	0.019 ± 0.01 (51.05%)	ND (100%)	ND (100%)
Benzo(a)pyrene*	0.082 ± 0.01	ND (100%)	ND (100%)	ND (100%)
Dibenzo(a,h)anthracene*	0.041 ± 0.01	ND (100%)	ND (100%)	ND (100%)
Benzo(ghi)perylene	ND	ND	ND	ND
Indeno(1,2,3,-cd)pyrene **	ND	ND	ND	ND
Total PAHs	1.032	0.230 (77.71%)	0.063 (93.90%)	0.010 (99.03%)
Total carcinogenic PAHs	0.443	0.135 (69.62%)	0.063 (85.78%)	0.010 (97.74%)

ND: Not detectable. SD: Standard Deviation ***: Reduction percentage

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Table.4 Mean of PAHs levels (µg/kg) in meat and reduction percentage after boiling

PAHs compounds	Mean concentration (µg/kg) ± SD						
	Raw meat	Control	Boiling with food additives				
			1	2	3	4	5
2-Bromonaphtha-lene	ND	ND	ND	ND	ND	ND	ND
Acenaphthylene	ND	ND	ND	ND	ND	ND	ND
Acenaphthene	ND	ND	ND	ND	ND	ND	ND
Fluorene	0.072 ± 0.01	ND (100%)	ND (100%)	ND (100%)***	ND (100%)	ND (100%)	ND (100%)
Anthracene	0.052 ± 0.01	ND (100%)	ND (100%)	ND (100%)	ND (100%)	ND (100%)	ND (100%)
Phenanthrene	0.337 ± 0.04	0.086 ± 0.01 (74.44%)	0.184 ± 0.02 (45.32%)	0.247 ± 0.03 (26.60%)	0.188 ± 0.02 (44.13%)	0.100 ± 0.01 (70.28%)	ND (100%)
Pyrene	ND	ND	ND	ND	ND	ND	ND
Fluoranthene	ND	ND	ND	ND	ND	ND	ND
Chrysene**	1.069 ± 0.09	0.889 ± 0.05 (16.88%)	0.032 ± 0.01 (97.01%)	0.158 ± 0.02 (85.23%)	0.176 ± 0.01 (83.54%)	0.307 ± 0.03 (71.29%)	0.227 ± 0.02 (78.78%)
Benzo(a)anthracene*	0.977 ± 0.07	0.682 ± 0.06 (30.23%)	ND (100%)	ND (100%)	0.041 ± 0.01 (95.81%)	ND (100%)	ND (100%)
Benzo(k)fluorancene**	ND	ND	ND	ND	ND	ND	ND
Benzo(a)pyrene*	0.467 ± 0.05	0.379 ± 0.02 (18.93%)	0.241 ± 0.02 (48.45%)	0.588 ± 0.06 (-25.78%)	0.092 ± 0.01 (80.32%)	0.176 ± 0.02 (62.35%)	0.039 ± 0.01 (91.66%)
Dibenzo(a,h)anthracene*	0.091 ± 0.01	0.584 ± 0.05	ND (100%)	0.059 ± 0.01 (35.52%)	ND (100%)	0.159 ± 0.02	ND (100%)
Benzo(ghi) perylene	ND	ND	ND	ND	ND	ND	ND
Indeno(1,2,3,-cd)pyrene**	ND	ND	ND	ND	ND	ND	ND
Total PAHs	3.065	2.620 (14.52%)	0.457 (85.09%)	1.052 (65.68%)	0.497 (83.79%)	0.742 (75.80%)	0.266 (91.32%)
Total carcinogenic PAHs	2.604	2.534	0.273	0.805	0.309	0.642	0.266

ND: Not detectable. SD: Standard Deviation ***:Reduction percentage

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1 Boiling with salt, pepper and baharrat

2 Boiling with salt, pepper, baharrat and cumin

3 Boiling with salt, pepper, baharrat and rose marry

4 Boiling with salt, pepper, baharrat and onion

5 Boiling with salt, pepper, baharrat, cumin, rosemary and onion.

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