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GC-MS analysis of PAHs in charcoal grilled rabbit meat with and without additives

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Abstract

Rabbit meat is a good source of protein as compared to beef and chicken. The processing of rabbit meat gives attractive texture with a strong flavor. Charcoal grilling is a well-known cooking method which results in the formation of polycyclic aromatic hydrocarbons. Therefore, to determine the concentration of PAHs, rabbit meat samples were prepared with and without additives and analyzed for six PAHs (naphthalene, fluorene, phenanthrene, anthracene, fluoranthene, and pyrene) using gas chromatography-mass spectrometry (GC-MS). Naphthalene was dominant in all the samples and the highest naphthalene concentration (2.61 µg/g) was found in the foreleg sample (without additives) due to the presence of higher fat molecules. PAHs concentration was found to be significantly ($p \leq 0.05$) lower by the addition of antioxidants. Lowest naphthalene concentration was observed in the back sample of seekh kebab (0.77 µg/g) with 1 h marinating duration. The content of pyrene was only noticed in foreleg (0.01 µg/g) and hind leg (0.03 µg/g) samples from reshmi kebab. The content of fluorene was not observed in all the samples.

Keywords: polycyclic aromatic hydrocarbons; charcoal grilling; GC-MS; food samples; antioxidant spices; vegetables.

Practical Application: More distance between heat source and sample, use of antioxidants show a significant reduction in PAH.

1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) have hydrophobic that consists of two or more fused benzene rings. PAHs are environmental pollutants which present in raw food through contaminated air, soil, and water (Chen & Chen, 2001). Various PAHs have been identified, but some of them have been proved to be carcinogenic and mutagenic to mammals. PAHs are formed when meat is processed under high temperatures above 200 °C, such as charcoal grilling, grilling, and frying (Alomirah et al., 2011).

Rabbit meat is used as a source of nutrition because of its high protein content and minerals. Processing of rabbit meat gives attractive texture with mild flavor and aroma. Charcoal grilling is a cooking method that imparts magic flavor, improves the appearance and color of the food. In this method, meat is heated over the direct flame and it releases lipid drippings that strike the super-hot charcoal and yield a burst of flame (Chen & Chen, 2001). The wood smoke contains numerous chemicals including aldehyde, acetic acid, phenols, and a variety of PAHs (Lingbeck et al., 2014). From diversified PAHs, naphthalene was formed in large concentrations than other PAHs during grilling (Ferrarese et al., 2008). Naphthalene (group 2B) is considered as possibly carcinogenic to humans (World Health Organization, 2002).

The consumption rate of grilled meat is becoming high and caused health problems (Kao et al., 2014). Numerous studies have reported the occurrence of PAH in a variety of food including beef, chicken (Ahmad Kamal et al., 2018; Duedahl-Olesen et al., 2015; Farhadian et al., 2010; Kafouris et al., 2020), pork (Duedahl-Olesen et al., 2015), duck (Chen & Lin, 1997), fish (Kamankesh et al., 2015) and sausages (Lorenzo et al., 2011). These studies confirmed that charcoal-grilled food possessed the highest PAH concentration than in oven or flame gas grilled meat. PAHs content in traditionally home-prepared dishes mainly depends upon various factors, such as; fat content, cooking method and time, type of heat source (Purcaro et al., 2013).

Therefore, to contribute information to this field, this first survey on rabbit meat includes PAHs analysis. The purpose of this study was to evaluate the PAHs concentration in rabbit meat samples. The PAHs from cooked rabbit meat and its raw meat were identified and quantified for the first time in Pakistan and this study provided the needed guideline information on PAHs in rabbit meat. Various cooking recipes including kebabs (without additives), recipe I (seekh kebab), and recipe II (reshmi kebab) were used for meat preparation. PAH contents were evaluated *via* the GC-MS technique.

2 Materials and methods

2.1 Standards and reagents

PAH standards: fluorene was purchased from Alfa Aesar, anthracene from BDH, fluoranthene, naphthalene, and phenanthrene from Fluka and pyrene from Hopkin Williams

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LTD. HPLC grade *n*-hexane, methanol, potassium hydroxide, dichloromethane, and anhydrous sodium sulfate were purchased from Daejung and silica gel 60 (70-230 mesh ASTM) was from Scharlau. HPLC grade acetonitrile and sodium sulfide nonahydrate were purchased from Sigma Aldrich.

2.2 Samples

The list of traditional meat recipes is shown in Table 1. Samples of meat (hindleg, back, and foreleg) were cooked by charcoal grilling. For sample preparation, twelve rabbits (each weighing approx. 400 g) were bought from the local supermarket of Faisalabad, Pakistan. The meat samples (hindleg, back, and foreleg) were washed with water and removed fat layers and bones. After drying, the meat samples were separately minced for 2 min by using a Waring food blender (Milford, MA, USA). Each part of the meat sample was weighed equally, placed in nylon plastic bags and kept in the refrigerator at -20 °C (Duedahl-Olesen et al., 2015). Every sample including three replicates (total of 36 samples) was analyzed for PAHs.

2.3 Extraction and clean up

The sample preparation for the detection of PAHs concentration is presented in Figure 1. The clean-up method, used to separate PAHs fractions from the rabbit meat samples, is a modification of a method utilized by Chung et al. (2011). Sample of 30 g meat was homogenized and saponified with 2 M potassium hydroxide solution in the methanol-water ratio (9:1, v/v) (100 mL) followed by the addition of 2 g of $Na_2S.9H_2O$. The sample was refluxed for 2 h in a water bath at 70 °C. 100 mL of *n*-hexane was added through the condenser. After 15 min, the mixture was cooled by the addition of 100 mL of cold water. The sample mixture was kept in dark for the whole night. The sample was concentrated with 60 mL *n*-hexane and extracted the organic layer. The extraction procedure was repeated twice with 30 mL *n*-hexane and the *n*-hexane layer was dried over anhydrous sodium sulfate and then filtered. The *n*-hexane layer was concentrated to about 2 mL by using a rotavap at 35 °C. The concentrate was purified through column chromatography. This methodology used silica gel as the stationary phase and organic solvents as the mobile phase. To pack the column, the slurry of silica gel was made by adding 40 mL *n*-hexane in 20 g of silica gel and then poured into the column. When the slurry of silica gel was settled down, added the sample mixture on the column. The elution of PAHs was carried out by passing *n*-hexane (50 mL) followed by *n*-hexane-dichloromethane (3:1, v/v) (8 mL). The solvent was concentrated to approximately 1 mL using rotavap and filtered through a microporous syringe (0.45 µm)

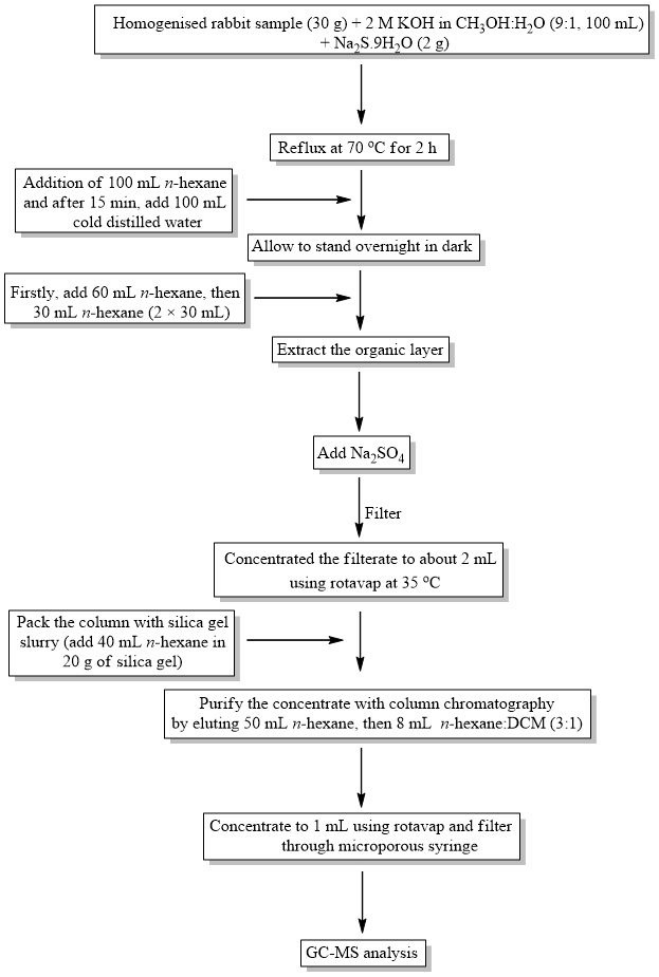

Figure 1. Experimental flowchart.

Table 1. Description of traditional meat recipes used in this study.

Food item	Description
Cooked	Minced meat (hindleg, back, and foreleg) without additives were grilled for 10 min on each side by using the grill (garden-type) fuelled with charcoal. After cooling, samples were packed in nylon plastic bags and used for the cleanup study
Recipe I (Seekh kebab)	Samples (hindleg, back, and foreleg) were minced and marinated with onion (4.48 g), ginger (0.26 g), garlic (0.26 g), mustard powder (0.07 g), turmeric powder (0.03 g), black pepper powder (0.15 g) and salt (0.27 g) for 1 h. The seekh kebabs were cooked for 10 min by using the grill (garden-type) fuelled with charcoal at a distance of 9 cm from heat source. After cooling, samples were packed in nylon plastic bags and used for the cleanup study.
Recipe II (Reshmi kebab)	Minced meat (hindleg, back, and foreleg) were mixed with garlic (0.17 g), ginger (0.17 g), and raw papaya (0.8 g). The ground mixture of fresh coriander leaves and mint (0.5 g), green chili (0.31 g), onion (4.48 g), butter (2 g), coconut powder (0.21 g), an aromatic mixture of spices (0.14 g), salt (0.27 g) and red chili powder (0.16 g) were added into the minced meat and then mixed. The reshmi kebabs were grilled for 10 min on each side by using the grill (garden-type) fuelled with charcoal at a distance of 9 cm from heat source. After cooling, samples were packed in nylon plastic bags and used for the cleanup study

in vials (Janoszka, 2011). Vials were kept in the refrigerator at -20 °C for gas chromatography-mass spectrometry analysis (Alomirah et al., 2011; Duedahl-Olesen et al., 2015).

2.4 GC-MS analysis

Quantification of PAHs in rabbit meat samples was done by using a GC-MS method presented by Kao et al. (2014) with minor modifications. An Agilent brand (7890B) Gas Chromatograph DB-5MS capillary column (30 m, 0.25 mm ID, 0.25 μm film thickness) coupled with Mass Spectrometer (5977A) was used to isolate six PAHs in meat samples. The sample solution (1 μL) was injected in a splitless mode by using helium (purity > 99.995%) as a carrier gas with a flow rate of 1 mL/min. The conditions of GC-MS operation for the separation of PAHs listed below: oven temperature 80 °C for 1 min, rate 25 °C/min to 260 °C/min, rate 10 °C/min to 300 °C for 6.3 min and detector temperature: 150 °C for ion source and 230 °C for quadrupole and 150 °C for ion source. While the minimum detection limit for each component during this study was 0.009 μg/g. The quantification was carried out by using the calibration method.

The stock solutions of naphthalene, fluorene, phenanthrene, anthracene, fluoranthene, and pyrene were prepared. The stock solution (100 μg/mL) was used to prepare by diluting 100 μg of each standard compound in acetonitrile (up to 1 mL). The working standard solution (20 μg/mL) was prepared from 1 mL of each stock standard solution and diluted up to 5 mL acetonitrile. These PAH standard solutions were used for GC-MS analysis to evaluate the concentration of these six PAH compounds in rabbit meat samples. Standard solutions were used to calculate the calibration curve.

To compare the concentration of PAHs in four types of samples (raw, cooked without additives, seekh kebab, reshmi kebab), the results of the evaluations were shown in the similar units, i.e., μg/g.

2.5 Statistical analysis

Two way ANOVA (Analysis of variance) was carried out for comparing different categories and food samples. The analysis was carried out using the statistical software Minitab 13.2.

3 Results and discussion

The quantitation of six *P*AHs, naphthalene, fluorene, phenanthrene, anthracene, fluoranthene, and pyrene in meat samples was carried out from the chromatograms which are shown in Figure 2.

The mean values of different PAHs (in μg/g) are given in Table 2. Samples were grouped according to the local kebab dishes listed in Table 2.

As shown in Table 2, the average contents of PAHs in raw samples (hindleg, back, and foreleg) ranged from 0.05 to 0.24 μg/g. Among six PAHs in raw samples, naphthalene was significantly ($p \leq 0.05$) higher than fluoranthene and phenanthrene. Napthalene concentration in the back raw sample was significantly ($p \leq 0.05$) lower than hindleg and foreleg. The level of anthracene concentration was only observed in back raw samples. Our findings are consistent with those obtained by Kao et al. (2014) who investigated the concentration of few PAHs (naphthalene, fluoranthene, phenanthrene, and fluorene) in raw poultry meats. Raw food can be contaminated with PAH through the air, soil, and water (Janoszka, 2011).

After the charcoal grilling, the content of naphthalene was significantly ($p \leq 0.05$) higher. This is because of fat dripping on fire which resulted in the highest PAH formation (Aaslyng et al., 2013). The grilling of sample meat in an open flame or direct flame also produced NPAHs (nitrated PAHs) which are more carcinogenic to humans than PAHs (Haiba et al., 2019). Besides the direct flame, the distance between a heat source and the sample is another significant factor. The analyzed sample grilled at a distance of 9 cm from a charcoal source produced high content of PAHs. An earlier study has demonstrated that smoking under a distance of 5 m contained more PAH levels (Hokkanen et al., 2018). The mean concentration of naphthalene in foreleg samples (without additives) was significantly ($p \leq 0.05$) higher, whereas naphthalene concentration was 1.91 μg/g in hindleg samples and 1.18 μg/g in back samples. Because the foreleg rabbit meat sample contains more fat than hindleg and back (Pla et al., 2004). The content of fluoranthene in the hindleg and foreleg sample was significantly ($p > 0.05$) the same.

The use of a variety of additives, a recipe I (seekh kebab), and recipe II (reshmi kebab) were prepared. Additives are used in kebab dishes for enhancing the flavor and taste of food. Results indicated that the addition of additives causes a decrease of naphthalene concentration in seekh and reshmi kebab (Janoszka, 2011). By comparing both the recipes, naphthalene concentration was significantly ($p \leq 0.05$) lower in rabbit samples from recipe I. Results showed that marination effect is responsible for the lowest PAH generation (Alomirah et al., 2011; Farhadian et al., 2012). The meat of recipe I marinated with highly antioxidant spices and herbs including garlic, ginger, pepper, onion, turmeric, and the mustard powder was possessed low PAH levels (El-Badry, 2010). However, the anthracene concentration in hindleg, back (grilled) and foreleg of recipe I (seekh kebab) samples was significantly ($p > 0.05$) same.

The foreleg sample from recipe II (reshmi kebab) contained 1.94 μg/g naphthalene concentration which is significantly ($p \leq 0.05$) higher than hindleg and back grilled reshmi kebab samples. In recipe II, spices as well as vegetables such as fresh mint and coriander leaves were used. Vegetables waxy surface exhibit low molecular weight PAHs molecules (European Commission, 2002). In another study, 19 PAHs as contaminants were determined in mint leaves (European Food Safety Authority, 2015). So, the use of vegetables especially green vegetables is responsible for increasing the PAH levels in recipe II. Phenanthrene concentration in investigated meat samples also varied, such as for recipe II hindleg samples (reshmi kebab) (0.51 μg/g), foreleg (0.36 μg/g) and back (0.23 μg/g) samples. However, the fluoranthene content in back grilled samples (reshmi kebab) was significantly ($p \leq 0.05$) lower, while in foreleg and hindleg samples, the concentration of fluoranthene was not significantly ($p > 0.05$) different. The pyrene concentration was observed only in foreleg and hindleg samples from recipe II (reshmi kebab).

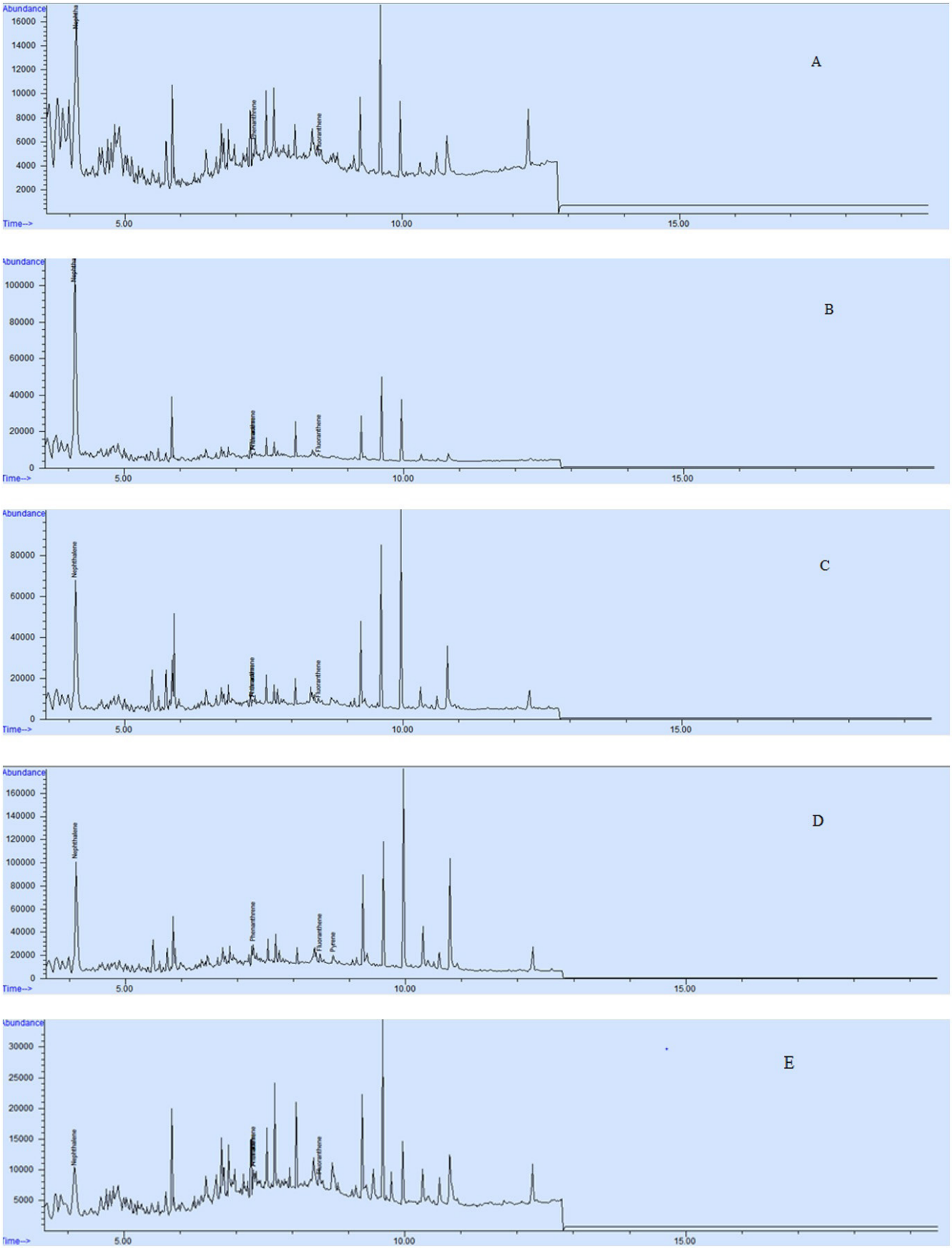

Figure 2. GC-MS chromatograms (A) Raw foreleg (B) grilled foreleg sample (C) seekh kebab of the foreleg (D) reshmi kebab of foreleg sample (E) mixed standards.

Table 2. Mean ± SE values of concentrations of different PAHs in rabbit meat samples (µg/g of grilled. Meat).

Category	Food Samples	Total Samples	Naphthalene (µg/g)	Fluorene (µg/g)	Phenanthrene (µg/g)	Anthracene (µg/g)	Fluoranthene (µg/g)	Pyrene (µg/g)
Raw Samples	Hindleg	3	$0.23 \pm 0.02_{b}^{c}$	BDL*	$0.13 \pm 0.01_{a}^{b}$	BDL	$0.05 \pm 0.003_{b}^{a}$	BDL
	Back	3	$0.15 \pm 0.012_{a}^{c}$	BDL	$0.19 \pm 0.012_{b}^{d}$	0.01_{a}^{b}	$0.05 \pm 0.006_{b}^{a}$	BDL
	Foreleg	3	$0.24 \pm 0.01_{b}^{c}$	BDL	$0.11 \pm 0.01_{a}^{b}$	BDL	$0.04 \pm 0.003_{a}^{a}$	BDL
Cooked	Hindleg	3	$1.91^{a} \pm 0.007_{f}^{d}$	BDL	$0.21 \pm 0.003_{bc}^{c}$	0.01_{a}^{a}	$0.06 \pm 0.003_{c}^{b}$	BDL
	Back	3	$1.18 \pm 0.65_{d}^{d}$	BDL	$0.18 \pm 0.013_{b}^{c}$	0.01_{a}^{a}	$0.04 \pm 0.003_{a}^{b}$	BDL
	Foreleg	3	$2.61 \pm 1.23_{g}^{c}$	BDL	$0.22 \pm 0.03_{c}^{b}$	0.05_{c}^{a}	$0.06 \pm 0.007_{bc}^{a}$	BDL
Recipe I (Seekh kebab)	Hindleg	3	$1.26 \pm 0.003_{d}^{d}$	BDL	$0.26 \pm 0.003_{d}^{c}$	0.04_{b}^{a}	$0.06 \pm 0.003_{c}^{b}$	BDL
	Back	3	$0.77 \pm 0.003_{c}^{d}$	BDL	$0.28 \pm 0.003_{e}^{c}$	0.05_{c}^{a}	$0.07 \pm 0.003_{d}^{b}$	BDL
	Foreleg	3	$1.37 \pm 0.007_{d}^{d}$	BDL	$0.21 \pm 0.007_{bc}^{c}$	0.01_{a}^{a}	$0.06 \pm 0.003_{c}^{b}$	BDL
Recipe II (Reshmi kebab)	Hindleg	3	$1.66 \pm 0.003_{e}^{d}$	BDL	$0.51 \pm 0.007_{g}^{c}$	BDL	$0.13 \pm 0.003_{e}^{b}$	0.03_{b}^{a}
	Back	3	$1.66 \pm 0.303_{e}^{d}$	BDL	$0.29 \pm 0.007_{e}^{c}$	0.06_{d}^{a}	$0.07 \pm 0.003_{d}^{b}$	BDL
	Foreleg	3	$1.94 \pm 0.003_{f}^{c}$	BDL	$0.36 \pm 0.03_{f}^{b}$	BDL	$0.11 \pm 0.003_{e}^{a}$	0.01_{a}^{a}

*below detection limit (0.009 µg/g). Different letters in subscript represent significant ($p < 0.05$) differences within the same column. Different alphabet letters in superscript represent significant different ($p \leq 0.05$) with the same row.

In a previous study by Janoszka (2011) charcoal-grilled chicken breast contained fluoranthene concentration of 0.012 µg/g. Furthermore, Alomirah et al. (2011) found that naphthalene, phenanthrene, and anthracene were present at concentrations of 0.02 µg/g, 0.01 µg/g, and 0.05 µg/g in grilled foods respectively. While comparing different studies with our study, it can be observed that the charcoal-grilled rabbit meat contains the highest concentration of PAHs. Generation of PAHs in higher amount is due to the presence of higher fat molecules (Pla et al., 2004), fat dripping on charcoal, a distance of meat from a heat source (Aaslyng et al., 2013; Reinik et al., 2007) and the use of green vegetables.

4 Conclusions

The present study reports, for the first time, the formation of PAHs in charcoal grilled rabbit meat. Charcoal grilled samples exhibit the highest PAH contents. The results obtained from this study strongly specified the effect of heat source, fat molecules, distance between food sample and heat source, the use of antioxidant spices as well as green vegetables on PAH generation. Among six PAHs, naphthalene content was dominated in examined samples. Higher the fat molecules in foreleg samples, the more PAH concentration was produced. By the addition of antioxidant additives with 1 h marinating duration, there was a significant ($p \leq 0.05$) PAH reduction in grilled samples. The use of green vegetables, PAH level in recipe II was increased. Content of fluorene was not determined in all the samples. Concentration of pyrene was only observed in hindleg and foreleg samples from reshmi kebab. However, conducting more studies is in process to find out the best cooking treatment which are responsible for PAH reduction in rabbit meat.

References

Aaslyng, M. D., Duedahl-Olesen, L., Jensen, K., & Meinert, L. (2013). Content of heterocyclic amines and polycyclic aromatic hydrocarbons in pork, beef and chicken barbecued at home by Danish consumers. *Meat Science*, 93(1), 85-91. http://dx.doi.org/10.1016/j.meatsci.2012.08.004. PMid:22910802.

Ahmad Kamal, N. H., Selamat, J., & Sanny, M. (2018). Simultaneous formation of polycyclic aromatic hydrocarbons (PAHs) and heterocyclic aromatic amines (HCAs) in gas-grilled beef satay at different temperatures. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment*, 35(5), 848-869. http://dx.doi.org/10.1080/19440049.2018.1425553. PMid:29334335.

Alomirah, H., Al-Zenki, S., Al-Hooti, S., Zaghloul, S., Sawaya, W., Ahmed, N., & Kannan, K. (2011). Concentrations and dietary exposure to polycyclic aromatic hydrocarbons (PAHs) from grilled and smoked foods. *Food Control*, 22(12), 2028-2035. http://dx.doi.org/10.1016/j.foodcont.2011.05.024.

Chen, B. H., & Chen, Y. C. (2001). Formation of polycyclic aromatic hydrocarbons in the smoke from heated model lipids and food lipids. *Journal of Agricultural and Food Chemistry*, 49(11), 5238-5243. http://dx.doi.org/10.1021/jf0106906. PMid:11714310.

Chen, B., & Lin, Y. S. (1997). Formation of polycyclic aromatic hydrocarbons during processing of duck meat. *Journal of Agricultural and Food Chemistry*, 45(4), 1394-1403. http://dx.doi.org/10.1021/jf9606363.

Chung, S., Yettella, R. R., Kim, J., Kwon, K., Kim, M., & Min, D. B. (2011). Effects of grilling and roasting on the levels of polycyclic aromatic hydrocarbons in beef and pork. *Food Chemistry*, 129(4), 1420-1426. http://dx.doi.org/10.1016/j.foodchem.2011.05.092.

Duedahl-Olesen, L., Aaslyng, M., Meinert, L., Christensen, T., Jensen, A., & Binderup, M.-L. (2015). Polycyclic aromatic hydrocarbons (PAH) in Danish barbecued meat. *Food Control*, 57, 169-176. http://dx.doi.org/10.1016/j.foodcont.2015.04.012.

El-Badry, N. J. (2010). Effect of household cooking methods and some food additives on polycyclic aromatic hydrocarbons (PAHs) formation in chicken meat. *World Applied Sciences Journal*, 9(9), 963-974.

European Commission. (2002). *Opinion of the scientific committee on food on the risks to human health of polycyclic aromatic hydrocarbons in food*. Luxembourg: Health & Consumer Protection Directorate-General, Scientific Committee on Food.

European Food Safety Authority – EFSA. (2015). Statement on the benefits of fish/seafood consumption compared to the risks of methylmercury in fish/seafood. *EFSA Journal*, 13(1), 3982. http://dx.doi.org/10.2903/j.efsa.2015.3982.

Farhadian, A., Jinap, S., Abas, F., & Sakar, Z. I. (2010). Determination of polycyclic aromatic hydrocarbons in grilled meat. *Food Control*, 21(5), 606-610. http://dx.doi.org/10.1016/j.foodcont.2009.09.002.

Farhadian, A., Jinap, S., Faridah, A., & Zaidul, I. J. (2012). Effects of marinating on the formation of polycyclic aromatic hydrocarbons (benzo[a]pyrene, benzo[b]fluoranthene and fluoranthene) in grilled beef meat. *Food Control*, 28(2), 420-425. http://dx.doi.org/10.1016/j.foodcont.2012.04.034.

Ferrarese, E., Andreottola, G., & Oprea, I. A. J. (2008). Remediation of PAH-contaminated sediments by chemical oxidation. *Journal of Hazardous Materials*, 152(1), 128-139. http://dx.doi.org/10.1016/j.jhazmat.2007.06.080. PMid:17689010.

Haiba, N. S., Asaal, A. M., El Massry, A. M., Ismail, I., Basahi, J., & Hassan, I. A. (2019). Effects of "Doneness" Level on PAH concentrations in charcoal-grilled beef and chicken: an Egyptian study case. *Polycyclic Aromatic Compounds*. In press. http://dx.doi.org/10.1080/10406638.2019.1602062.

Hokkanen, M., Luhtasela, U., Kostamo, P., Ritvanen, T., Peltonen, K., & Jestoi, M. J. (2018). Critical effects of smoking parameters on the levels of polycyclic aromatic hydrocarbons in traditionally smoked fish and meat products in Finland. *Journal of Chemistry*, 20, 2160958. http://dx.doi.org/10.1155/2018/2160958.

Janoszka, B. (2011). HPLC-fluorescence analysis of polycyclic aromatic hydrocarbons (PAHs) in pork meat and its gravy fried without additives and in the presence of onion and garlic. *Food Chemistry*, 126(3), 1344-1353. http://dx.doi.org/10.1016/j.foodchem.2010.11.097.

Kafouris, D., Koukkidou, A., Christou, E., Hadjigeorgiou, M., & Yiannopoulos, S. (2020). Determination of polycyclic aromatic hydrocarbons in traditionally smoked meat products and charcoal grilled meat in Cyprus. *Meat Science*, 164, 108088. http://dx.doi.org/10.1016/j.meatsci.2020.108088. PMid:32092623.

Kamankesh, M., Mohammadi, A., Hosseini, H., & Modarres Tehrani, Z. (2015). Rapid determination of polycyclic aromatic hydrocarbons in grilled meat using microwave-assisted extraction and dispersive liquid-liquid microextraction coupled to gas chromatography-mass spectrometry. *Meat Science*, 103, 61-67. http://dx.doi.org/10.1016/j.meatsci.2015.01.001. PMid:25618021.

Kao, T. H., Chen, S., Huang, C. W., Chen, C. J., & Chen, B. H. (2014). Occurrence and exposure to polycyclic aromatic hydrocarbons in kindling-free-charcoal grilled meat products in Taiwan. *Food and Chemical Toxicology*, 71, 149-158. http://dx.doi.org/10.1016/j.fct.2014.05.033. PMid:24932919.

Lingbeck, J. M., Cordero, P., O'Bryan, C. A., Johnson, M. G., Ricke, S. C., & Crandall, P. G. (2014). Functionality of liquid smoke as an all-natural antimicrobial in food preservation. *Meat Science*, 97(2), 197-206. http://dx.doi.org/10.1016/j.meatsci.2014.02.003. PMid:24583328.

Lorenzo, J. M., Purriños, L., Bermudez, R., Cobas, N., Figueiredo, M., & García Fontán, M. C. (2011). Polycyclic aromatic hydrocarbons (PAHs) in two Spanish traditional smoked sausage varieties:"Chorizo gallego" and "Chorizo de cebolla". *Meat Science*, 89(1), 105-109. http://dx.doi.org/10.1016/j.meatsci.2011.03.017. PMid:21501932.

Pla, M., Pascual, M., & Ariño, B. J. (2004). Protein, fat and moisture content of retail cuts of rabbit meat evaluated with the nirs methodology. *World Rabbit Science*, 12(3), 149-158.

Purcaro, G., Moret, S., & Conte, L. S. (2013). Overview on polycyclic aromatic hydrocarbons: occurrence, legislation and innovative determination in foods. *Talanta*, 105, 292-305. http://dx.doi.org/10.1016/j.talanta.2012.10.041. PMid:23598022.

Reinik, M., Tamme, T., Roasto, M., Juhkam, K., Tenno, T., & Kiis, A. J. F. (2007). Polycyclic aromatic hydrocarbons (PAHs) in meat products and estimated PAH intake by children and the general population in Estonia. *Food Additives and Contaminants*, 24(4), 429-437. http://dx.doi.org/10.1080/02652030601182862. PMid:17454117.

World Health Organization – WHO, International Agency for Research on Cancer – IARC. (2002). *Some traditional herbal medicines, some mycotoxins, naphthalene and styrene*. Geneva: WHO.