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Identification and Quantification of Polycyclic Aromatic Hydrocarbons in Processed Chicken Meat Products

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ABSTRACT

Experiments were carried out to identify and quantify the concentrations of polycyclic aromatic hydrocarbons (PAHs) in tandoor chicken, chicken tikka and smoked chicken and to evaluate the impact of different cooking methods on the levels of PAH compounds. The PAH's viz., Acenaphthylene, Acenaphthene, Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Chrysene, Benz (a) anthracene, Benzo (k) fluoranthene, Benzo (b) fluoranthene, Benzo (a) pyrene, Indeno (1,2,3-cd) pyrene, Dibenzo (a,h) anthracene, Benzo (g,h,i) perylene were separated and quantified using GC-MS. The calibration curves for 15 different compounds of PAHs at various concentrations were found to be linear with standard regression value. Test results showed that all the PAH compounds were below the limit of quantification in chicken tikka samples. However, 2 out 3 tandoor chicken samples showed presence of pyrene at 0.01 ppm level. Smoked chicken samples also showed the presence of pyrene and anthracene at 0.01 to 0.02 ppm level. None of the products showed the presence of carcinogenic PAH compounds viz., benzo[a]anthracene, benzo[b]fluoranthene, chrysene, and benzo[a]pyrene. Analysis of seekh kebabs cooked under different cooking methods for PAH indicated that, the levels were below the limit of quantitation (0.01mg/kg). Current study indicates that, the level of carcinogenic PAH compounds are below the detection level in routinley consumed meat products, however, more samples need to be screened.

Keywords: *Meat Products, Cooking Methods, PAH Compounds, GC-MS MS*

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INTRODUCTION

Cooking of meat enhances the taste and flavor, helps in inhibition of microorganisms while extending shelf life (Broncano et al., 2009). However, the cooking method as well as the high temperature cooking condition may modify the chemical composition of meat with consequent changes in nutritional value, leading to the formation of chemical carcinogens, specifically, heterocyclic amines (HCA) and polycyclic aromatic hydrocarbons (PAH). Polycyclic aromatic hydrocarbons are a group of organic compounds containing two or more fused aromatic rings and are derived from the incomplete combustion of organic matter, including oil, gas, coal, wood, or other organic substances, such as charbroiled meat (Codex Alimentarius Commission 2004). They have relatively low solubility in water but are highly lipophilic. The PAH compounds include Benzo (a) pyrene, Dibenz (a,h) anthracene, Benzo (b) fluoranthene, Indeno (1,2,3 cd) pyrene, dibenzo (a,l) pyrene, Benz (j) aceanthrylene, Dibenzothiophene, Benzo (c) fluorene, Benzo (k) fluoranthene, Phenanthrene and Chrysene (Fetzer, 2007).
In red meat, PAH's can be generated through pyrolysis of organic matter that can occur under intense and direct heating conditions or direct contact of lipids dripping directly over the flame generating volatile PAHs. PAHs formed in the flames adhere to the surface of the meat (European Commission, 2002; Farhadian et al., 2012). The amount and types of PAHs that accumulate in cooked meats are dependent on the meat type, the cooking methods, and the temperature and the duration of cooking (Knize et al., 1994).

There are several reports on the presence of PAHs in various meat products (Knize et al., 1994; Vu-Duc et al., 2007; Farhadian et al., 2012; Rose et al., 2015; Mohammadi and Valizadeh-Kakhki, 2016). Kazerouni et al. (2001) opined that PAH is mostly associated with barbecued meats. Similarly, Chen and Lin (1997) and Dost and Ideli (2012) observed higher levels of PAHs in grilled meat. Hitzel et al. (2012) reported total PAHs of 8.22 and 9.03 g/kg, respectively, in the Frankfurters sausages and mini-salamis smoked with sundry woods. Higher levels are found in foods that have been exposed to combustion products and foods that have been charred or burned when cooked at high-temperatures (Jagerstad and Skog, 2005).

Polycyclic aromatic hydrocarbons may induce the formation of DNA adducts and interfere with apoptosis and that may increase the risk of colorectal, pancreatic and prostate cancer (Baird et al., 2005). Out of 16 PAHs, benzo [a] anthracene, benzo [b] fluoranthene, benzo [k] fluoranthene, chrysene, benzo [a] pyrene, dibenzo [a,h] anthracene, and indeno [1,2,3-cd] pyrene have been categorized as probable human carcinogens (USEPA, 1994). The European Union has setup maximum levels of 2ppb wet weight for benzo[a]pyrene and considered to be a marker for carcinogenic risk (European Commission, 2005). In 2008, the European Food Safety Authority recommended that relying merely on benzo (a) pyrene as a marker is unsuitable for detecting the occurrence of PAH in food. Rather, an approach is recommended relying on an analytical system with either four specific substances (PAH4 – benzo (a) pyrene, benz (a) anthracene, benzo (b) fluoranthene and chrysene) or with eight (PAH8 – PAH4 plus benzo (k) fluoranthene, benzo (g,h,i) perylene, dibenz (a, h) anthracene, and indeno (1,2,3-c,d) pyrene) (European Commission 2011).

There are certain possible interventions and innovations to prevent or reduce PAHs formation. Cooking at lower temperatures, using cooking methods like baking, broiling, using slow cookers, sous-vide cooking, employing microwave oven to cook meat prior to exposure to high temperatures, use of spices and herbs in the marinade are few ways to lower the level of formation of PAH

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compounds (Hitzel et al., 2012; Ledesma et al., 2015; Lee et al., 2016). Charcoal grilling contributes to greater PAH formation resulting from incomplete combustion of charcoal, compared with that generated by gas grilling (Viegas et al., 2012; Gorji et al., 2016). Hence, studies focusing to prevent or reduce PAHs formation need to be carried out.

Grilled and smoked meat products represent a significant part of everyday diet in India. However, there is a paucity of information on the levels of PAHs in various processed meat products commonly consumed in India. Periodical monitoring of foodstuffs for the presence of chemical residues is necessary to ensure public health (Muthukumar et al., 2015). Hence, the current study was conducted with the objectives to identify certain PAH compounds and their levels in processed meat products commonly consumed in India viz, Tandoori chicken, Chicken Tikka and Smoked chicken and also to determine the impact of different cooking methods viz. grilling, roasting and smoking on the levels of PAH compounds in seekh-kebabs.

MATERIAL AND METHODS

A. Determining the type and level of PAH compounds in commonly consumed processed meat products: Chicken meat products viz., tandoor chicken and chicken tikka were procured in different batches from various restaurants in Hyderabad and smoked ckicken processed at ICAR-NRC on Meat was collected and analyzed for the presence of 15 different PAH compounds (n=10). The modified quick, easy, cheap, effective, rugged, and safe (QueChers) method was employed for extraction, which involves initial extraction and extraction /partitioning, followed by clean up procedure for extraction of PAH. Analysis of the PAHs in meat samples were carried out with the gas chromatographic method with mass selective detector (GC/MS).

Extraction and clean-up: The AOAC method was used for extraction and clean-up of samples. Homogenized sample 2 g, was mixed with 5 mL distilled water and shaken vigorously by hand with 10 mL ethyl acetate in a 50 mL polypropylene centrifuge tube for 1 min. Subsequently, 4 g anhydrous magnesium sulfate and 2 g sodium chloride were added to the mixture to induce phase separation and force the analytes into the ethyl acetate layer. The tube was again shaken by hand for 1 min followed by centrifugation for 10 min. One mL aliquot of the ethyl acetate layer was separated and collected into vials for injection into GC (AOAC , 2012)

Instrumentation and Chromatographic conditions: The extract was analyzed on Agilent 7890A gas chromatograph interfaced to Waters Quattro Micro GC mass spectrometer with Electron impact ionization (EI+ Ion mode). The gas chromatograph was connected with a 30 m HP-5MS capillary column with 0.25 mm × 0.25 µm film. Helium was used as the carrier gas. The column was maintained at a constant flow rate of 1.0 ml/min, and 2 µl of aliquot was injected in a split-less mode. Ion source and interface temperature were set at 180°C and 250°C, respectively. Inlet temperature was maintained at 275°C. The column temperature programmed for PAH analysis was set initially at 50°C hold for 2 min, then 100˚C/min to 90°C, 65˚C/min to 190˚C, 50˚C/min to 265˚C, 40˚C to 310˚C and then held for 8.5 min. Blank sample run was carried out and matrix-matched standards employed. Mixed standard was used at concentrations ranging from 1 to 50 PPB of each PAH compound (Figure 1 and 2).

Total run time was set for 15 min. Various PAHs viz., Acenaphthylene, Acenaphthene, Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Chrysene, Benzo(a)anthracene, Benzo(k)fluoranthene, Benzo(b)fluoranthene, Benzo(a)pyrene, Indeno (1,2,3-cd) pyrene, Dibenzo (a,h) anthracene, Benzo (ghi) perylene were identified by comparing retention times and mass spectra of unknown peaks with those of reference standards (Figure 3). After GC-MS analysis, the standard curve of each PAH was obtained by plotting concentration against area. The regression equations and correlation coefficient were determined automatically with an Excel software system. Used MassLynx software (system software) where calibration curve is not forced through origin. Calibration standards were run once.

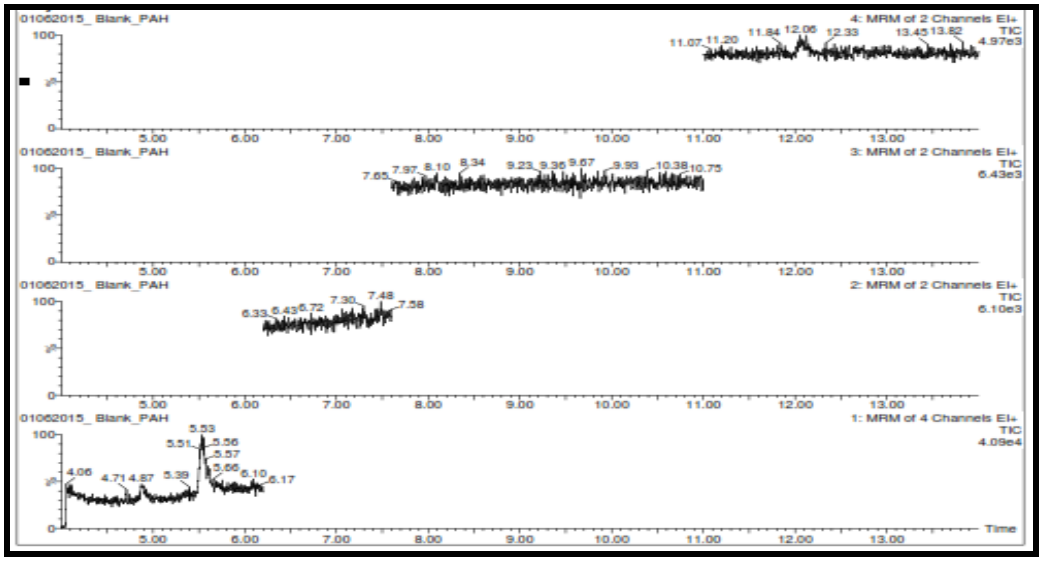

Fig. 1 Chromatograms for blank run

The concentrations of various PAHs were calculated as follows:
Concentration of PAH (ng/g) = (A-b/a) X V X dilution factor ÷ recovery/ Ws
Where A: peak area of PAH
b: intercept of regression equation
a: slope of regression equation
V: volume of extract
Ws: weight of sample (g)

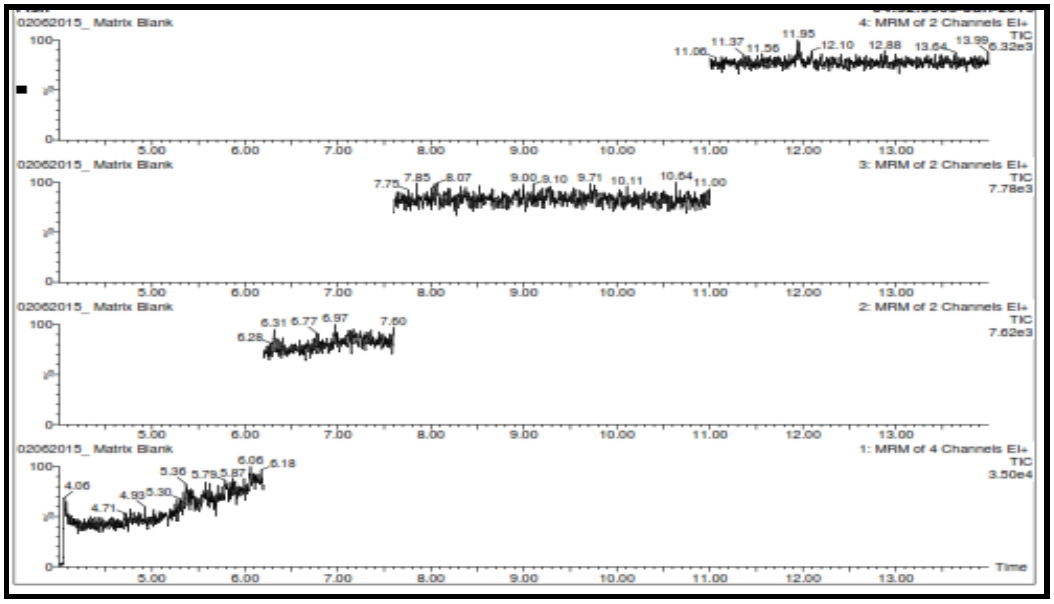

Fig. 2 Chromatograms for matrix blank run

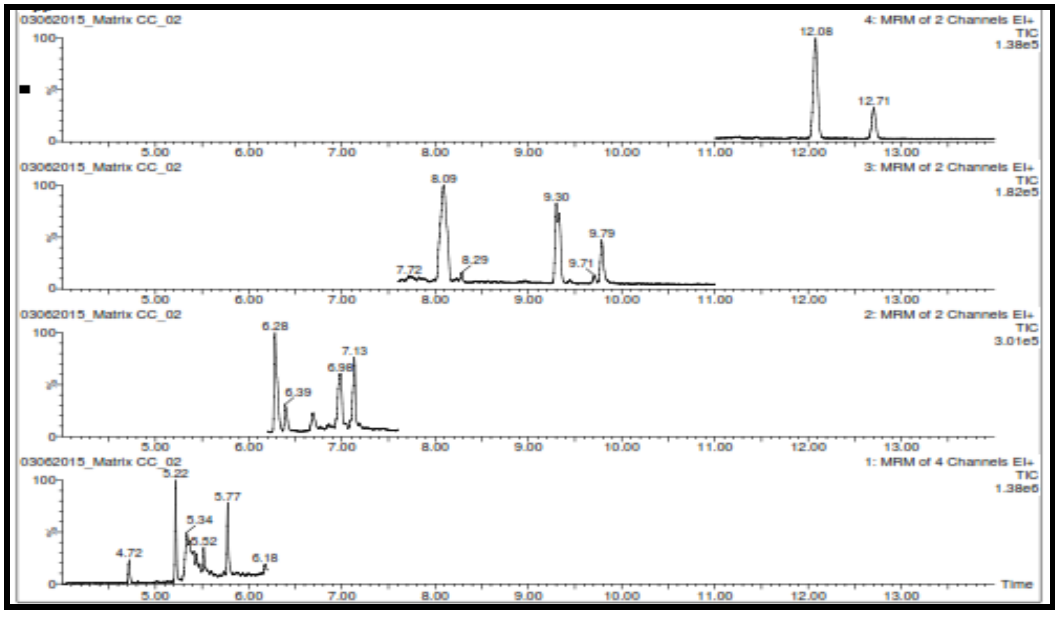

Fig. 3 Chromatograms for matrix matched standards run

B. Studying the effects of different cooking methods on the type and level of PAH compounds in chicken seekh kebab

Preparation of seekh kebabs: Boneless chicken meat procured from a local retail shop (Sneha Fresh Chicken, Hyderabad, India) was chilled overnight at 4±1°C and was minced in a meat mincer (Scharfen, Model X70, 58413 Witten, Germany). The minced meat was pre-blended with common salt and sodium tripolyphosphate followed by mixing with butter, refined wheat flour (maida), spice mix and condiments in a planetary mixer (Smaky, France) for 10 min. About 70 g of batter was manually moulded on stainless steel skewers and divided into three groups for processing.

Three different cooking methods – charcoal grilling, oven roasting and smoking – were chosen, as representative of those most common cooking method employed domestically and also by the caterers. For each group, duplicate batches were prepared. Grilling was carried out in a consumer type outdoor grill (45.8x28.8x13.2 cm). Approximately 2 kg of charcoal was placed in the bottom of the grill. The kebab samples were grilled for 12 min until well done, and the internal temperature reached a minimum of 80°C.

The samples were turned four times at each quarter (every 3 min) during the total cooking time. No oil was applied to the kebabs before or after grilling. Roasting of the kebab samples was done in an oven (Retigo Combi Oven, Model B1011, Czech Republic) for 16 min at 160°C till the core temperature reached a minimum of 80°C. The kebab samples were placed in the same position in the direct smoking chamber (Smoke Rite Ovens, France) at the same distance from the smoke source and processed for 30 min. The temperature in the smoking chamber ranged between 75–100°C during processing. After cooling, the samples were randomly collected from grilled, roasted and smoked kebabs and put in low density polyethylene (LDPE) pouches and stored in a refrigerator (4 ± 1°C) for estimating PAHs. The extraction, clean-up of the sample and estimation of PAHs in chicken kebab was carried out in the same way as that of market samples. The various PAHs in cooked kebabs samples were identified by comparing retention times and mass spectra of unknown peaks with those of reference standards as explained earlier.

RESULTS AND DISCUSSION

The calibration curves for 15 different compounds of PAH at various concentrations were found to be linear with standard regression values as shown in Table 1 along with retention time. The analysis of processed meat products collected from markets revealed that all 15 different compounds of PAHs were below the limit of quantification in chicken tikka samples. However, 2 out 3 tandoor chicken samples showed presence of pyrene at 0.01 ppm level (Figure 4). The smoked chicken samples sourced from NRC on Meat, Hyderabad contained compounds viz., pyrene and anthracene at 0.01 to 0.02 ppm level (Table 2). None of the processed meat products showed presence of carcinogenic compounds viz., Benzo (a) anthracene, Benzo (b) fluoranthene, Chrysene, and Benzo (a) pyrene. However, more samples need to be screened for assessing the PAH residues level in processed meat products to arrive any conclusion.

Table 1: Retention time (RT) and regression coefficient (r^2) of 15 PAH Standards

Name of the PAH Compound	RT (min)	R^2 Value
Acenaphthylene	5.5	0.993
Acenaphthene	5.5	0.991
Fluorene	5.8	0.981
Phenanthrene	6.3	0.990
Anthracene	6.3	0.992
Fluoranthene	7.0	0.993
Pyrene	7.1	0.995
Chrysene	8.1	0.995
Benzo(a)anthracene	8.1	0.998
Benzo(k)fluoranthene	9.3	0.989
Benzo(b)fluoranthene	9.3	0.995
Benzo(a)pyrene	9.8	0.995
Indeno(1,2,3-cd)pyrene	12.1	0.997
Dibenzo(a,h)anthracene	12.1	0.998
Benzo(ghi)perylene	12.7	0.998

Table 2: Levels (ppm) of various polyaromatic hydrocarbons in processed chicken meat products

Name of the PAH Compound	Tandoor chicken	Chicken tikka	Smoked chicken
Acenapthalene	BLQ	BLQ	BLQ
Acenapthene	BLQ	BLQ	BLQ
Fluorene	BLQ	BLQ	BLQ
Phenanthrene	BLQ	BLQ	BLQ
Fluoranthene	BLQ	BLQ	BLQ
Pyrene	0.01 (2)	BLQ	0.013 (3)
Anthracene	BLQ	BLQ	0.01 (2)
Chrysene	BLQ	BLQ	BLQ
Benz(a)anthracene	BLQ	BLQ	BLQ
Benzo(k)fluoranthene	BLQ	BLQ	BLQ
Benzo(b)fluoranthene	BLQ	BLQ	BLQ
Benz(o)pyrene	BLQ	BLQ	BLQ
Indeno(1,2,3 cd) pyrene	BLQ	BLQ	BLQ
Dibenz (a,h) anthracene	BLQ	BLQ	BLQ
Benzo (ghi) perylene	BLQ	BLQ	BLQ

Values in parenthesis indicate number of positive samples; BLQ – Below the level of quantitation; Quantification Limit: 0.01mg/kg

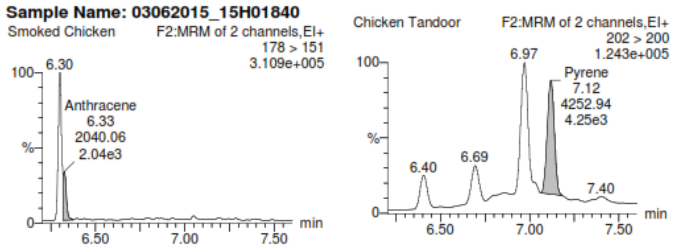

Fig. 4 Chromatograms showing various polyaromatic hydrocarbons in processed chicken meat products

The findings of the present study is in accordance with earlier studies, where several workers have observed varying levels of PAHs in processed meat products (Cross and Sinha, 2004; Knize et al.,1994; Vu-Duc et al., 2007; Farhadian et al., 2012; Rose et al., 2015; Mohammadi and Valizadeh-Kakhki, 2016). This could be due to the fact that, the amount and types of PAHs that accumulate in cooked meats are dependent on several factors such as meat type, fat content of the food, the cooking methods, temperature and duration of cooking, type of fuel used and others (Knize et al., 1994; Mohammadi and Valizadeh-Kakhki, 2016). Similarly, Hussain Al-Thaiban et al. (2018) detected fluorene and anthracene in smoked chicken breast and fluorine and pyrene in prime smoked chicken breast. Jagerstad and Skog (2005) reported that higher levels are found in foods that have been exposed to combustion products and foods that have been charred or burned when cooked at high-temperatures.

In the second experiment, the analysis of seekh kebab samples for polycyclic aromatic hydrocarbons indicated that slightly higher levels of low molecular weight PAHs were noticed in smoked kebabs when compared to charcoal grilling and oven roasting.

These results are in accordance with our earlier study on the market samples indicating presence of PAHs at very low concentration. The slightly higher level of PAHs observed in smoked kebabs compared to charcoal grilled and oven roasted kebabs could be due to long duration of smoking (30 minutes), no application of oil (fat) while charcoal broiling (less smoke produced due to less dripping of fat from the meat onto the charcoal fire) and low temperature of oven roasting. Ledesma et al. (2015) had established the impact of duration of smoking and the levels of PAHs in smoked Spanish meat products. Comparatively low level of PAH compounds recorded in the oven roasted seekh kebab (150°C) in the current study is in accordance with Knize et al. (1994), who reported the lowest concentrations of PAHs at 150°C and the formation of PAHs have increased with temperature.

CONCLUSION

The processed meat products evaluated in our study contained little or no PAH compounds and does not pose any threat to the health of consumer as per the current toxicological data. However, detailed studies with large number of samples covering wide varieties of processed meat products consumed in different parts of the country should be carried out to establish a baseline data on the levels of PAH compounds. Further, studies should focus on possible interventions and innovations to prevent or reduce PAHs formation, which forms a major research area to work out.

COMPETING INTERESTS: The authors have no known competing interests either financial or personal between themselves and others that might bias the work.

ETHICS STATEMENT: Not applicable

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