Research Article

# Determination of Polyaromatic Hydrocarbon Levels in Barbecued Mutton and Beef with Different Grilling Procedures

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**Abstract** | This study examined the levels of polycyclic aromatic hydrocarbons (PAHs) in different barbecued mutton and beef samples available in district Peshawar, Pakistan. In addition, the effect of various grilling methods on PAH levels was evaluated. Mutton grilled with three grilling procedures, were analyzed for its PAHs content. A total of 120 samples were collected from local food vendor spots or general restaurants, and control samples of gas and electric grilled meat samples were also prepared and analyzed. Significant difference sp≤0.05 in PAHs levels were observed in different grilling procedures. Among the mutton samples, the highest concentration of PAHs was recorded in mutton grilled on coals. The chrysene, naphthalene, flourenthene and DBAHA concentrations were assessed high in mutton samples grilled on coals. Furthermore, this research observed the factors that might be helpful in minimizing the level of meat contaminants if practiced with traditional grilling procedures.

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## Introduction

Barbecued meat or grilled meat is quite famous and consuming food worldwide in general and particularly in Peshawar, Khyber Pakhtunkhwa. The study area is well-known city for its barbecued meat all across Pakistan. Grilling is a method of cooking that comprises of applying dry heat to the surface of food, commonly from above or below. Meat grilling or cooking of other foods with intense heat over a direct flame result in fat dripping on the hot fire and yielding flames containing a number of PAHs and they are potent carcinogenic substances. Presence of various PAHs in food is gained much attention in present time, due to the health-related hazards generated by polycyclic aromatic hydrocarbons (Akpambang *et al.*, 2008). Various researches have been conducted for detection of PAHs in processed and unprocessed foods. Polycyclic aromatic hydrocarbons are a group of chemical compounds composed of two or more fused aromatic rings that are formed during the incomplete combustion or high-temperature pyrolysis of coal, oil,

gas, wood, fossil fuels, garbage, or other substances, such as tobacco and charbroiled meat (Mottier *et al.*, 2000) PAHs Due to their carcinogenic potential, have been studied widely in mammals (WHO, 2006) and concern for human population has stimulated interest in knowledge of their distribution as well as accumulation in the environment and in food items.

This research study was aimed to serve as baseline for the future researches on barbecued meat, as limited research work has been conducted on barbecued meat in Pakistan.

In Khyber Pakhtunkhwa, mutton and beef are the most favorite choices of barbecued meats among other meat choices and grilling procedures are comprised of traditional barbecuing of meat on the smoke produced by coals which is direct heat processing. Direct grilling gives meat a characteristic color, improves its flavor and preservation because smoke contains phenols, aldehydes, acetic acid and other carboxylic acid but, on the other hand produces toxic and carcinogenic compounds like PAHs.The food contamination may transfer diseases and infection to human beings. Number of pollutants exist in the atmosphere one of them is heavy metals which can be harmful for wellbeing and are a common cause of spreading diseases in humans. Meat Contamination of food follows during the treatment and processing of food and particularly when food is treated at high thermal treatments, like in drying and smoking, roasting and baking (Ishizaki, *et al.*, 2010). Three PAHs including, Benz [a] Pyrene, anthracene and DBAHAare the most hazardous PAHs documented in various researches in relation to health and safety. According to carcinogenicity they are grouped as 2A. PAHs can enter water courses through industrial discharges and waste water treatment plants and can be found in soils as a result of their escape from segregate containers close to hazardous water sites (FAO/WHO, 2008). PAHs can also contaminate foods during heating and drying processes that allow combustion products to come into direct contact with food. PAHs are group of chemical compounds known to be possible carcinogens and they are wide spread in the environment, in water, air, and soil and also in the traces of these substances and may be found in various food products. Benz [a] Pyrene is extensively studied and assessed PAHs because of its noticeable cancer causing nature and due to its relatively easy analysis and presence in food items Benz [a] Pyreneis a good indication for other PAHs in food items and amount of 5ug/kg has been observed for Benz[a] Pyrenein grilled meats and smoked meat products (European Commission, 2001).

The Highest quantities of PAHs were formed in meat and meat products barbecued on a grill over an open charcoal of wooden chip flame (Kazerouni *et al.*, 2001). PAHs are pervasive environmental pollutants that have been identified worldwide in various matrices, such as water soil or dust particles. PAHS have also been found as contaminants in various foods such as dairy products vegetables, fruits oils roasted or smoked meat (Simko, 2002).

## Materials and Methods

Different samples of barbecued as well as raw mutton and beef samples were collected from four locations of Peshawar and were evaluated for their PAHs contents. The study comprised of the following sections.

1. Collection of samples.
2. Storage of samples.
3. Treatment of samples.
4. Proximate analysis of samples.
5. Analysis of PAHs in barbecued meat.
6. Analysis of data

*Sample*

All collected samples were stored in clean polythene bags and labeled according to their type and transported to the laboratory for analysis. Different samples for PAHs analysis of barbecued meat (beef, and mutton) were collected from various shops of local markets, raw as well as cooked with common local procedures includes: grilled on coals, grilled on gas and grilled on electricity. Samples of barbecued mutton, and beef, used for the study were collected from four randomly selected popular selling spots of District Peshawar.

Total no of samples taken for PAHs analysis were = 120

The criteria for the selection of samples was based upon the following factors;

- Type of meat (2 types)
- Location (4 Locations) Raw samples along with coal grilled samples were collected from four major areas of Peshawar which comprised of Namak Mandi, Ring Road Hayatabad, University Road and Saddar.

- Marinated and unmarinated samples.
- Type of Heat Processing Applied for barbecuing of meats (Grilling on coals, Grilling on Gas, Grilling on electricity total= 3 types of processing techniques).

The samples taken were in duplicate. Electric grilling and Gas grilling of samples was carried out in laboratory as it is not practiced in local commercial meat grilled vendors. All the meat samples were wrapped in plastic bags and stored in refrigerator for analysis. The storage of samples was kept at 20°C till the final analysis was done.

*Chemicals*

Solvents used in this research were of high grade and quality in addition were according to certified HPLC grade and bought from Sigma Aldrich. Standard solutions were purchased from Merck Germany and Sigma Aldrich. All solvents used were of HPLC quality. Mixed standard solutions of the six PAHs were prepared, from these stock solutions, individual PAHs solutions with various concentrations were prepared up to 300 µg/l in an appropriate solvents of HPLC grade acetonitrile. Mixed standard solutions for six PAHS were made of 50, 100, 200 and 300 µg/l. After preparation solutions were kept and stored at 4°C.

*Post-extraction cleanup*

The concentrated 5ml extracts were purified by column chromatography on silica gel, as described by Hossain and Fujita (2009) with some modifications. The cleanup column with an internal diameter of 1cm (i.e., =1cm) was filled with cotton in the bottom. An activated silica gel (17g) soaked with dichloromethane was then loaded into the cleanup column (5cm), which was thereafter topped with 1.5cm of anhydrous sodium sulphate. A volume of 5ml of dichloromethane was added to wash the sodium sulphate and the silica gel. A volume of 50ml of dichloromethane was then added to the glass column and allowed to flow through the column at a rate of 5ml/minute, and the eluent was then collected.

*Analysis by HPLC*

All cleaned samples of meat were evaluated by The HP series ll00 LC system equipped with gradient pump (Model 600), with an auto sampler (Model 717 plus) with 20 uL injection loop and ultraviolet detector at 240 nm was used. In view of data quality assurance, each sample was analyzed in duplicate. Therefore, the average value of each sample was used for further interpretation. Standard solutions of all elements were prepared by dilution of 1000 mg/L certified standard solutions The analysis for PAHs in the meat samples was carried out using a HPLC apparatus equipped with a Model 600 controller pump, an in-line degasser, a Model 717 plus auto-sampler, a Model 474 fluorescence detector with an excitation wavelength of 290mm and emission wavelength of 43 Onm and a Millenniurn 32 data processor. The mobile phase consisted of 40% acetonitrile and 60% Water at a flow rate of 1.8ml/minute. The injection volume used was 5 µl. The recoveries (a mean of three replicate analyses) were calculated by comparing the differencebetween spiked and unspiked samples with the known amount of PAHs added (Choe *et al*., 2009; Kazerouni *et al*., 2001). For any PAH the concentration [C] in ug/100 g.

$$C = A \times F \times 6.0 \times 1.2$$

Where A= Area; F= Conversion factor; 6= Dilution Factor; 1.2= Recovery Co-efficient 100/83; Therefore [C] = 7.2×A

## Results and Discussion

The data regarding this research study on revealed that PAHs generated during the thermal food processing depends on several parameters such as temperature, duration of the treatment, distance from the source of heating, oxygen accessibility, fat content, and the type of combustible used (Alonge, 1988). Most PAHs are chemically inert, hydrophobic, and soluble in organic solvents. This study investigated the effects of firewood smoking on the PAHs levels in Peshawar. The exact mechanism of PAHs development in food processing or cooking is not exactly known, however, it is might be associated with incomplete combustion is involved. Food processing or cooking steps such as roasting, grilling, barbecuing and smoking generate PAHs and increase the level of PAHs in the food being cooked. The findings of this research are fairly supported by work done by (Farhadian *et al*., 2011).

Significant ($p<0.05$) differences were found in the levels of PAHs in raw and processed meat samples evaluated are presented in Table 3. Findings of present research are supported by several studies and documented that PAHs are frequently found in

cooked food such as fish and meat, with concentrations ranging from 0.17 to 78mg/kg these ranges are within the values reported by (Choe *et al.*, 2009; Kazerouni *et al.*, 2001).

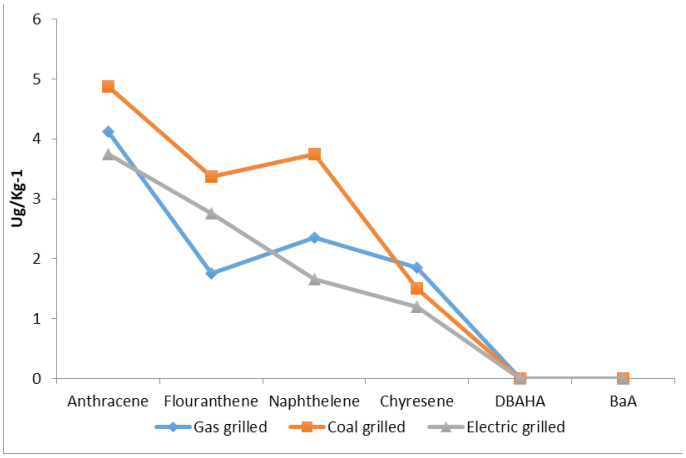


**Figure 1:** *PAHs level determined in coal grilled beef meat.*

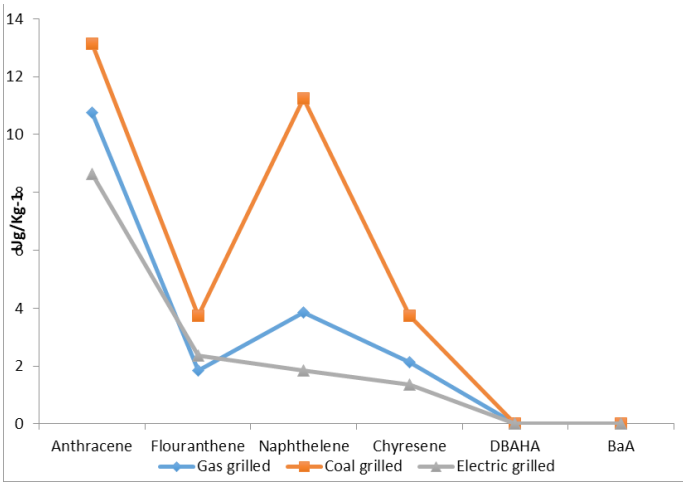


**Figure 2:** *PAHs level determined in coal grilled mutton meat.*

**Table 1:** *Local cooking procedures for various BBQ meats.*

| S. No | Type of meat | Procedure and ingredients |
|---|---|---|
| 1. | Mutton | Mutton chops cut into small pieces and salt sprinkled on chopped mutton pieces and chopped lard pieces and after 10-15 minutes screwed on skewers and barbecued on coals for 25-35 minutes. |
| 2. | Beef | Beef pieces chopped into small pieces and salt, red pepper and coriander powder sprinkled on the chopped pieces and barbecued on coals for 25-30 minutes. |

Result revealed that the coal grilled samples contained the high concentrations of PAHs than gas grilled and electric grilled samples chromatograms no 1,2,3 and 4. The coal grilled beef contains 13.125g/kg anthracene, 3.75µg/kgfln, 1.25µg/kg, Naphthalene, 3.75µg/kg chrysene and 1.35µg/kg DBAHA, whereas BaA was not detected in the analyzed beef samples graph no1.

**Table 2:** *Temperature ranges for different grilling procedures.*

| Description | Gas grilling | Coal grilling | Electric grilling |
|---|---|---|---|
| Low | 150°F | 250°F | 150°F |
| Moderate | 250°F | 350°F | 180°F |
| Hot | 350°F | 450°F | 250°F |
| Very Hot | 400°F | 550°F | 350°F |

*Note: These temperature ranges are taken from the given temperature ranges in electric and gas ovens available for cooking. The temperature ranges of coal grilling is taken from average temperature range of heated coals in grilling operations.*

**Table 3:** *PAHs content of raw and processed meat.*

| | Processing | Mean | sig |
|---|---|---|---|
| Anthracene | raw meat | 5.1 | |
| | cooked meat | 9.2 | Significant |
| Flouranthene | raw meat | 1.4 | |
| | cooked meat | 2.4 | |
| Naphthalene | raw meat | 1.1 | Significant |
| | cooked meat | 2.6 | |
| Chrysene | raw meat | 1.4 | |
| | cooked meat | 2.2 | Significant |
| DBAHA | raw meat | 1.3 | |
| | cooked meat | 3.2 | Significant |
| BaA | raw meat | .0531 | |
| | cooked meat | 1.3396 | Significant |

**Table 4:** *PAHS level of barbecued meat (n=120).*

| S. No. | Name of PAHs | Mutton (µg/kg-1) N=60 Mean±SD | Beef (µg/kg-1) N=60 Mean±SD | P value |
|---|---|---|---|---|
| 1 | Anthracene | 14.5±12.50 | 13.22±9.05 | 0.097 |
| 2 | Flouranthene | 5.44±3.85 | 5.36±4.30 | 0.703 |
| 3 | Naphthalene | 3.65±1.35 | 4.45±1.44 | 0.001 |
| 4 | Chrysene | 7.22±5.90 | 5.86±3.20 | 0.000 |
| 5 | DBAHA | 4.55±2.6 | 3.65±1.0 | 0.026 |
| 6 | BaA | ND | ND | N/A |

*ND: Not Detected. Mean followed are not significantly different at p ≤ at p ≤ 0.05*

The samples were grilled with three common grilling procedures and the highest concentrations were observed in coal gilled samples and their detection limits were above the permissible detection limits settled by codex alimentarious and the possible cause for this was observed grilling on very high temperatures Table 2. The PAHs levels found in grilled mutton meat available in District Peshawar revealed that


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the coal grilled mutton samples contained the high concentrations of PAHs than gas grilled and electric grilled samples the present values are fairly in line with work reported by (Janoszka *et al*., 2005). The coal grilled mutton comprises 13.125ug/kg anthracene, 3.75ug/kg flourenthene, 1.25ug/kg Naphthalene, 3.75ug/kg chrysene and 1.35ug/kg DBAHA, whereas BaA was not detected in the analyzed mutton samples graph no 2. The lowest concentrations were found in the samples grilled with an electric medium, though their heat levels were also higher above the detection limits. Significant differences were detected in PAHs levels in various samples of mutton and beef which are displayed in Table 4.

## Conclusions and Recommendations

The main findings of this study reveals that the PAH content of different smoked or grilled meat products commonly consumed in Khyber Pakhtunkhwa Pakistan and that were smoked or grilled using traditional systems, which use a wood fire, were observed heavily contaminated. Considerably lower contamination levels were found in samples smoked or grilled in the laboratory using a gas grilling and electric grilled procedures. Results shown that grilling on coals produces high amounts of PAHs as compared to other grilling procedures like electric and gas grilling. The occurrence of PAHs in food has been widely studied in countries all over the world. Data from surveys show that the most abundant PAHs in foodstuffs are the low molecular weight PAHs from the EPA list (particularly those of two or three benzene rings). These PAHs are less significant from the toxicological point of view and do not contribute to the genotoxic and carcinogenic potential of PAHs. Use of lean cuts of meat and removing of fat and skin before cooking will probably reduce dripping fat, pyrolysis and PAHs formation. Practice of safe grilling procedures like use of indirect heat source will minimize the development of PAHs, instead of direct heat source which is in practice in local grilling practices.

## Novelty Statement

This study examined the levels of polycyclic aromatic hydrocarbons (PAHs) in different barbecued mutton and beef samples. In addition, effect of various grilling methods on PAH levels were also evaluated.

## Author's Contribution

**Zahin Anjum**: Designed the study, conducted the research, performed experiments and analysis.
**Farhat Shehzad**: Major supervisor who provided guidance in research
**Shaista Ali**: Helped in statistical analysis & tabulation.
**Sumbla Yousaf**: Helped in manuscript write-up
**Amina Rahat**: Provided facilitation in samples treatments.
**Hamid Ullah Shah**: Helped in provision of laboratory and other technical support.

*Supplementary material*
There is supplementary material associated with this article. Access the material online at: https://dx.doi.org/10.17582/journal.sja/2022/38.3.1044.1050

*Conflict of interest*
The authors have declared no conflict of interest.

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