Research Article

Effect of Marination and Grilling Techniques in Lowering the Level of Polyaromatic Hydrocarbons and Heavy Metal in Barbecued Meat

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Abstract | This study was designed to assess the levels of polycyclic aromatic hydrocarbons (PAHs) and heavy metals in different types of marinated meat, such as beef, chicken, mutton and fish, and different grilling techniques were applied to see their effectiveness in lowering the levels of these toxicants. Polyaromatic Hydrocarbons and heavy metals refer to a large group of chemicals that may be present in the environment as pollutants and can enter the food chain and might be carcinogenic to humans. The lead content was found high in fish with the mean value of 3.18mg/kg and the lowest values were observed in chicken with mean value of 1.38mg/kg. The chrysene, naphthalene, flourenthene and Dibenz(a,h) anthracene concentrations were also found high in fish and mutton. The observed values were greater than permissible limits determined by Codex Alimentaurius and World Health Organization. High concentration of Polyaromatic Hydrocarbons was observed in coal grilled meats. Anthracene content was found high in mutton meat and mean value was 12.50 µg/kg-1; flouernthene was assessed high in fish meat with mean value of 5.35 µg/kg-1; naphthalene content of chicken meat was found high with mean value of 1.85 µg/kg-1. The chrysene and DBAHA contents were observed high in mutton and fish with mean values of 5.90 and 3.25 µg/kg-1 respectively and Benz a anthracene was not detected in all meat samples tested. It was concluded that factors like marination of meat and covered grilling significantly reduced the level of meat contaminants if, practiced with traditional grilling procedures.

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Keywords | Proximate analysis, Polyaromatic Hydrocarbons, Contaminants, Chrysene, Naphthalene, Flourenthene, Dibenz (a, h) anthracene (DBAHA)

Introduction

Meat is one of the most favorite food items and an integral part of human diet in Pakistan, particularly in Khyber Pakhtunkhwa. It was hypothesized that meat contains different toxicants (PAHs and heavy metals) that adversely affect the health of people. Meat, being comprised of many essential micro and macro nutrients, is an integral part of human diet but on the other hand, it may also furnish many poisonous substances (Nkansah M. et al., 2014). Contamination of food occurs during the treatments such as high thermal treatments, like in drying and smoking, roasting and baking and processing of food. Ishizaki et al. (2010). Pollution of meat by heavy metals is alarming for food safety and human health. Presence of various PAHs in food has gained much attention in present time, due to the health related hazards generated by PAHs (Mottier, 2000). With the increase in heat the PAHs level in meat

increases (Larsson, T., 2000). International Agency for Research on Cancer (IARC) has estimated and evaluated carcinogenicity of various PAHs. Cooking methods in grilling can produce marked differences in the concentrations of carcinogens. As a result of fat drippings while grilling foods over direct flame, flames containing a number of PAHs are yielded. The selling of meat in Pakistan is mostly done in open markets and even near road sides and due to this practice meat becomes contaminated and unhealthy due to deposition of various heavy metals which puts consumer at risk and great alarm for food safety. Even in a very small amount present in food may cause hostile effect. Different investigators had stated the instances of heavy metal pollution while treating the meat products. (Bilal Aslam, 2010).

Grilled meat or barbecued meat is a very famous type of food and is consumed worldwide and also in Peshawar, Khyber Pakhtunkhwa. Peshawar is famous for its barbecued meat all across Pakistan. This research study will be helpful and will serve as baseline for future research on barbecued meat. In addition, it will pave way for policies to be formulated regarding the consumption as well as processing safe guards for grilled meat. It is important to screen out the health risk issues, regarding common and traditional grilling procedures for meat and also to determine the safety measures to be carried out for conservation of important meat nutrients during cooking and find out the factors that might be helpful in minimizing the level of meat contaminants if practiced with traditional grilling procedures. The present investigation is an endeavor to identify the major toxicants found in the grilled meat available and sold in District Peshawar. It will also analyze the relationship between the type of PAHs found in local grilled meat and will examine how the local grilling procedures increase the level of meat toxicants. Furthermore, it will conclude how to prevent meat toxicity and enhance food safety measures.

Materials and Methods

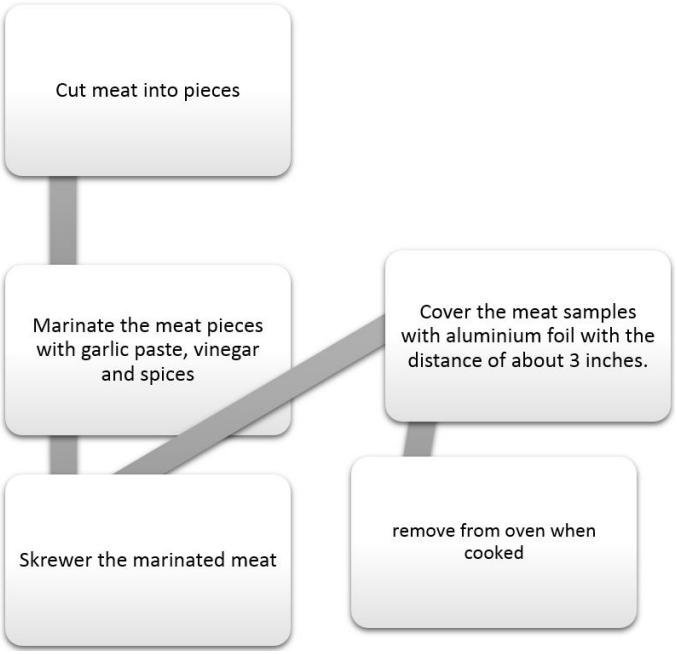
The objective of the research conducted was to investigate about PAHs concentrations in the locally available meat as well as to find out the specific heavy metals in cooked meat sold at various popular food vender spots.

Collection of samples

Different samples of barbecued meat (fish, beef, chicken and mutton) were collected from various shops of local markets, raw as well as cooked with common local procedures, grilled on coals was collected from local market, whereas, gas grilled and electric grilled samples were prepared in the laboratory for PAHs analysis. Common local marinades for the barbecuing of meat were salt, dried coriander powder, red pepper, garlic paste, lemon or vinegar. Samples were collected from four different locations viz. (A) Namak mandi, (B), Sadder Bazar (C) Ring road and (D) University road, Peshawar. These were popular food spots and mostly located on roadside, near heavy vehicular load and road construction activities.

Marination

Before grilling procedures, the chicken, fish and beef meat was marinated with commonly consumed spices like red chili powder, coriander, salt and cumin along with garlic paste, whereas for mutton only salt was sprinkled before grilling.



Flow chart for barbequing of meat in an electric and gas oven.

Heavy metal analysis

The heavy metal analysis was carried out at University of Agriculture, Peshawar and Central Resource Laboratory, University of Peshawar. The amount of heavy metals measured by Atomic Absorption Spectroscopy (AAS) Perkin Almer (model A-700), 'These instruments were calibrated with respective metals standards for the analysis of Lead, Cadmium, and Nickel.' Graphs of each standard solution were drawn by establishing a curve of absorbance against concentration. On the basis of respective standard

curve, each metal was analyzed for their absorbance reading. For the analysis of Copper (Cu), Zinc (Zn), Lead (Pb) and Nickel (Ni) the powdered samples were prepared by method as described by Association of Official Analytical Chemist.

$$\text{Amount of Heavy metal (mg/kg)} = \frac{\text{Absorbance reading} \times \text{dilution factor} \times 100}{\text{Weight of sample}}$$

Samples were prepared by wet digestion method and for this purpose 5 gm of meat was taken into crucibles and kept for about three hours in an oven and temperature range was 105 ^{0}C after moisture evaporation the samples were cooled and 5 ml of nitric acid was added and kept for 24 hours. Digested samples were heated and after cpping 100 ml of deionized water was added for dilution and prepared mixture was

Assessment of Polyaromatic hydrocarbons

PAHs are ever-present environmental pollutants that have been identified worldwide in various matrices such as water soil or dust particles there are more than a hundred PAHs which are recognized as contaminants to the environment in the composite fusions. The highest quantities of PAHs are formed in meat and meat products barbecued on a grill over an open charcoal of wooden chip flame (Kazerouni et al., 2005). Samples of barbecued fish, mutton, beef, and chicken used for the study were obtained from four randomly selected popular sales spots of District Peshawar. The raw meat was purchased from the market and the same meat was used for coal grilling. The meat collected was grilled in the laboratory with certain preventive measures to see the effect on formation of PAHs. The coal grilled samples were purchased from local shops and analyzed for comparison with the laboratory prepared gas and electric grilled samples.

Preparation of analytical standards

Standard solutions were purchased from Sigma Aldrich and other standard chemical agencies and these included; Anthracene analytical standard (code 31581), PAHs mix 3 (code no 861291).

PAHs extraction

The 100 ml of prepared sample was transported to a soxhlet extractor thimble. About 200 ml of the extraction solvent (methylene chloride) was measured into a 500-ml round bottom flask containing two clean boiling chips. The extract was allowed to cool after the extraction was complete. The extraction and clean-up procedures applied in this study were used as described by Farhadian et al. (2011). Extracted proficiency was 70 to 80 percent on the whole as designated by Hajeb et al. (2014).

Analysis by HPLC

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 nm and a Millenniurn 32 data processor. For separation, a C18 column stable at 30°C was used. The mobile phase consisted of 40% acetonitrile and 60% water at a flow rate of 1.8ml/minute. The injection volume used was 5 ul.

Statistical analysis

The collected data was analyzed statistically for the results by using a SPSS version 20 and Analysis of variance (ANOVA) was applied by CRD (completely randomized design) and descriptive analysis was calculated with the help of mean and standard deviation for factors like marinated and non-marinated meat samples, covered and non-covered meat samples and covering of meat was achieved by wrapping of meat samples with aluminum foil but direct contact with samples was avoided. Different types of meat samples were analyzed. Mean values were calculated from triplicate values and significant differences were tested by LSD (least significant difference) at 0.05%probability. All graphs and bars were prepared with the help of latest version of Excel.

Results and Discussion

The current study was conducted on locally available barbecued meat and total of 240 samples were analyzed which comprised of raw as well as barbecued meat samples for various parameters and findings of this study reveals that the coal grilled meat contains more PAHs as compared to the meat grilled in gas and electric grilled medium. A similar study was conducted by Kazerouni et al. (2001) were highest quantities of PAHS were formed in meat and meat products barbecued on a grill over an open charcoal of wood chips flame. The results of this research work also suggests that the level of PAHs and heavy metals can be minimized by the addition of spices and marinades in the meat before barbecuing.

Table 1: *PAHs detected in meat available in district Peshawar with various grilling procedures (n=144).*

S. No.	Name of PAHs	Gas grilled (µg/kg-1) N=48 Mean±SD	Coal grilled (µg/kg-1) N=48 Mean±SD	Electric grilled (µg/kg-1) N=48 Mean±SD	P-Value
1	Anthracene	0.510±0.14	0.564±0.33	0.491±0.27	0.001
2	Flouranthene	0.302±0.49	2.473±0.76	1.471±1.46	0.000
3	Naphthalene	0.252±0.14	3.581±1.07	1.969±2.00	0.001
4	Chrysene	0.285±0.21	3.533±1.05	1.933±1.99	0.001
5	DBAHA	0.194±0.12	3.250±1.01	1.748±1.79	0.000
6	BaA	ND	ND	ND	

ND: *Not detected Mean followed are not significantly different at p ≤ at 0.05; Mean followed are not significantly different at p ≤ 0.05Note: The values were taken in (µg/kg-1).*

Table 2: *PAHs level of meat barbecued in un-covered medium (N=240).*

S. No.	Name of PAHs	Chicken (µg/kg-1) N=60 Mean±SD	Fish (µg/kg-1) N=60 Mean±SD	Mutton (µg/kg-1) N=60 Mean±SD	Beef (µg/kg-1) N=60 Mean±SD	P-Value
1	Anthracene	0.317±0.31	0.306±0.30	0.305±0.30	0.330±0.30	0.992
2	Flouranthene	0.583±1.03	0.815±1.32	0.543±0.98	0.423±0.78	0.001
3	Naphthalene	0.708±1.24	1.037±1.88	0.890±1.57	0.705±1.26	0.002
4	Chrysene	0.742±1.36	0.975±1.80	0.792±1.49	0.817±1.34	0.057
5	DBAHA	0.750±1.33	0.855±1.55	0.760±1.43	0.662±1.22	0.302
6	BaA	ND	ND	ND	ND	

ND: *Not detected Mean followed are not significantly different at p ≤ at 0.05;* **Note:** *The values were taken in (µg/kg-1).*

Numerous investigations on carcinogenic compounds from heterocyclic amines group have shown that it is possible to decrease their concentrations in food by applying synthetic and natural antioxidants and vegetables containing antioxidants as food additives.

Marination of meat before grilling will be helpful in minimizing the levels of meat toxicants, as marination mixture contains herbs and spices which have shown possible role in minimizing the levels of heavy metals and PAHs in grilled meat. Before grilling or cooking meat at high temperature, the meat can be cooked partially by a method which employs a lower cooking temperature such as boiling.

Results shown that grilling on coals produces high amounts of PAHs as compared to other grilling procedures like electric and gas grilling as indicated in Table 1. Among the meats the highest concentrations of PAHs were detected in fish and mutton meat.

Preventive measures for minimizing PAHs in grilled meat

PAHs create smoke that settles on food and these compounds are associated with high risk of developing cancers. There are more than a hundred PAHs which are recognized as contaminants to the environment in composite fusions (Moret et al., 2002). There are certain preventive measures which should be carried out for minimizing the PAHs in grilled meat. Some of these measures include covered grilling, which is helpful in lowering the formation of PAHs in meat as it prevents the meat from direct contact with the heat; marination of meat before grilling will be helpful in decreasing the levels of pollutants in meat, as the mixture of marinade contains a number of spices and herbs which have revealed apparent role in diminishing the levels of heavy metals and PAHs in grilled meat.

Lower temperatures should be used for meat grilling rather than high temperature and over cooking should be avoided. The meat should be cooked thoroughly to kill food borne pathogens.

The data regarding the heavy metal content of meat revealed that the lead content was found high in fish with the mean value of 3.18 and the lowest values were observed in chicken with mean value of 1.38, whereas the mean values in beef were 1.81 µg/kg and 1.55 µg/kg in mutton Figure 1. Similar work was reported by. Excessive intake of nickel produces severe allergic reactions, bronchial asthma, dermatitis, eczema and myocardial infarction (Chowdhury, 2011). The nickel content of fish was found higher

with mean value of 3.15 µg/kg and lowest values were recorded in beef with mean value 1.70 and the mean values of mutton was 1.44 µg/kg and 1.33 µg/kg in chicken respectively. Cadmium content of fish was higher with mean value of 2.49 µg/kg and lowest values were observed in chicken with mean value of 1.22. The mean values of cadmium for mutton and beef was 1.39 µg/kg and 1.44 µg/kg respectively. On the whole the heavy metal content of all meats was higher and above the permissible heavy metal doses according to the codex international standards for meat contaminants.

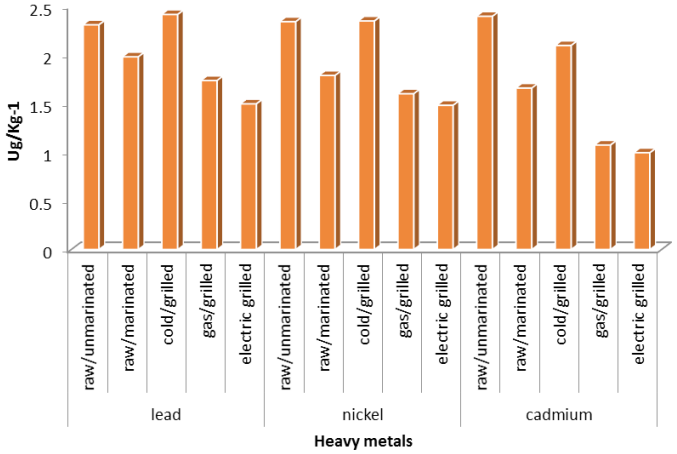


Figure 1: *Heavy metal content of meats (with different grilling procedures) Total no of cases-240.*

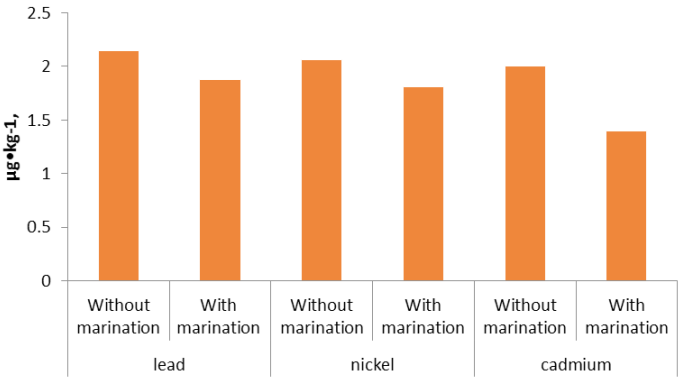


Figure 2: *Comparison of marinated and un-marinated beef samples: Total no of cases taken-60(30=marinated and 30 without marination).*

Figures 2, 3, 4 and 5 represents the comparison of marinated and unmaintained meat samples and the values revealed that the heavy metal content was found high in the unmaintained meat samples. Marinated samples comprised of low heavy metal concentration. This proves possible preventive effect of marination lowering the heavy metal contents in the meat samples as the marinades consists of herbs and spices holding antioxidant properties and medicinal effects. According to the findings of numerous conducted research work on the toxic compounds in foods, the role of marination is proven to be effective in minimizing the heavy metals in meat and other foods as well (P. Hajeb et al., 2014).

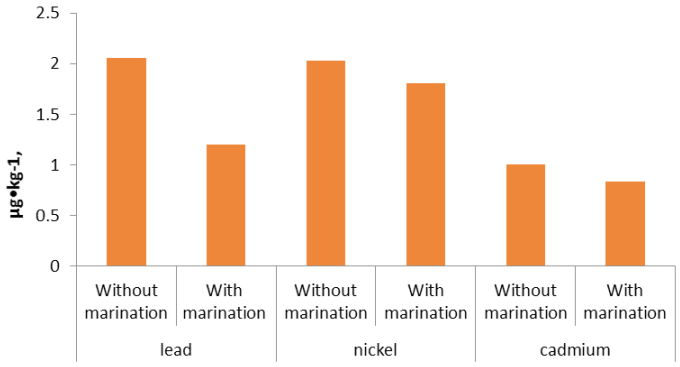


Figure 3: *Comparison of marinated and un-marinated chicken samples. Total no of cases taken-60(30=marinated and 30 without marination.*

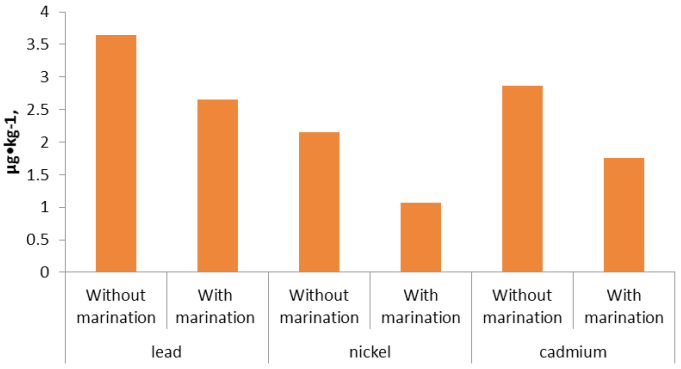


Figure 4: *Comparison of marinated and un-marinated mutton samples. Total no of cases taken-60(30=marinated and 30 without marination.*

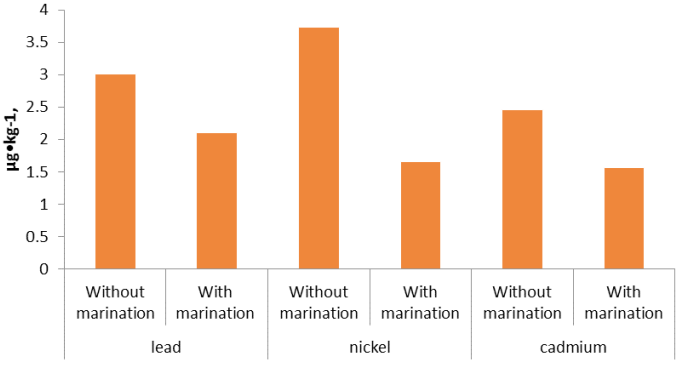


Figure 5: *Comparison of marinated and un-marinated fish samples. Total no of cases taken-60(30=marinated and 30 without marination.*

PAHS content of barbecued meat

The results of this research work show that the concentrations of each single PAH in both raw and processed samples were found higher according to international codex dose determined for PAHs Table

3. Formation of PAHs occur through pyrolysis of fat at temperatures of above 200 ^{0}C and it is favored at temperature range of 500-900 ^{0}C, especially above 700 ^{0}C These temperature ranges are taken from the given temperature ranges in electric and gas ovens available for cooking and the temperature ranges of coal grilling is taken from average temperature range of heated coals in grilling operations.

There is a relationship of PAHs with higher cooking temperatures and due to fat pyrolysis. When food is in direct contact with a flame, pyrolysis of fats in the meat generates PAHs. Alternatively, the melted fat from food dropping on the heat source generates PAHs and the PAHs will in turn be deposited on the meat surface as the smoke rises. PAHS profile of meats according to the nature of food matrix Figure 6, the anthracene content of fish was higher and lowest value was found in beef meat. Flourenthene was observed high in fish and lowest values were observed in beef. Naphthalene content was found higher in fish and lowest values were recorded in beef chrysene content was also was found higher in fish and the lowest value was recorded in chicken. DBAHA was found in higher quantities in fish and lowest value was observed in beef. The BaA level of fish was higher with and lowest value was observed in chicken. On the whole, it was concluded that the PAHs content of all meats was above the permissible dose limits settled by CODEX international and other agencies Table 3. Among meats the PAHs content of fish was observed higher and possible contributing factor for this was pyrolysis of fish fat dripping on the coals during the coal grilling, as the fish available in most of shops was grilled with skin and fats, thus, there are reservations on its intake safety and may place consumers at potential health risks.

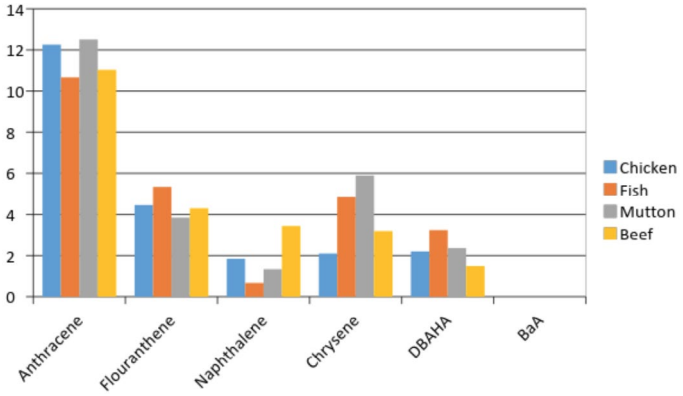


Figure 6: *PAHs level of various meats grilled on coals with un- covered medium*

Table 1 indicates the PAHs levels found in grilled meat available in District Peshawar. The major findings revealed that the coal grilled samples contained the high concentrations of PAHs than gas grilled and electric grilled samples. The coal grilled meat contains 4.875 µg/kg anthracene, 3.75 µg/kg flouranthene, whereas BaA was not detected in the analyzed meat samples.

The results of meat grilled with uncovered medium show that open flame or direct grilling produces more PAHs in meat Table 2 and less amounts are generated when meat is covered and covering of meat was accomplished with the help of aluminum foil, which was used as a medium for covering. Table 3 shows the values of covered and uncovered meat samples. Covering of meat samples minimized the rate of PAHs deposition in grilled meat. Covered grilling is helpful in lowering the formation of PAHs in meat, as it prevents the meat from direct contact with the heat. The results show that there were significant differences in level of PAHs in covered and uncovered grilling procedures.

Table 3: *Limits of detection for various PAHs in meat.*

S.No	Polyaromatic Hydrocarbons	Limit of Detection(ug/kg)
1.	Acenapthene	0.2
2.	Acenapthylene	0.1
3.	Anthracene	0.7
4.	Benz(a)anthracene	0.1
5.	Benzo(b)flouranthene	0.3
6.	Benzo(k) flouranthene	0.3
7.	Benzo(ghi)perylene	0.2
8.	Benzo(a)pyrene	0.3
9.	Chrysene	0.1
10.	Dibenz(a,h)anthracene	0.2
11.	Flouranthene	0.2
12.	Pyrene	0.2
13.	Indeno(1,2,3-cd) pyrene	0.2
14.	Phenantherne	0.1

Source: *GAIN (Global Agricultural Information Network).*

The meat grilled on direct flame produced significantly ($p \leq 0.05$) higher concentrations of PAHs in all types of meats. Anthracene content was found high in mutton meat and mean value was 12.50 µg/kg , flouernthene was assessed high in fish meat with mean value of 5.35 µg/kg , naphthalene content of chicken meat was found high with mean value of 1.85, the chrysene and DBAHA contents were observed high in mutton and

fish with mean values of 5.90 µg/kg and 3.25 µg/kg respectively and BaA was not detected in any meat samples. Grilling meat or other foods with intense heat over a direct flame results in fat dripping on the hot fire and yielding flame containing a number of PAHs.

The comparison of marinated and unmarintained meat samples values revealed that the PAHs content was found high in the unmarintained meat samples. Marinated samples comprised of low PAHs concentration and common local marinades for the barbecuing of meat were salt, dried coriander powder, red pepper, garlic paste, lemon or vinegar. This proves possible preventive effect of marination on lowering the PAHs contents in the meat samples.

The result of the mean concentrations of lead, cadmium and total nickel in the food samples analyzed are presented in Figure 1. On the contrary, the raw food samples contain lower levels of heavy metals than in the grilled samples. The effect of processing was found to be significantly correlated with the increase in the level of heavy metals in processed meat. Among the processing procedures, coal grilling was found to be responsible for the increase in toxic heavy metals whereas electric grilled samples found with decreased heavy metal concentrations as compared to coal grilled and gas grilled samples. The heavy metal content of raw unmarinated meat was found higher when compared with raw marinated meat samples, so it shows possible preventive effect of marination in minimizing the toxicants levels in meat.

The Figure 2, 3, 4 and 5 shows the role of marination in lowering the heavy metal content of mutton samples. The result has depicted the effective role of marination in minimizing the heavy metal contents in the observed mutton samples.

The heavy metal content of raw unmarinated meat was found higher when compared with raw marinated meat samples, so it shows possible preventive effect of marination in minimizing the toxicants levels in meat. Marinating foods is effective in reducing the amount of carcinogens like PAHs and heavy metals and in some cases by as much as 92 to 99%. The amount of marinating time is very important in this regard even for few minutes may help to get the full cancer-preventing effect.

Conclusions and Recommendations

The present study investigated the levels of PAHs in different barbecued meat available in District Peshawar and the effect of cooking methods on PAH levels. The purpose of this study was to investigate PAH levels in some grilled meat which is very popular and commonly consumed type of meat in the province Khyber Pakhtunkhwa and also to estimate the potential risk associated with the consumption of such traditionally processed food stuff. Four types of barbecued meats, namely beef, mutton, chicken and fish were studied. A total of 240 barbecued meat samples were analyzed and some of them were collected from local food vendor spots or general restaurants, and control samples of gas and electric grilled meat samples were prepared in the laboratory and analyzed for their PAHs content. Moreover, results have depicted the effective role of marination in minimizing the PAHs contents.

Author's Contribution

Zahin Anjum: Designed the study, conducted the research, performed experments and analysis.
Farhat Shehzad: Major supervisor who provided guidance in research.
Amina Rahat: Helped in data analysis
Hamid Ullah Shah: Provided technical input and helped in experiments.
Saleem Khan: Helped in writing the manuscript.

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