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Evaluations on Presence of Polycyclic Aromatic Hydrocarbons (PAHs) in Grilled Beef, Chicken and Fish by Considering Dietary Exposure and Risk Assessment

Abstract

Polycyclic aromatic hydrocarbons (PAHs) are dangerous chemical compounds that can be formed by cooking foods at high temperatures. The aim of this study is to determine the level of contamination of PAH compounds with HPLC on heat treated meat samples and the consumption of PAH compounds in meat samples, as well as the dietary exposure status and possible health risk estimation. In five different heat treated meat samples (meat doner, chicken doner, meatballs, grilled chicken and fish), the total PAH (Σ16PAH) contamination level was 6.08, 4.42, 4.45, 4.91 and 7.26 µg/kg, respectively. Benzo[a]pyrene (BaP) in meatballs and grilled fish samples had a level between 0.70 and 0.73 µg/kg. All of the samples analyzed were found to be below the EU permitted limit (5 µg/kg) in terms of BaP. Estimates of daily intake (EDI) for a total of 16PAH in heat treated meat doner, chicken doner, meatballs, grilled chicken and fish samples were 3.41, 3.71, 2.49, 4.12 and 1.77 ng/kg bw/day, respectively. In this study, the average margin of exposure (MOE) value calculated was found in the range of 179.487 and 425.000 for BaP and PAH4. This study is the first study to provide important information in terms of evaluating the possible health risk that PAH compounds can create in people's diets due to heat treatment of meat and meat products
in Sivas, Turkey.

Keywords: Polycyclic aromatic hydrocarbons (PAHs), Benzo[a]pyrene, Grilled meats, Dietary exposure, Risk assessment.

Introduction

Polycyclic aromatic hydrocarbons (PAH) are hydrophobic organic compounds composed of carbon and hydrogen atoms containing two or more aromatic rings. It is stated that there are 200 different PAH compounds in this group (ATSDR, 1995; Singh et al., 2016). PAH compounds are evaluated by the European Food Safety Authority (EFSA), the Food Scientific Committee (SCF) and the Committee of Food Additives Experts (JECFA). Of these, 16 PAH compounds are considered to be a priority. This 16 compounds are as follows; Naphthalene (Nap), Acenaphthene (Ace), Acenaphthylene (Acy), Fluorene (Fle), Phenanthrene (Phe), Anthracene (Ant), Fluoranthene (Flu), Pyrene (Pyr), Benzo[b]fluoranthene (BbF), Benzo[k]fluoranthene (BkF), Benzo[a]anthracene (BaA), Chrysene (Chr), Benzo[a]pyrene (BaP), Indeno[1,2,3-cd]pyrene (IcdP), Dibenzo[a,h]anthracene (DahA) and Benzo[ghi]perylene (BghiP) (EFSA, 2008; Sing and Agarwal, 2018).

To better understand the health risks associated with people being exposed to PAHs through consuming contaminated foods, the European Food Safety Authority (EFSA, 2008) was divided PAHs into three according to carcinogenic, mutagenic, and toxic activities. These include PAH2 (BaP and Chr), PAH4 (BaA, BaP, BbF and Chr), and PAH8 (BaA, BaP, Chr, BkF, BbF, IcdP, DahA, BghiP). Studies conducted on experimental animals as in vivo reported that these compounds have a mutagenic/genotoxic effect in somatic cells (EFSA, 2008). It is also reported that these compounds may be considered potentially genotoxic and carcinogenic in humans (Domingo and Nadal, 2015; Lee and Shim, 2007; Yoon et al., 2007).

It is very difficult to determine quantitatively due to the wide variety of PAH compounds and the variability of the amounts in the samples. Therefore, the most important PAH compound known as the most potent carcinogenic, BaP (Group 1 Carcinogen) has been chosen for evaluation. The European Food Safety Authority (EFSA, 2008) stated that BaP analyses alone are not sufficient and instead of the conduction of using the four PAH (PAH4) system (BaA, BaP, BbF and Chr) or the eight PAH (PAH8) system (BaA, BaP, Chr, BkF, BbF, IcdP, DahA, BghiP) recommended. Due to its effects on human health, the maximum incidence of BaP and PAH4 compounds in foodstuffs has been determined to control people's exposure to these compounds in many countries, including Turkey. According to the Turkish Food Codex Food Contaminants Regulation (TFC, 2011) and the European Union Directive (EC 1881/2006), the maximum limit for PAH compound of BaP is determined as 5 µg/kg and the sum of PAH4 is determined as 30 µg/kg.

In recent years, the importance given to PAH has increased due to the large number of sources of transmission. People's exposure to PAH type molecules is mostly through diet. (Alomirah et al., 2011; Bansal and Kim, 2015). There are two ways in which PAHs are transmitted to food. The first is environmental contamination caused by air, water and soil, and the other is when food is processed and cooked. The process of processing of food (smoking and drying), cooking at high temperature (frying, grilling and roasting) are the main reason for the formation of PAHs (Bansal and Kim, 2015; Jiang et al., 2018; Singh and Agarwal, 2018). In foods cooked at high temperatures (above 200°C), pyrolysis occurs as a result of oil dripping into the flame and the fumes that are generated and the PAHs infect the food through the fumes. In grilled meats cooked in charcoal flames, PAHs are formed depending on the amount of fat contained in the meat, the cooking temperature and duration (Aydın and Şahan, 2018; Duedahl-Olesen et al., 2015; Farhadian et al., 2010).

PAHs that appear in smoked, grilled or grilled meat over open heat can damage DNA and increase the risk of cancer (IARC, 2015; Lee et al., 2016). There is no direct evidence as to whether meat consumption directly triggers this mechanism. Therefore, there is no proven information about which is the safest method of cooking meat. In addition, cooking methods such as barbecue (direct contact with fire), grilling and barbecue reveal more carcinogenic compounds such as PAH in red meat (IARC, 2015).

It is a well-known fact that meat and meat products are important in nutritions (Arslan, 2013). Changing consumer habits, economic conditions and increased diversity of processed food are increasing consumption of meat and meat products (MMB, 2018). There have been some studies examining the relationship between different types of meats, meats with different cooking methods and the chemical structure of meats for the determination of PAH compounds (Aydın and Şahan, 2018; Aygün and Kabadayi, 2005). In addition to the formation of PAH compounds, there was no data on the dietary exposure to PAH compounds and the evaluation of possible health risk. The main purpose of this study was to investigate the types and quantities of PAH components contained in the samples of doner (meat and chicken), chicken, meatballs and fish and with heat treatment by grilling method. Using literature support and universal acceptances based on measured PAH values, it is the estimation of dietary exposure and possible health risk along with the consumption of these food products.

Materials and Methods

Chemicals and standards

In this study, chemicals that were used as follows: a mixture of PAH calibration standards (Sigma-Aldrich; CRM 47940, St. Louis, Missouri, USA), Acetonitrile (Merck; 1.00030, Darmstadt, Germany) and Methanol (Merck; 1.06007, Darmstadt, Germany). 1, 2, 5, 10 and 20 mg/L calibration standards were prepared using the main stock solution of 2000 mg/L (methanol/acetonitrile) of PAH calibration standard blends. Prepared stock standard solutions were stored in dark glass vials and at 4℃.

Sampling and sample treatment

In this study, a total of 100 samples consisting of 20 randomly chosen meat doner, chicken doner, meatballs, grilled chicken and fish were used as materials. Each sample was analyzed in 3 repetitions. Samples were taken between March and June 2017 from different restaurants in Sivas, Turkey. Samples were brought to the laboratory under cold chain at 4℃ and samples that would not be analyzed immediately were stored in the freezer -20℃ (Table 1).

The method proposed by Farhadian et al. (2010) for sample preparation before HPLC analysis was applied with some minor modifications. Firstly, the main reagents of the method, Carrez I and Carrez II solutions were prepared according to literature. In chromatographic analysis for each sample type, a part of PAH calibration standard was spiked to control samples in order to determine the location of the peaks and the reliability of the analysis (Sigma-Aldrich, PAH Cationlibra Mix 47940, USA).

For Carrez solutions, 10.6 g potassium ferrocyanide [$K_4Fe(CN)_6.3H_2O$] (Merck; 1.04984) was weighed for Carrez I solution and dissolved in distilled water to 100 mL. For Carrez II solution, 21.9 g zinc acetate $Zn(CH_3COO)_2.2H_2O$ was weighed, 3 mL of acetic acid (CH_3COOH) was added and completed to 100 mL with distilled water. The prepared solutions were stored in dark glass bottles and at 20℃.

Then, about 50.0 g samples were homogenized and weighed 3.0 g from each sample. It was added from a mixture of 10 mL of 1 M KOH and 10 mL of Methanol/Acetonitrile (50:50) on samples. The tubes were closed and mixed, vigorously. Then, It was kept in ultrasonic water bath at 40℃ for 10 min. The tubes were then shaken at 2 (×g) for 30 min in orbital shaker and the organic content was transformed into the solution phase. After waiting for 10 min at 40℃ in ultrasonic water bath, the tubes were centrifuged at 4200 (×g) for 5 min. After this process, the solid and liquid phases were separated from each other and the liquid phase was taken to another tube. Then, 1.3 mL of 6 M HCl was added to the liquid phase and the pH was adjusted to 6 then 1 mL Carrez 1 and 1 mL Carrez II solutions were added. After the tubes were thoroughly shaken, they were centrifuged at 4200 (×g) for 5 min. The 1.5 mL sample from the upper phase after centrifuge was filtered with 0.45 μm syringe tip filters and transferred to HPLC vials.

HPLC analysis of PAH molecules

The HPLC system were consisted of Shimadzu HPLC High Performance Liquid Chromatography (Shimadzu Corporation, Japan) and SPD-10AD DAD Detector (Shimadzu Corporation, Japan) at a flow rate of 1 mL min^{-1} and column temperature of 30℃; The injection volume for the samples was set at 10 μL, and wavelength 256 nm. Luna Omega C18 HPLC column (250 mm x 4.6 mm, 5 μm) from Phonemex (California, USA chromatographic column was used to separate the analytes. Eluents were filtered through 0.45 μm microporous membrane and degassed under ultrasound for 15 min before use.

The mobile phase composition was MeOH:ACN:pH 6.0 phosphate buffer (40:10:50) at isocratic mode throughout analysis. The detector wavelength was operated at 256 nm for all PAH molecules. Peaks in the chromatograms were identified by comparison with retention times and UV spectra of standards. Peak area was considered for quantification. All data were recorded using the above described chromatographic conditions. The obtained chromatogram under the optimum conditions was given in Figure 1. The retention time of each compound was plotted with their names.

Method validation

Analytical validation of the analysis method was partly carried out by using experimental studies. Firstly, linear range, LOD and LOQ values were calculated for each PAH compounds by considering experimental observations. Linearity was checked by constructing the calibration curves using spiked model solutions at 6 concentration levels. Calibration curves were obtained by graphing peak area of each PAH molecules versus its concentration. Linear regression analysis was used to calculate the slope, intercept and the correlation coefficient of each calibration line. The results were presented in Table 2. Accuracy expressed as percentage recovery was calculated from the found mass of the analyte on the spiked sample toward added mass after replicate analysis of spiked samples at various concentrations. Recovery values were found in the range of 95.3-104.8% for PAH molecules.

Dietary exposure and health risk estimations

The exposure of consumers to PAH was assessed by daily meat consumption, depending on the levels of contamination obtained in this study. Data on meat consumption were obtained from the Meat and Milk Board of Turkey and the daily consumption amount was determined (MMB, 2018). The body weight of an adult person was taken as 70 kg and this value was used in the calculations (Turkmen et al., 2009). Estimated daily intake (EDI) was calculated using the following formula (Bogdanovic et al., 2019): (Eq. 1):

$$EDI = \frac{Ci \; x \; Cc}{BW}$$

Where;

EDI = ng/kg body weight per day

Ci = for the mean concentration of PAH or combination of PAHs (µg/kg)

Cc= the daily average consumption of meat per person (g)

BW = for the body weight (kg) of the consumer

The Toxic Equivalency Quotients (TEQ)

We calculated TEQ concentrations of 16 target PAHs with Eq. (2) as follows: The toxic equivalency factors (TEFs) suggested by Nispet and Lagoy, (1992) were used to calculate the TEQ_{BaP} value (Eq. 2).

$TEQ_{BaP} = C_i \times TEF_i$

Where;

C_i= is a PAH congener, (i) is the sample concentration (ng/kg)

TEF_i= is the BaP value relative to the potency value published for each individual PAH.

Margin of Exposure (MOE)

In the present study health risk characterization was performed by the margin of exposure (MOE) approach according to the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) (EFSA, 2008). Taking into account the findings of the EFSA study on PAHs in food, the BMDL10 for BaP was 0.07 mg/kg bw/day, and the BMDL10 for the PAH4 was 0.34 mg/kg bw/day. These values were used as a reference for the calculations of MOEs (EFSA, 2008).

$$MOE = \frac{BMDL_{10}}{EDI}$$

Where;

$BMDL_{10}$ = dose lower limit measurable response of 0.1 mg benzo[a]pyrene / kg BW / day

EDI = the chronic daily dietary PAHs exposure (mg/kg BW/ day)

Results and Discussion

Contamination levels of PAHs in grilled meats

In this study, five different meat samples consisting of meat doner, chicken doner, meatballs, grilled chicken and fish, which are offered for consumption by heat treatment at different restaurants in Sivas, were analyzed using HPLC method to determine the level of PAH compounds (Table 1). In this study, a developed and approved (validated) method was used for quantitative analysis of 16 PAH compounds in heat treated meat samples (Table 2).

Known as the most important PAH compound, BaP is considered an indicator in determining the level of PAH (EFSA, 2008). In this study, the level of BaP in meatball and fish samples was between 0.70 and 0.73 μg/kg, respectively. The presence of BaP was not detected in heat treated meat doner, chicken doner and grilled chicken samples (Table 3). The technique of cooking the meat can differ in terms of PAH formation (Lee et al., 2016). In one study, two different geometric cooking techniques were compared in meat and fish samples. It has been reported that the amount of PAH formed by the oil dripping directly into the source of fire in the horizontally cooked meat occurs more than cooked in a vertical position (Saint-Aubert, 1992). In this study, meatballs and fish samples were cooked horizontally and in the charcoal flame. Interestingly, although it was cooked horizontally on the grill and in a charcoal fire, there was no presence of BaP in the grilled chicken samples. It is thought that this is due to the lack of skin on the breast used in the grilled chicken. During grilled cooking, the oils, which are primarily located on the outer surfaces of the meat and come into direct contact with flame, melt and contribute to the formation of PAH by dripping on the fire. Lee et al. (2016) in their research in Korea were cooked beef on a barbecue and used a design to prevent meat oil from dripping onto the flame. As a result, 3.23 μg/kg BaP was detected as a result of cooking the beef on the grill on the charcoal fire, while 0.78 μg/kg BaP was determined in the system where the oil dripping was prevented. In a study conducted in

Turkey, Terzi et al. (2008) investigated BaP levels in samples of doner kebabs cooked in coal and gas fire. In the samples cooked in coal fire, they found the average amount of BaP as 24.2 µg/g and 5.7 µg/g in samples cooked in the gas flame. In this study, meat and chicken doner samples were cooked vertically on the electric grill and the presence of BaP was not detected.

When roasted beef, turkey, lamb and chicken meats were evaluated in terms of PAH4 (BaP, BaA, BbF and Chr) concentration, it was reported that differences were observed according to the type of meat and PAH type (Aydın and Şahan, 2018). Aydın and Şahan, (2018) in their study evaluated meat samples in terms of PAH4; they determined the highest amount of PAH4 in chicken meat (3.30 ppb), followed by turkey (3.14 ppb), lamb (1.74 ppb) and beef (1.10 ppb). They stated that the reason for this difference may be due to the difference in the chemical composition of meats. In this study, PAH4 (BaP, BaA, BbF and Chr) concentration was evaluated according to meat products; the lowest PAH4 amount (1.95 µg/kg) was determined in meatballs samples, and the highest PAH4 (3.30 µg/kg) was determined in grilled fish samples. Olatunji et al. (2013) stated that total PAH concentrations in grilled chicken fillets and beef stripes were ranged 0.99, 9.29 µg/kg, respectively. In accordance with this study, Kim et al. (2014) in their study in Korea were reported that they found the amount of PAH4 in fish samples as 0.19 µg/kg. Researchers reported that levels obtained from fish samples were below the EU's limit in terms of both BaP (5 µg/kg) and PAH4 (30 µg/kg). The results of this study were evaluated in accordance with the Turkish Food Codex Food Contaminants Regulation and the European Union Directive (EC 1881/2006; TFC, 2011). In the relevant regulation, the maximum limit for PAH compounds of BaP in the meat and meat products analyzed is 5 µg/kg and for PAH4 is given as 30 µg/kg. The analyzed meat doner, chicken doner, meatballs, chicken and fish samples were all found to be below the permitted limits for BaP and PAH4 compounds (Table 3).

When the PAH compounds were evaluated individually, the presence of Ant, which is one of the light PAHs (LPAHs include: Nap, Ace, Ane, Fle, Phe, Ant, Flu, and Pyr), was found in all meat samples. In meat doner, chicken doner, meatballs, grilled chicken and fish samples, the presence of Ant was found to be 0.95, 1.07, 0.93, 0.87, and 0.97 µg/kg, respectively. A study by Hamzawy et al. (2016) in Egypt reported a similar result in meat (0.90 µg/kg) and grilled chicken (0.67 µg/kg) samples related to the presence of Ant.

The presence of BkF, BbF, and Chr, which are heavy PAH (HPAHs include: BaA, BaP, BbF, BkF, Chr, DahA, BghiP and IcdP) were detected in all meat samples. Among the heavy PAH compounds, the amount of BkF in grilled fish samples was found to be highest at 0.91 µg/kg, while the amount of BbF was 0.77 µg/kg and Chr was 0.63 µg/kg. In grilled fish samples, BaP concentration was found as (0.77 µg/kg) and did not exceed the maximum allowable limit of 5 µg/kg. PAH8 which includes heavy PAHs, also known as genotoxic PAH, was found to be above 5 µg/kg. In a study conducted by Alomirah et al. (2011), the average BaP level in smoked fish samples was 0.50 µg/kg and did not exceed the limit value, but genotoxic PAH8 was found to be 10.3 µg/kg (7.58-12.9 µg/kg) which was above the allowable (5 µg/kg) value. These results also showed that BaP is not a good indicator as a carcinogenic and genotoxic PAH compound alone (Alomirah et al., 2011; EFSA, 2008).

The formation of each PAH compound and the average level of contamination in all heat treated meat products (meat doner, chicken doner, meatballs, grilled chicken and fish) are given in Table 3. In five different meat samples (meat doner, chicken doner, meatballs, grilled chicken and fish), the total level of PAH (Σ16PAH) was 6.08, 4.42, 4.45, 4.91 and 7.26 µg/kg, respectively. Jiang et al. (2018) in their study in China were found the contamination level of Σ15PAH between 12.0-341.0 µg/kg and the average level was 80 µg/kg in grilled meat samples. Duedahl-Olesen et al. (2015) in their study in Denmark were found the concentration of Σ16PAH in barbecued beef (steak) and chicken breast as 10.2 and 6.3 µg/kg. In a study conducted by Alomirah et al. (2011), Σ16PAHs level was in the range of 48.2-342.0 µg/kg in grilled chicken meat, with an average of 222 µg/kg. PAHs are caused by pyrolysis or incomplete combustion of organic material during cooking of meat at high temperatures (Alomirah et al., 2011, Farhadian et al., 2010; Jiang et al., 2018). Chung et al. (2011) were investigated that roasted and baked cattle, chicken and pork meat contained PAH, and reported that the charcoal grill caused the highest PAH level. They found that the samples of chicken meat grilled in a charcoal fire contained the highest level of Σ PAHs (9.46 µg/kg). For this reason, in grilled meats cooked in a charcoal fire, PAHs are formed depending on the amount of fat contained in the meat, the cooking temperature and duration (Aydın and Şahan, 2018; Chung et al., 2011; Duedahl-Olesen et al., 2015; Jiang et al., 2018; Lee et al., 2016).

Dietary exposure estimation

Estimates of the daily dietary PAH, PAH4, PAH8, and Σ_{16}PAH intakes of adult people from meat products (meat doner, chicken doner, meatballs, grilled chicken and fish) were given in Table 4. Daily intake estimates of individual PAH compounds in meat doner samples were 0.13 to 0.72 ng/kg bw/day on average, while exposure for PAH4, PAH8, and Σ16PAH was estimated as 1.24, 1.78, and 3.41 ng/kg bw/day, respectively. For grilled fish samples, daily intake estimates of individual PAH compounds were 0.03 to 0.24 ng/kg bw/day, PAH4, PAH8, and Σ16PAH exposure were 0.80, 1.25 and 1.77 ng/kg bw/day. In addition, the average daily BaP intake for adults was 0.39 ng/kg bw/day and 0.18 ng/kg bw/day in meatball and grilled fish samples. According to the EFSA (2008) stated that the average amount consumers will be exposed to for meat and meat products for member states is 132 g/day, while for BaP, PAH4, and PAH8, it is 42, 195 and 279 ng/day.

Estimated dietary intake of PAH compounds has been compared with previous studies. In one study, the average dietary intake of BaP and PAH8 in grilled and smoked meat products by Kuwaiti people was found to be 9.20 and 95.7 ng/day (Alomirah et al., 2011). Kim et al. (2014) were stated in their evaluation that the average dietary intake of Korean people in all age groups from fish and shellfish for BaP, PAH4 and PAH8 was 0.01 ng-TEQBaP/kg/day, 0.01 ng-TEQBaP kg/day and 0.01 ng TEQBaP kg/day. In the same study, exposure to meat products was determined as 0.15 TEQBaP/kg/day, 0.29 TEQ BaP/kg/day and 0.54 TEQBaP/kg/day for BaP, PAH4 and PAH8. Jiang et al. (2018) were reported in their study which was conducted in China that exposure to BaP, PAH4, PAH8 and Σ15PAH in grilled meat samples of the adult population was as 0.49, 3.96, 4.99 and 120 ng/kg bw/day, respectively. Researchers were stated that the dietary intake of PAHs is in relation to the dietary habits of the consumers and the PAH contamination load in foods. Also it has been stated that the consumers would be exposed to more than average levels of PAHs as the consumption rate increases.

Risk assessment of PAHs

In this study, risk assessment of heat treated meat products was carried out with TEQ and MOE approach. The TEQ approach has been applied to directly assess the carcinogenity of PAH contamination to heat treated meat products (meat doner, chicken doner, meatballs, grilled chicken and fish). BaP-like TEQs for individual PAH compounds were given in Table 4. In this study, the average TEQ value calculated for the Σ16PAH compound was 328.46, 570.9, 1,746.48, 310.46 and 1,116.42 ng/kg in heat treated meat doner, chicken doner, meatballs, grilled chicken and fish samples, respectively. As a matter of fact, it has been reported that PAHs are formed in grilled meats cooked in charcoal fire depending on the amount of fat contained in the meat, the cooking temperature and duration (Lee et al., 2016).

In this study, the MOE value calculated for BaP and PAH4 was given in Table 5. The MOE value calculated for PAH4 in heat treated meat products was found as lowest at 165.048

in chicken doner samples and as highest at 425.000 in fish samples. EFSA (2008) stated that if the MOE value was less than 10.000, it could be a potential concern for human health. Rozentale et al. (2018) stated that MOE was 11.602 for BaP and 8486 for PAH4, which could be a risk for middle-aged consumers (39-50 years). A study by Duedahl-Olesen et al. (2015) in Denmark found a health concern with 7080 and 8500 of more value, respectively, with excessively contaminated barbecue and home cooked meat consumption on a daily basis in a worst-case scenario. Kim et al. (2014) found that MOE for PAH4 was 485.437 for fish and shellfish, 25.634 for meat and 265.957 for smoked products, and stated that the risk assessment results were at safe intervals. As a matter of fact, the data obtained in this study showed that the risk assessment results were within a reliable range.

Conclusion

Consequently, the contamination levels of the Σ16PAH compound were determined by HPLC method in heat treated meat doner, chicken doner, meatballs, grilled chicken and fish samples in Sivas, Turkey. The level of BaP in meatball and fish samples was between 0.70 and 0.73 µg/kg. The average MOE value calculated in this study was found in the range of 179.487 and 425.000 for BaP and PAH4. The fact that there were less than 10.000 critical limits reported by the EFSA has shown that the results of the study are within a reliable range. However, there is a need for MOE evaluation of individuals in different age groups. The data from this study were used to predict consumers' dietary exposure to PAH compound. This study provides important information in terms of evaluating the possible health risk that PAH compounds due to heat treatment of meat and meat products can create in people's diets.

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Table 1. Description of heat treated meat samples collected from Turkey and the cooking methods

Cooking method	**Meat samples or (Food item)**	**Description**
Electric grilled (direct heat)	Meat doner (Beef meat, Turkish kebab) Chicken doner (Chicken meat, Turkish kebab)	Meat and chicken doner kebab is made from fillets of meat stacked on a vertical spit and roasted on a vertical grill. The production of doner beef or poultry meat is seasoned with pepper, onion, tomatoes and some spices. The beef meat and some animal fats are shredded or ground, after then mixed with seasoning materials and molded to give a cone like shape. Doner means turning of the vertical spit is rotated in front of the heat source (electric or charcoal). The cooked meat parts of the doners cut into thin slices.
Charcoal grilled (direct heat)	Meatball (Beef or sheep meat)	Meatball are used from beef or sheep meat as raw material. Salt is added for each of the mixture prepared and it is ground in mincing machine. The mixture is sliced in to 25 g slices and an oval shape is given to the meat by hand. The meatballs prepared are grilled over an intense charcoal grilled fire by turning upside down in short intervals to cook both sides.
Charcoal grilled (direct heat)	Grilled chicken (Chicken breast)	Grilled chicken is made from breast of chicken (without skin) and also some spices with marinated. Chicken are grilled for 8-10 min on each side using charcoal.
Charcoal grilled (direct heat)	Grilled fish (Whole anchovy fish)	Grilled fish is made from whole anchovy (*Engraulis encrasicolus*, L.1758) fish. Fish cooked over charcoal for to cook both sides.

Table 2. Validation parameters of HPLC method for determination of target compound PAHs

Target compound (PAHs)	**Peak Number**	**Range (µg/ kg)**	**LOD (µg/k^{g})**	**LOQ (µg/ kg)**	**r^2**
Naphthalene	2	0.125–250	0.050	0.100	0.993
Acenaphthene	3	0.100–250	0.035	0.070	0.994
Acenaphthylene	4	0.100–250	0.035	0.070	0.995
Anthracene	5	0.125–250	0.050	0.100	0.987
Benz[a]anthracene	6	0.125–250	0.050	0.100	0.989
Benzo[a]pyrene	7	0.125–250	0.050	0.100	0.985
Benzo(k)flouranthene	8	0.125–250	0.050	0.100	0.999
Bezo[b]fluoranthene	9	0.125–250	0.050	0.100	0.991
Chrysene	10	0.100–250	0.035	0.070	0.986
Flouranthene	11	0.100–250	0.035	0.070	0.985
Phenanthrene	12	0.100–250	0.035	0.070	0.995
Dibenzo(a,h)anthracene	13	0.100–250	0.035	0.070	0.993
Pyrene	14	0.200–250	0.065	0.175	0.997
Benzo[ghi]perylene	15	0.200–250	0.065	0.175	0.984
Flourene	16	0.200–250	0.065	0.175	0.986
Indenol[1,2,,3-cd]pyrene	17	0.200–250	0.065	0.175	0.990

Table 3. Determination of PAHs levels in grilled meats (µg/kg) (n:3)

PAHs	Meat Doner (µg/kg)	Chicken Doner (µg/kg)	Meatball (µg/kg)	Grilled Chicken (µg/kg)	Grilled Fish (µg/kg)
Nap*	0.26±0.02	ND	0.18±0.02	ND	0.14±0.02
Ace*	0.41±0.03	ND	0.39±0.02	ND	0.33±0.04
Ane*	1.29±0.06	ND	ND	0.12±0.02	0.21±0.02
Fle*	ND	0.20±0.01	0.21±0.02	0.24±0.02	0.24±0.03
Phe*	ND	ND	ND	ND	ND
Ant*	0.95±0.05	1.07±0.05	0.93±0.04	0.87±0.04	0.97±0.05
Flu*	ND	ND	ND	ND	0.24±0.03
Pyr*	ND	ND	ND	ND	ND
BaA**	0.94±0.04	1.13±0.05	ND	0.89±0.04	1.17±0.06
BaP**	ND	ND	0.70±0.03	ND	0.73±0.04
BkF**	0.72±0.03	0.70±0.04	0.62±0.04	0.63±0.03	0.91±0.02
BbF**	0.71±0.04	0.62±0.04	0.74±0.04	0.71±0.04	0.77±0.03
Chr**	0.56±0.03	0.70±0.03	0.51±0.04	0.53±0.02	0.63±0.04
DahA**	ND	ND	ND	ND	ND
BghiP**	ND	ND	ND	0.74±0.04	0.74±0.04
IcdP**	0.24±0.02	ND	0.17±0.01	0.18±0.02	0.18±0.02
PAH4	**2.21 ± 0.06**	**2.45 ±0.07**	**1.95 ±0.06**	**2.13 ±0.06**	**3.30 ±0.09**
PAH8	**3.17 ±0.07**	**3.15 ± 0.08**	**2.74 ± 0.08**	**3.68 ± 0.08**	**5.13 ± 0.10**
Σ_{16}PAH	**6.08 ± 0.11**	**4.42 ±0.10**	**4.45 ± 0.09**	**4.91 ± 0.09**	**7.26 ± 0.13**

ND: Not detected
Lihgt PAH: (Nap, Ace, Ane, Fle, Phe, Ant, Flu, and Pyr)
Heavy PAH (BaA, BaP, BbF, BkF, Chr, DahA, BghiP and IcdP)
PAH4: BaA, Chr, BbF, BaP
PAH8: BaA, Chr, BbF, BkF, BaP, DahA, BghiP and IcdP

Table 4. Estimated daily intake (ng/kg bw/day) and toxicity equivalency quotient concentrations (TEQ_{BaP} ng/kg) of PAHs in grilled meats

PAHs	TEFs*	Meat Doner		Chicken Doner		Meatball		Grilled Chicken		Grilled Fish	
		EDI (ng/kg)	**TEQ (ng/kg)**	**EDI (ng/kg)**	**TEQ (ng/kg)**	**EDI (ng/kg)**	**TEQ (ng/kg)**	**EDI (ng/kg)**	**TEQ (ng/kg)**	**EDI (ng/kg)**	**TEQ (ng/kg)**
Nap	0.001	0.15	0.26	ND	ND	0.10	0.18	ND	ND	0.03	0.14
Ace	0.001	0.23	0.41	ND	ND	0.22	0.39	ND	ND	0.08	0.33
Ane	0.001	0.72	1.29	ND	ND	ND	ND	0.10	0.12	0.05	0.21
Fle	0.001	ND	ND	0.17	0.2	0.12	0.21	0.20	0.24	0.06	0.24
Phe	0.001	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Ant	0.01	0.53	9.5	0.90	10.7	0.52	9.3	0.73	8.7	0.24	9.7
Flu	0.001	ND	ND	ND	ND	ND	ND	ND	ND	0.06	0.24
Pyr	0.001	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
BaA	0.1	0.53	94	0.95	113	ND	ND	0.75	89	0.29	117
BaP	1	ND	ND	ND	ND	0.39	700	ND	ND	0.18	730
BkF	0.1	0.40	72	0.59	70	0.35	62	0.53	63	0.22	91
BbF	0.1	0.40	71	0.52	62	0.41	74	0.60	71	0.19	77
Chr	0.1	0.31	56	0.59	70	0.29	51	0.45	53	0.15	63
DahA	5	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
BghiP	0.01	ND	ND	ND	ND	ND	7.4	0.62	7.4	0.18	7.4
IcdP	0.1	0.13	24	ND	ND	0,10	17	0.15	18	0.04	18
PAH4		**1.24**	**221**	**2.06**	**245**	**1.09**	**825**	**1.79**	**213**	**0.80**	**987**
PAH8		**1.78**	**317**	**2.65**	**315**	**1.54**	**911.4**	**3.09**	**301.4**	**1.25**	**1 103.4**
Σ_{16}PAH		**3.41**	**328.46**	**3.71**	**570.9**	**2.49**	**1746.48**	**4.12**	**310.46**	**1,77**	**1116.42**

ND: Not detected.

*The toxicity equivalency factors relative to BaP for PAHs (Nisbet and Lagoy, 1992).

Table 5. Estimates of daily intake (EDI) ng/kg bw/day and risk assessment (MOE) in different meat products groups

Country	Foodstuffs	BaP		PAH4[1]		Reference
		EDI	MOE	EDI	MOE	
Turkey	Meat doner[a]			1.24	274.193	In this study
	Chicken doner[a]			2.06	165.048	
	Meatball[a]	0.39	179.487	1.09	311.926	
	Grilled chicken[a]			1.79	189.944	
	Grilled fish[a]	0.18	388.888	0.80	425.000	
Croatia	Meat products[b]	14.35		62.56	66.213	Bogdanovic et al. (2019)
	Shellfish products[a]	4.44		24.35	298.000	
Egypt	Grilled meat[a]	290.45				Darwish et al. (2019)
	Pan-Fried meat[a]	109.36				
Latvia Lithuania Estonya	Smoked meat products	5.4	11602	35.89	8486	Rozentale et al. (2018)
China	Grilled meat[c]	0.49		3.96		Jiang et al. (2018)
	Fried meat[c]	0.58		2.12		
Egypt	Grilled meat	0.20				Hamzawy et al. (2016)
	Grileld chicken	0.89				
Denmark	Restaurant (Beef, pork, chicken) barbecued meat			48	7080	Duedahl-Olesen et al. (2015)
	Home (Beef, pork, chicken) grilled meat			40	8450	
Korea	Fish and Shell-fish	0.18	549.451	0.20	485.437	Kim et al. (2014)
	Meat	2.00	49.900	3.90	25.634	
	Smoked products	0.15	666.667	0.37	265.957	
Kuwait	Grilled and smoked meat[d]	9.20				Alomirah et al. (2011)
France	Foodstuffs	0.191		1.48	230,000	Veyrand et al. (2013)

[a,b,c,d] Bw considered as 70, 79.09, 60, 80.7 kg, respectively.
PAH4[1] = BaP, BaA, BbF, Chr

Fig. 1. Chromatogram of PAH mix standart