Analysis of some kinds of polycyclic aromatic hydrocarbons in different meat products using high-performance liquid chromatography with fluorescence detector

Soma Faeq Muhammed Rashid*, Khulod Ibraheem Hassan

Department of Food Science and Quality Control, College of Agricultural Engineering Sciences , University of Sulaimani, Kurdistan Region of Iraq.
***Corresponding author e-mail: soma.muhammed@univsul.edu.iq.**
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Abstract

Polycyclic aromatic hydrocarbons (PAHs) in meat products have raised serious issues for scientists over the past few decades because of their highly toxic effects on human health, hence, monitoring their levels in this food are very important. The aim of this study is to estimate five kinds of PAHs Benzo[a]pyrene (B[a]P), Benzo[a]anthracene (B[a]A), Benzo[b]fluoranthene (B[b]F), chrysene (CHR), and Indenol[1,2,3-cd] pyrene (IND) in some meat products (kabab, teka, burger, and shawarma) in local markets of the Sulaymaniyah / Kurdistan region of Iraq. High-performance liquid chromatography (HPLC) with a fluorescence detector (FLD) was applied for their analysis after validation of the technique in terms of linearity, specificity, sensitivity, and accuracy. The results show good linearity ($R^2 = 0.9918$) with the limit of detection (LOD) ranging from 0.1 to 0.5 µg kg^{-1} and the limit of quantification (LOQ) ranging from 0.3 to 1.5 µg kg^{-1} with recovery values ranging from 90 to 105% for the selected PAHs. Due to its carcinogenicity, B[a]P is used as a biomarker with PAH4 (B[a]P, B[a]A, CHR, and B[b]F). The highest level of B[a]P (2.56 µg kg^{-1}), PAH4 (13.65 µg kg^{-1}), and IND (2.60 µg kg^{-1}) was recorded in the shawarma sample, which exceeded the EU maximum limit of B[a]P (2 µg kg^{-1}) and PAH4 (12 µg kg^{-1}), and makes it the most contaminated type of meat product with the selected PAHs used in this study, followed by teka samples. Further research is suggested to expand the study to a wider range of samples and investigate methods for mitigating PAHs levels in these products.

Keywords: Polycyclic aromatic hydrocarbons (PAHs), meat products, high-performance liquid chromatography (HPLC), fluorescence detector (FLD).

Introduction

Meat and its products are extensively consumed worldwide through various preparation methods, owing to their nutritional importance, particularly their supply of essential amino acids, essential fatty acids, vitamins, and minerals crucial for human health [1]. Red meat has additional nutritional advantages over other meat varieties,

including iron, zinc, vitamin B12, selenium, and omega-3 fatty acids, all of which are vital for bodily functions [2]. The goal of processing meat is to raise its digestibility, quality, and desired sensory qualities while simultaneously prolonging its shelf life. Polycyclic aromatic hydrocarbons (PAHs) are among the hazardous byproducts that meat may produce as a result of its thermal processing. PAHs are a broad class of extremely hydrophobic chemical molecules with two or more aromatic rings bonded together [3]. The bulk of PAHs are colorless, pale yellow, or white, lipophilic, with low volatility, and dissolve readily in organic solutions like acetone and cyclohexane. They can be divided into two groups according to the total number of aromatic rings: light PAHs, which have two to three rings and volatile, and heavy PAHs, which have four to six rings which are more stable, poisonous, and less volatile, they are present in a variety of environmental matrices [4]. PAHs are common environmental pollutants that can be created from natural sources such as wildfires and volcanic eruptions or human activities like industrial emissions, vehicle exhaust and cooking with fossil fuels such as wood or coal, oil and gas. Food is the primary source of PAH exposure, accounting for about 90% of the general population's exposure to PAHs across all countries [5]. Meat processing techniques that release PAHs, include roasting, toasting, barbecuing, baking, drying, and frying, which results from the incomplete combustion of charcoal and the pyrolysis of fat especially when heated to temperatures higher than 200 °C.[6]

The length and form of exposure, the concentration of PAHs to which one is exposed, and the relative toxicity of the type of the PAHs are some of the variables that contributes to how PAHs affect human health. The harmful effects of human exposure to PAHs, include genotoxicity, immunological toxicity, neurotoxicity, disruption of the reproductive and endocrine systems, carcinogenicity, and teratogenicity [7]. Hence it is very important to determine their concentration in food in order to reduce their side effects on human health.

About 10,000 different forms of PAHs have been identified to far; of these, over 100 are measurable, and 16 have been designated by the European Food Safety Authority (EFSA) and the International Agency for Research on Cancer (IARC) as both carcinogenic and genotoxic [8]. B[a]P and PAH4 (B[a]P, B[a]A, CHR, and B[b]F) have been used as biomarkers by JECFA (Joint FAO/WHO Expert Committee on Food Additives), which established the maximum permitted level of 2 μg kg^{-1} for benzo[a]pyrene (B[a]P) and 12 μg kg^{-1} for PAH4 in smoked meat and meat products [9] As a result, these compounds have been widely selected as target pollutants in studies investigating PAH contamination in meat products. [3, 10,11]. Highly sensitive and selective analytical technique are used in the detection and quantification of PAHs, such as gas chromatography [12,13], high-performance liquid chromatography (HPLC) combined deferent detection methods such as flame ionization detectors (FID) [14], UV detector [10], diode array detector [15,16], fluorescence detector [17,11], and mass spectrometry [18]. Recently MS/MS (Tandem Mass Spectrometry) was used as sensitive detector [19] with HPLC that also combined with GC [20] for the same purpose. Among them, high-performance liquid chromatography combined with fluorescence detection (HPLC-FLD) is a widely applied technique for quantification of PAHs

due to its high selectivity and sensitivity, with low detection limits, toward these compounds, since PAHs exhibit strong native fluorescence due to their polyaromatic ring structures. As a detector, FLD offers improved signal-to-noise ratios and reduced matrix interference; moreover, it is cost-effective. Hence, many studies used HPLC-FLD for analysis of PAHs in meat products [17, 21].

It is worth mentioning that a crucial stage in the measurement of PAHs is sample preparation, particularly in complex food matrices like meat products, where effective extraction and purification procedures are needed to separate PAHs from coexisting matrix components before their quantification [22]. Due to the limited data on PAH4 occurrence in the most popular meat products consumed in the Kurdistan region of Iraq including Kabab, teka, burger, and shawarma, particularly in relation to the cooking method used in their preparation, there is variability in the type of meat, thermal treatments, cooking time, and environmental conditions, which can significantly influence PAH formation in the meat products. Therefore, analysis of PAHs is essential to evaluate their compliance with safety standards. The aim of this study was to determine the concentration of five kinds of PAHs, the more harmful PAH kinds to human health in meat products, including PAH4 (B[a]P, B[a]A, CHR, and B[b]F) and IND, in some meat's products (Kabab, teka, burger, and shawarma) using HPLC-FLD instrument.

Materials and Methods

Sample collection

Twenty samples of the different types of grilled meat products including (Kabab, Teka, burger, Shawarma) five sample of each type were collected in Sulaymaniyah city/Kurdistan region of Iraq. A sterilized polyethylene bag was used for sample collection, and 50 g of each of the processed meat samples were stored at −20 °C in the dark prior until analysis.

Reagents, Chemicals, and Materials

The standard of the five selected PAH types (B[a]A, CHR, B[b]F, B[a]P, and IND) used in the current study (≥99% purity), syringe filters (0.25 and 0.45 µm), and capillary columns C18 (150 x 4.6 mm, 5 µm) were bought from Supelco (USA). Acetonitrile (ACN) (99.5%), acetic acid (99.9%), primary secondary amine (PSA) 40 µm particle size, hexane, sodium chloride (NaCl), and anhydrous magnesium sulphate ($MgSO_4$) were all purchased from Merck. PAH standards and stock solutions were kept in sealed amber glass vials, ideally in a freezer between -18 and -20 °C.

Sample Preparation using Quencher's method

The Quenchers (Quick, Easy, Cheap, Effective, Rugged, and Safe) method was used for PAH extraction and purification according to Hamad amin and Hassan [23] with a few modifications. The samples were thawed overnight at 4°C. The mixture was then homogenized using a high-speed blender until a uniform consistency was achieved and transferred to a 50 mL Falcon tube flask for the first step of extraction, adding 5 mL of hexane to the 2 g sample in the flask and agitating it in a vortex mixer for 1 min. Subsequently, 10 mL of acetonitrile (ACN) containing 1% (v/v) acetic acid was added, and the mixture was vortexed for one minute. After adding 0.5 g of NaCl and 1.6 g of anhydrous $MgSO_4$, the liquid was homogenized in a vortex for one minute.

To separate the phases (liquid-liquid partition), the mixture was centrifuged for 3 min at 3000 rpm. The top phase, which corresponds to the organic solvent hexane, was removed using a pipette. The subsequent stage, which corresponded to the organic solvent ACN, was transferred to a tube containing 150 mg of MgSO4 and 70 mg of the adsorbent Primary Secondary Amine, which was used as a sample clean-up. The tube was centrifuged at 3000 rpm for 1 min after manual shaking for 30 s. Syringe filters (0.45 and 0.20 µm) were used to filter the supernatant. Each filtered supernatant sample was evaporated under a nitrogen stream at 45 °C until it was 1 mL and then stored at 4 °C till subjected to HPLC-FLD analysis.

HPLC-FLD condition

The PAH concentrations were estimated on an HPLC System (Shimadzu, Nexera-iLC2040C, Tokyo, Japan) consisting of a binary pump, a degasser, an auto sampler, and a column oven, combined with a fluorescence detector (FLD) (Shimadzu RF-550), used for analyzing PAH levels in meat product samples. Applying a reverse-phase C18 column (150 x 4.6 mm, 5 µm), separation was accomplished. The injection volume is 20 µL; the analytical column temperature was maintained at 30°C. The mobile phase consisted of a mixture of acetonitrile and water (80:20 to 100:0) over 10 min, at a flow rate of 1.2 mL/min. The FLD was set at an excitation and an emission wavelength of 275 nm and 420 nm.

Validation Method

The validation method for determining PAH in meat products by HPLC-FLD regarding parameters of Method validation for PAH determination in some meat products using HPLC-FLD was performed by evaluating some parameters such as linearity, accuracy, precision, and sensitivity that include the limits of quantification (LOQ) and detection (LOD). The linearity was evaluate using a sequence of dilutions of PAH standard solutions ranged from 0 to50 ug kg^{-1}. Calibration curves were created by plotting the peak area against the PAH concentration. The linearity estimated by applying linear regression: Equation: $Y = mx + b$, in which Y= peak area, x= PAH concentration, m= slope and b= intercept.

Recovery values were assessed by conducting an entire analytical procedure for PAH determination on blank meat samples spiked with a combination of standards (B[b]F, B[a]A, CHR, B[a]P, and IND) at varying concentrations of (0.2, 0.6,1,3 and 5) µg kg^{-1}. Then PAH content in in spiked and blank samples was performed. The technique's accuracy was assessed by calculating the recovery percentage. (Percentage recovery % = concentration from the chromatogram/spiked or added concentration x 100] [24]. The sensitivity expressed as the limits of detection (LOD) and quantitation (LOQ), these were identified using a signal-to-noise ratio; concentrations exhibiting peak intensity of signal-to-noise ratios of 3 and 10 were classified as LOD and LOQ, respectively. They are estimated using the following formula: LOD = 3 × N/B and LOQ = 10 × N/B, where N is the standard deviation of the PAH's peak area and B is the calibration curve's slope. The standard deviation of the response and the slope of the calibration curve served as the foundation for these findings. Intra-day, at-the-same-run (repeatability), and inter-day in triplicate assays of a contaminated meat sample were carried out to assess precision. The method's selectivity for every type of

PAHs used in this study was confirmed by the lack of interfering peaks at the PAH retention period.

Calibration Curve and Quantification

A stock solution of PAH (1000 µg/kg) was produced in deionized water that serially diluted to obtain standard solutions at concentrations of 0, 1, 3, 5, 10, 15, 25, and 50 µg/kg. For every standard, note the peak area. Plotting the peak area (Y-axis) against the matching PAH concentration (X-axis), which was expressed in µg/kg, allowed for calibration. By using linear regression, the linearity was estimated to be the formula Y = mx + b, where x is the PAH concentration, Y is the peak area, M is the slope, and B is the intercept. The peak area following injection was measured. Then the calibration curve calculation was used to determine the PAH concentration in the samples under study using the peak area value. Sample concentration (µg kg-1) is calculated by (Area of sample)/ (Area of standard) concentration. Standard dilution factor.

Results and Discussion

Validation Method

Validation method in HPLC is a crucial stage in ensuring the precision and reliability of results, which verifies that the procedure is appropriate for its intended use and satisfies legal and scientific requirements. The results demonstrate excellent linearity with a correlation coefficient (R^2) equal to 0.9918, verifying a steady correlation between PAH concentration and detector response and demonstrating the method's capability in measuring PAH in meat matrices over a wide concentration range. According to the sensitivity of the technique, which is represented by the limit of detection (LOD), The lowest PAH concentration at which background noise can be reliably separated, it ranged between 0.1to 0.5 µg kg^{-1} (Table 1) for the studied PAHs. The sensitivity of the technique also includes the limit of quantification (LOQ), which is the lowest concentration of analyte that can be determined with an acceptable amount of accuracy. It was ranged between 0.3 to 1.5 µg kg^{-1} (Table 1) for the studied PAHs. It is worth to mention that the importance of being the LOD of the technique for the tested PAHs be below their respective Minimal Risk Levels, MRLs. The specificity of the HPLC-FLD technique was evidenced by the distinct separation of PAHs peaks from matrix constituents without interfering peaks.

The method's accuracy was assessed by recovery values, which were estimated using different concentrations (5, 3, 1, 0.6, and 0.2 µg kg^{-1}) of each studied kind of PAHs. These value ranged between 90–105% (Table 2) , which are within the acceptable range of recovery values (70–120%) set by regulatory agencies of the European Union's criteria for food contaminant control (SANTE document No. 11312/2021).The recovery values of the same technique differ slightly in other studies it was 96 to 99% in the study of Gutiérrez-Valencia and de Llasera [25], who quantify four kinds of PAHs in bovine tissues, and ranged between 83.83 to 100.94% in the study of Zachara et al [17], in determine PAHs in different kinds of commercial smoked meat and fish products. Whereas Onopiuk et al [11] the recovery of HPLC- FLD, that used in determining PAH in smoked and grilled meat products, ranged between 87.66 to 88.04 µg

kg^{-1}. Obtaining high recovery values in this study can be attributed to the efficiency of the Quenchers extraction technique that applied to the meat products samples. Since acetonitrile, promotes partitioning of PAHs while limiting the other co-extraction of lipids and proteins. Moreover salting-out by MgSO4 enhances phase separation and clean-up using PSA effectively removes meat interferences such as fatty acids or pigments and improved method accuracy which reflected by the acceptable recovery values.

Recording lower recoveries (below 70%) using HPLC-FLD might suggest that some PAH is being lost during the extraction or analysis process, potentially due to degradation or incomplete extraction [26]. Moreover, matrix effects may be taken into consideration in determining the recovery values, particularly in analytical methods that rely on external calibration, which assumes that the instrument's response to the analyte is identical in a complicated sample matrix, and a standard solution, which is often not the case [27].

Table (1): Optimum parameters for separation and quantitation BAHS in five types of meats.

PAH	Linearity equation	R2	LOD µg/kg	LOQ µg/kg
B[a]A	Y=16613X +15945	0.9908	0.1	0.3
CRY	Y=14549X +28109	0.990	0.11	0.33
B[b]F	Y=14438X +20253	0.991	0.23	0.69
B[a]P	Y= 13488 X +17686	0.9923	0.5	1.5
Ind	Y=13905 X + 19600	0.9918	0.4	1.2

Explanation: R^2, coefficients; LOD, limit of detection; LOQ, limit of quantification; B[a]A, benzo[a]anthracene; CRY, chrysene; B[b]F, benzo[b]fluoranthene; B[a]P, benzo[a]pyrene; Ind, indeno[1,2,3-cd] pyrene.

Table (2): Recovery values of PAHs added with different concentrations to the blank samples

Matrices	recovery%				
Add Concentration (µg kg^{-1})	**B[a]P**	**B[a]A**	**CHR**	**B[b]F**	**IND**
5	97.8	95.6	97.6	100.4	93.4
3	105	91.66	104.6	101.6	93.65
1	105	103	98	97	99
0.60	98.3	95	101.6	93.33	103.3
0.20	95	105	95	105	90

Explanation: B[a]P; benzo[a]pyrene, B[a]A; benzo[a]anthracene, CHR; chrysene, B[b]F; benzo[b]fluoranthene, and IND; indeno[1,2,3-cd] pyrene

Concentration PAH in Kabab sample

Table 3 shows the concentrations of the studied PAHs, that were selected in the current study in different meat products (kabab, teka, shawarma, and burger).

Generally, in PAHs analysis in meat products, the most hazardous and an indicator compound in food safety regulations is B[a]P, due to its carcinogenicity. According to the European Union Regulation (EU) 2023/915, the maximum limit for B[a]P is 2 μg kg^{-1}. It is used as a biomarker with PAH4, which includes B[a]P, B[a]A, CHR, and B[b]F. Their concentration level must not exceed 12 μg kg^{-1}.

In the comparison between kabab samples that were collected in this study, the highest level of PAH4 (8.78 μg kg^{-1}) was recorded in S2, which means that all the samples remain below the EU maximum limit (12 μg kg^{-1}) for PAH4 and within the regulatory limits. In the study of El Husseini et al., PAH4 levels were also detected in 62 charcoal-grilled restaurants, in which 35.2% exceeded the MRLs for PAH4 of smoked meat established by the EU [28]. The highest concentration of B[a]P recorded in the current study was in S2 (1.946 μg kg^{-1}) which was very close to the allowable limit of this compound in meat. Highest concentrations of CHR (2.386 μg kg^{-1}) and B[b]F (2.453 μg kg^{-1}) were also recorded in this sample. Whereas S5 was the least contaminated sample with the lowest level of PAH4 (5.54 μg kg^{-1}) .

Regarding the concentration of each type of PAH, higher concentrations of B[a]P were recorded in S4 (2.295 μg kg^{-1}), which slightly exceeds the EU maximum limit, and S1 (2.008 μg kg^{-1}) and S3 (2.099 μg kg^{-1}), are very close to this limit, raising potential concerns about consumer safety. B[b]F exhibited the highest concentrations among the analyzed PAHs in kabab, with values ranged between (1.583 to 2.453) μg kg^{-1}, followed by B[a]A. High concentrations of PAHs were also detected in Kabab in other studies [29, 30].

PAHs levels were significantly higher in kabab using charcoal as a heating source compared to electric, gas, and wood [9]. This is due to the fact that more types of kababs in the current study have high percentage of fat [31]and charcoal used in their grilling, which entails direct flame exposure, fat dripping on fire and pyrolysis, which contributes to PAH formation followed of PAHs produced from incomplete charcoal combustion because of the uneven distribution of heat in the burning portions of the charcoal mass [32,33].

Another reason for PAH formation may be the higher temperature (above 200 °C) used during charcoal burning [34]. The variations in PAH concentrations may be explained by variations in the amount of fat, the heat treatment time, and the grill's type/shape, The heat source's distance, and even the kind of wood or fuel used during processing [35,36]. Overall, this analysis shows that although the majority of kabab samples met EU legal limitations, a sizeable percentage went above the maximum B[a]P level; this raises the possibility of a public health issue for kabab consumers. Reducing fat content, switching to gas or electric heat sources, and avoiding direct exposure to heat sources are three mitigation techniques that may greatly lower PAH exposure [37].

Table (3): concentration of polycyclic aromatic hydrocarbons (µg kg-¹) in kabab samples analyzed in this study.

Analyte(kabab)	B[a]p	B[a]A	CHR	B[b]F	IND	∑4 PAH
S.1	2.008	1.728	1.531	1.583	1.157	6.85
S.2	1.946	1.994	2.386	2.453	2.223	8.78
S.3	2.099	2.118	1.322	2.057	2.137	7.60
S.4	2.295	2.067	1.807	2.390	1.549	8.56
S.5	1.302	1.781	0.693	1.763	2.315	5.54

Explanation: B[a]P; benzo[a]pyrene, B[a]A; benzo[a]anthracene, CHR; chrysene, B[b]F; benzo[b]fluoranthene, and IND; indeno[1,2,3-cd] pyrene, ∑4 PAH (benzo[a]pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene)

PAHs Concentration in Teka sample

The concentrations of selected PAHs in the teka sample used in this study are represented in table 4. In the comparison between the samples, the concentrations of each studied PAH type varied notably among the samples. The highest concentration of B[a]P (2.936 µg kg⁻¹) recorded in S4, which exceeded the EU limit of 2 µg kg⁻¹, Since B[a]P is of particular concern due to its carcinogenicity, and this suggests this sample was subjected to higher temperatures or longer cooking durations. B[a]A exhibited the highest level (4.084 µg kg⁻¹) in SI, and S5 recorded the highest concentration of IND (3.191 µg kg⁻¹). Highest level of PAH4 in teka samples used in the current study was also observed in S5 (9.181 µg kg⁻¹), which were below the EU maximum limit (12µgkg⁻¹) set by the European Union. PAHs were also detected in the grilled meat in other studies [3,33].

Among the types of PAHs selected in the current study, B[a]A was recorded the highest concentration in all teka samples. Karslıoğlu and Kolsarıcı [38] reported that grilling meat using charcoal resulted in the highest accumulation of both B[a]P and PAH4 concentrations. Similarly, to Kabab, teka samples are grilled directly on a charcoal open flame and have similar proximity to the heat source, which results in the formation of high concentration of PAH4 in these samples. The variation between the levels of PAHs between samples may be related to level of fat composition, which is considered important factor affecting the formation of PAHs. When meat products undergo grilling processes, foods with high-fat content are more susceptible to contamination with PAHs due to their affinity for lipophilic PAHs [39, 40], hence, fatter meat accumulates higher PAH concentrations due to more intense exposure to pyrolysis products during processing [41].

Table (4): Concentration of polycyclic aromatic hydrocarbons (µg kg⁻¹) in teka samples analyzed in this study.

Analyte (teka)	B[a]p	B[a]A	CHR	B[b]F	IND	∑4PAH
S.1	1.480	5.084	1.604	0.738	1.205	8.91
S.2	0.911	1.940	2.361	1.701	2.145	6.91
S.3	1.817	2.313	1.948	2.363	2.83	8.44

S.4	1.982	2.222	2.190	2.184	1.986	8.58
S.5	2.936	2.044	2.051	2.15	3.191	9.18

Explanation: B[a]P; benzo[a]pyrene, B[a]A; benzo[a]anthracene, CHR; chrysene, B[b]F; benzo[b]fluoranthene, and IND; indeno[1,2,3-cd] pyrene, ∑4 PAH (benzo[a]pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene).

Concentration of PAHs in Shawarma sample

Regarding to the shawarma samples used in this study. The results in table 5 show different levels of the selected analytes in every sample. Highest concentrations of B[a]P, CHR, and B[b]F were shown in S3. Consequently, highest level of PAH4 (13.65 µg kg^{-1}) also recorded in S3 of shawarma samples used in the current study, all values exceeding the maximum limit for PAH4 in meat products. Regarding the types of PAHs, chrysene concentrations were the highest, ranging from 2.607 to 3.090 µg kg^{-1}, while B[a]A had the lowest concentration of all these PAHs, (2.033 to2.919) µg kg^{-1}). B[b]F concentration ranged from 2.183 to 3.833 µg kg^{-1}, this compound is typically linked to traffic emissions and incomplete combustion of organic matter [42].

The most concerning levels were observed for B[a]P, the most hazardous PAH, which ranged from 2.113 to 4.185 µg kg^{-1}, all of which surpass the maximum limit of 2 µg kg^{-1} for B[a]P in meat products. High levels of PAHs were also recorded in shawarma in other studies of [29,42]. The reason for detecting high concentrations of PAHs in shawarma in these studies may be attributed to the fact that it is prepared using a large amount of tail fat, which increases PAH formation. Moreover, shawarma is grilled using gas (not charcoal). While there are intrinsic advantages to gas grills over charcoal grills, such as easier lighting, quicker heating, and no ash residues to deal with [34], PAHs are also associated with petrol genic sources (coming from petroleum products such as crude oil and gasoline) as they are associated with pyrogenic sources that result from the incomplete burning of fossil fuels or organic substances like wood [29]. Furthermore, the shawarma preparation method involves prolonged vertical roasting and continuous fat dripping on the heat source that favors PAH formation. Hence, the variation between the levels of these compounds in different samples may be due to differences in cooking time, temperature, and fat-to-lean ratio.

Table (5): Concentration of polycyclic aromatic hydrocarbons (µg kg^{-1}) in shawarma samples analyzed in this study.

Ana-lyte(shawarma)	B[a]P	B[a]A	CHR	B[b]F	IND	∑4PAH
S.1	1.113	2.919	3.041	2.183	2.237	9.26
S.2	1.723	2.568	2.743	3.098	2.644	10.13
S.3	4.185	2.538	3.090	3.833	2.938	13.65
S.4	2.305	2.033	2.80	2.239	2.547	9.38
S.5	3.485	2.442	2.607	2.230	2.609	10.76

Explanation: B[a]P; benzo[a]pyrene, B[a]A; benzo[a]anthracene, CHR; chrysene, B[b]F; benzo[b]fluoranthene, and IND; indeno[1,2,3-cd] pyrene, ∑4 PAH (benzo[a]pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene).

Concentration PAHs in Burger sample

Table 6 represents the level of PAH types that were detected in burger samples used in the current study. The greatest concentration of B[a]P and PAH4 (2.699 µg kg^{-1} and 8.77 µg kg^{-1}) was shown in S1. While the lowest concentration of PAH4 (6.93 µg kg^{-1}) and the lowest concentration of B[a]P (0.854 µg kg^{-1}) were recorded in S4. 4PAH did not exceed the EU maximum limit (12µg kg^{-1}), while B[a]P exceeded the maximum permissible limit (2.0 µg kg^{-1}) for B[a]P in processed meat. The PAH4 concentration exceeded the EU maximum limit in the study of Bulanda et al. [43] and da Silva et al, [39] who detected high PAH levels in Brazilian smoked meats. Since B[a]P is commonly used as a marker for PAH contamination in food due to its established role in carcinogenicity with PAH4, S1 was the most contaminated sample in the current study.

Among the PAH types quantified in burger samples used in the current study, chrysene showed the highest concentrations (1.893 to 2.210) µg kg^{-1} whereas IND recorded the lowest concentration (1.469 to 2.093 µg kg^{-1}). High level of IND was also detected in roasted pork products in previous study [44].

Detection of PAHs in burgers may be attributed to more intense thermal processing, since burgers are cooked using a high-heat flat-top gas grill, which induces PAH formation. And the burning of previous debris left on the surface of the top flat, the blackening of the surfaces, and the sticking to the burger's during cooking is another factor for PAH formation. Hence, implementing mitigation strategies such as grilling burgers on flat-top griddles generally produces lower PAH levels than open-flame grilling because they lack direct flame contact; Moreover, the heat should not be so high (above 300°C) that it causes incomplete combustion of fat, leading to PAH formation. Furthermore, using different types of meat, such as chicken meat with less amounts of fat, reduces PAH formation [28], or the use of antioxidant-rich spices (such as garlic, pepper, and herbs) may help prevent or reduce the production of PAHs in commercial processing [45]. Hence, when prepared under controlled settings, burgers could be a safer alternative to grilled meat.

Table (6): Concentration of polycyclic aromatic hydrocarbons (µg kg^{-1}) in burger samples analyzed in this study.

Analyte(burger)	B[a]P	B[a]A	CHR	B[b]F	IND	∑4PAH
S.1	2.699	2.06	1.893	2.12	1.775	8.77
S.2	1.345	1.703	2.210	2.077	2.093	7.34
S.3	2.428	1.927	2.071	1.327	1.558	7.75
S.4	0.854	2.235	2.087	1.749	1.557	6.93
S.5	1.793	1.620	2.001	1.994	1.469	7.41

Explanation: B[a]P; benzo[a]pyrene, B[a]A; benzo[a]anthracene, CHR; chrysene, B[b]F; benzo[b]fluoranthene, and IND; indeno[1,2,3-cd] pyrene, ∑4 PAH (benzo[a]pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene)

Comparison of PAH levels in the types of meat products

In the comparison of PAH levels in the types of meat products that were selected in this study, shawarma samples had the highest concentrations of PAH4 (13.65 µg kg^{-1}),

with the highest level of B[a]P (4.185 µg kg^{-1}). Followed by teka samples with concentrations of 9.18 µg kg^{-1} for PAH4 and a B[a]P level of 2.936 µg kg^{-1}. Shawarma also had the highest concentration of IND (3.191 µg kg^{-1}), all these make shawarma the most contaminated samples with PAHs, followed by teka. The kabab sample contained the lowest concentration of PAH4 and IND (5.54 µg kg^{-1}, 1.157 µg kg^{-1}). These findings may be ascribed to some factors, like the influence of cooking technique, having a lot of fat content, and the usage of high grilling temperatures. Direct-heat temperatures from gas grilling can consistently reach extremely high levels, and longer cooking times and staying more exposed to heat sources result in the creation of more PAHs, since overheating meat—especially when fat is dripping—produces more pyrolysis, that increase the formation of PAHs. In a study on gas-grilled beef PAHs level increases with increasing heating temperature from 150 to 350°C, suggesting that shawarma poses a comparatively higher dietary risk due to its elevated PAHs content [46].

Widely Using HPLC-FLD in determination of PAHs in foods is due to its excellent sensitivity; accuracy, high efficiency, low cost, and ease of use. Using a fluorescence detector (FLD) to quantify PAHs content is often preferred due to its variable excitation and emission wavelengths which meet the measurement requirements of PAHs [47, 48]. Unlike absorbance-based measurements, FLDs detect very weak light signals, allowing highly sensitive PAH detection. [49].

HPLC-FLD successfully determined and quantified PAHs in some meat products collected from the markets of Sulaymaniyah-Kurdistan region of Iraq. The analysis verified the existence of different levels of five PAHs—B[a]P, B[a]A, B[b]F, CHR, and IND in 4 types of meat Products samples ((kabab, teka, burger, and shawarma) analyzed in this study. Highest concentration of B[a]P was found in shawarma samples, which exceeded the EU's maximum limit (2 µg kg^{-1}). Shawarma also exhibited the highest concentrations of IND and PAH4 (B[a]P, B[a]A, CHR, and B[b]F), which exceeded the EU maximum level (12 µg kg^{-1}) for PAH4 in meat products, which make shawarma the most contaminated meat products type with the analyzed PAHs, due to their higher fat content and longer cooking times, followed by teka, kababs, and burgers. These findings highlight how crucial it is to monitor PAH levels in ready-to-eat meat products and the necessities to regulate cooking techniques .

References

1) Aoudeh E., Oz E., Oz F. (2023). Effect of beef patties fortification with black garlic on the polycyclic aromatic hydrocarbons (PAHs) content and toxic potency. Food Chemistry. 428: 136763.
2) Ruxton C., Gordon S. (2024). Animal board invited review: The contribution of red meat to adult nutrition and health beyond protein. Animal. 18(3): 101103.
3) Kafouris D., Koukkidou A., Christou E., Hadjigeorgiou M., Yiannopoulos S. (2020). Determination of polycyclic aromatic hydrocarbons in traditionally smoked meat products and charcoal grilled meat in Cyprus. Meat Science. 164: 108088.

4) Mogashane T.M., Mokoena L., Tshilongo J. (2024). A review on recent developments in the extraction and identification of polycyclic aromatic hydrocarbons from environmental samples. Water. 16(17): 2520.
5) Alomirah H., Al-Zenki S., Al-Hooti S., Zaghloul S., Sawaya W., Ahmed N., et al. (2011). Concentrations and dietary exposure to polycyclic aromatic hydrocarbons (PAHs) from grilled and smoked foods. Food Control. 22(12): 2028–2035.
6) Ostadgholami M., Zeeb M., Amirahmadi M., Daraei B. (2023). Multivariate optimization and validation of a modified QuEChERS method for determination of PAHs and PCBs in grilled meat by GC-MS. Foods. 13(1): 143.
7) Sun K., Song Y., He F., Jing M., Tang J., Liu R. (2021). A review of human and animals' exposure to polycyclic aromatic hydrocarbons: Health risk and adverse effects, photo-induced toxicity and regulating effect of microplastics. Science of the Total Environment. 773: 145403.
8) Pirsaheb M., Dragoi E.-N., Vasseghian Y. (2021). Polycyclic aromatic hydrocarbons (PAHs) formation in grilled meat products—analysis and modeling with artificial neural networks. Polycyclic Aromatic Compounds. 42(1): 156–172.
9) Akkaya E., Colak H., Hampikyan H., Cakmak Sancar B., Akhan M., Engin A.S., et al. (2024). Determination of 16 European Priority Polycyclic Aromatic Hydrocarbons in Doner Kebab Varieties Cooked Under Different Heating Sources. Foods. 13(23): 3725.
10) Karslıoğlu B., Kolsarıcı N. (2023). The effects of fat content and cooking procedures on the PAH content of beef doner kebabs. Polycyclic Aromatic Compounds. 43(4): 3291–3304.
11) Onopiuk A., Kołodziejczak K., Marcinkowska-Lesiak M., Poltorak A. (2022). Determination of polycyclic aromatic hydrocarbons using different extraction methods and HPLC-FLD detection in smoked and grilled meat products. Food Chemistry. 373: 131506.
12) Mohammadi M., Valizadeh-Kakhki F. (2018). Polycyclic aromatic hydrocarbons determination in grilled beef and chicken. Polycyclic Aromatic Compounds. 38(5): 434–444.
13) Rascón A.J., Azzouz A., Ballesteros E. (2019). Trace level determination of polycyclic aromatic hydrocarbons in raw and processed meat and fish products from European markets by GC-MS. Food Control. 101: 198–208.
14) Agus B.A.P., Rajentran K., Selamat J., Lestari S.D., Umar N.B., Hussain N. (2023). Determination of 16 EPA PAHs in food using gas and liquid chromatography. Journal of Food Composition and Analysis. 116: 105038.
15) Wang S.-W., Hsu K.-H., Huang S.-C., Tseng S.-H., Wang D.-Y., Cheng H.-F. (2019). Determination of polycyclic aromatic hydrocarbons (PAHs) in cosmetic

products by gas chromatography-tandem mass spectrometry. Journal of Food and Drug Analysis. 27(3): 815–824.

16) Sharma N., Jain A., Verma K.K. (2021). Extraction strategies of PAHs from grilled meat for their determination by HPLC–DAD. Chemical Papers. 75(8): 3859–3871.

17) Zachara A., Gałkowska D., Juszczak L. (2017). Contamination of smoked meat and fish products from Polish market with polycyclic aromatic hydrocarbons. Food Control. 80: 45–51.

18) Aresta A.M., Zambonin C. (2023). Determination of polycyclic aromatic hydrocarbons (PAHs) in coffee samples by DI-SPME-GC/MS. Food Analytical Methods. 16(6): 1009–1016.

19) Soursou V., Campo J., Picó Y. (2023). Revisiting the analytical determination of PAHs in environmental samples: An update on recent advances. Trends in Environmental Analytical Chemistry. 37: e00195.

20) Ma T.-T., Shen X.-F., Yang C., Qian H.-L., Pang Y.-H., Yan X.-P. (2019). Covalent immobilization of covalent organic framework on stainless steel wire for solid-phase microextraction GC-MS/MS determination of sixteen polycyclic aromatic hydrocarbons in grilled meat samples. Talanta. 201: 413–418.

21) Adeyeye S.A.O., Ashaolu T.J. (2022). Polycyclic aromatic hydrocarbons formation and mitigation in meat and meat products. Polycyclic Aromatic Compounds. 42(6): 3401–3411.

22) Chen B.-H., Inbaraj B.S., Hsu K.-C. (2022). Recent advances in the analysis of polycyclic aromatic hydrocarbons in food and water. Journal of Food and Drug Analysis. 30(4): 494.

23) Hamad Amin A.Y., Hassan K.I. (2020). Gas chromatography–mass spectrometry based sensitive analytical approach to detect and quantify non-polar pesticides accumulated in the fat tissues of domestic animals. Saudi Journal of Biological Sciences. 27(3): 887–893.

24) Saleh S.K.H., Hassan K.I., Mahmud P.H.S., Abdalla S.S. (2024). Assessment of fungal species in some spices using molecular study with detection of their mycotoxins using chromatography analysis. Biomedical Chromatography. 38(7): e5889.

25) Gutiérrez-Valencia T.M., de Llasera M.P.G. (2017). On-line MSPD-SPE-HPLC/FLD analysis of polycyclic aromatic hydrocarbons in bovine tissues. Food Chemistry. 223: 82–88.

26) Krüger O., Kalbe U., Meißner K., Sobottka S. (2014). Sorption effects interfering with the analysis of polycyclic aromatic hydrocarbons (PAH) in aqueous samples. Talanta. 122: 151–156.

27) Hiwa H.M.H., Khulod K.I.H. (2024). Optimization and validation of high-performance liquid chromatography using modified QuEChERS to determine anthelmintic drugs in mutton. Biomedical Chromatography. 38(3): e5814.

28) El Husseini M., Mourad R., Rahim H.A., Al Omar F., Jaber F. (2021). Assessment of polycyclic aromatic hydrocarbons (PAH4) in the traditional Lebanese grilled meat products and investigation of Broasted frying cooking method and meat size on the PAH4 formation. Polycyclic Aromatic Compounds.

29) Shawish R., Hamed R., Elbayoumi Z. (2022). Recognition of aromatic hydrocarbon compounds in some grilled beef products on the Egyptian market by gas chromatography-mass spectrophotometry technique. Advances in Animal and Veterinary Sciences. 10(8): 1769–1773.

30) Gorji M.Eh., Ahmadkhaniha R., Moazzen M., Yunesian M., Azari A., Rastkari N. (2016). Polycyclic aromatic hydrocarbons in Iranian Kebabs. Food Control. 60: 57–63.

31) Hassan K.I., Mohamed Ali B.A., Mohammed N.A. (2019). Detection of the origin of animal species in kebab meat using mitochondrial DNA based - Polymerase Chain Reaction (mtDNA-PCR). Iraqi Journal of Veterinary Sciences. 33(1): 39–43.

32) Ciecierska M., Komorowska U., Bryła M., Roszko M. (2025). Formation of Polycyclic Aromatic Hydrocarbons on Grilled Pork Neck Loins as Affected by Different Marinades and Grill Types. Foods. 14(10): 1673.

33) Jiang D., Wang G., Li L., Wang X., Li W., Li X., et al. (2018). Occurrence, dietary exposure, and health risk estimation of polycyclic aromatic hydrocarbons in grilled and fried meats in Shandong of China. Food Science & Nutrition. 6(8): 2431–2439.

34) Ghorbani M., Najafi Saleh H., Barjasteh-Askari F., Nasseri S., Davoudi M. (2020). The effect of gas versus charcoal open flames on the induction of polycyclic aromatic hydrocarbons in cooked meat: a systematic review and meta-analysis. Journal of Environmental Health Science and Engineering. 18(1): 345–354.

35) Sumer G., Oz F. (2023). The effect of direct and indirect barbecue cooking on polycyclic aromatic hydrocarbon formation and beef quality. Foods. 12(7): 1374.

36) Duedahl-Olesen L., Aaslyng M., Meinert L., Christensen T., Jensen A., Binderup M.-L. (2015). Polycyclic aromatic hydrocarbons (PAH) in Danish barbecued meat. Food Control. 57: 169–176.

37) Rose M., Holland J., Dowding A., Petch S.R., White S., Fernandes A., et al. (2015). Investigation into the formation of PAHs in foods prepared in the home to determine the effects of frying, grilling, barbecuing, toasting and roasting. Food and Chemical Toxicology. 78: 1–9.

38) Karslıoğlu B., Kolsarıcı N. (2025). The impact of fat contents and different cooking processes on the potential health concerns of polycyclic aromatic hydrocarbons in chicken doner kebabs. Polycyclic Aromatic Compounds. 45(4): 645–664.

39) da Silva S.A., de Rossi G.Z., de Almeida A.P., Guizellini G.M., Torres E.A.F.d.S., Rogero M.M., et al. (2024). Occurrence and exposure to polycyclic aromatic hydrocarbons (PAHs) in traditional dry-cured or smoked meat products from Brazil. Food Production, Processing and Nutrition. 6(1): 82.

40) 40)Škaljac S., Jokanović M., Peulić T., Vranešević J., Kartalović B., Tomović V., et al. (2024). Content of polycyclic aromatic hydrocarbons in traditionally smoked meat products from North Serbia (Vojvodina). Fermentation. 10(2): 104.

41) Coroian C.O., Coroian A., Becze A., Longodor A., Mastan O., Radu-Rusu R.-M. (2022). Polycyclic aromatic hydrocarbons (PAHs) occurrence in traditionally smoked chicken, turkey and duck meat. Agriculture. 13(1): 57.

42) Salem S., Eltantawy E. (2020). Assessment of Polycyclic Aromatic Hydrocarbons in Heat Treated Meat and Fish. Suez Canal Veterinary Medical Journal SCVMJ. 25(1): 143–152.

43) Iwu G.I., Lajide L., Madu P.C., Isah I.A. (2024). Assessment of polycyclic aromatic hydrocarbon (PAH) profiles in heat-processed meat and fish: a study on health risk evaluation. Discover Food. 4(1): 46.

44) Bulanda, S., & Janoszka, B. (2023). Polycyclic aromatic hydrocarbons (PAHs) in roasted pork meat and the effect of dried fruits on PAH content. International Journal of Environmental Research and Public Health, 20(6), 4922.

45) Timon M.L., Manzano R., Martin-Mateos M.J., Sanchez-Ordonez M., Godoy B., Ramirez-Bernabe M.R. (2025). Reduction of polycyclic aromatic hydrocarbons in pork burgers using high-pressure processed white grape pomace. LWT. 218: 117492.

46) Ahmad Kamal N.H., Selamat J., Sanny M. (2018). Simultaneous formation of polycyclic aromatic hydrocarbons (PAHs) and heterocyclic aromatic amines (HCAs) in gas-grilled beef satay at different temperatures. Food Additives & Contaminants: Part A. 35(5): 848–869.

47) Kanu A.B. (2021). Recent developments in sample preparation techniques combined with high-performance liquid chromatography: A critical review. Journal of Chromatography A. 1654: 462444.

48) Li X., Gao Y., Deng P., Ren X., Teng S. (2023). Determination of four PAHs and formaldehyde in traditionally smoked chicken products. Molecules. 28(13): 5143.

49) Franz H., Jendreizik V. (2013). Fluorescence method development handbook. Thermo Fisher Scientific, Germering, Germany: 1–2.