

**ARTICLE**

Assessing the Formation of Polycyclic Aromatic Hydrocarbons in Grilled Beef Steak and Beef Patty with Different Charcoals by the Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) Method with Gas Chromatography–Mass Spectrometry

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Ali Samet Babaoğlu*

Department of Food Engineering, Faculty of Agriculture, Selçuk University, Konya 42100, Türkiye

*Corresponding author : Ali Samet Babaoğlu
Department of Food Engineering, Faculty of Agriculture, Selçuk University, Konya 42100, Türkiye
Tel: +90-332-223-2935
Fax: +90-332-241-0108
E-mail: asbabaoglu@selcuk.edu.tr

*ORCID
Ali Samet Babaoğlu
<https://orcid.org/0000-0003-4643-7454>

Abstract This study investigated the effects of different charcoals on the occurrence of 16 polycyclic aromatic hydrocarbons (PAHs) in grilled beef steaks and beef patties. Seven different charcoals were used as follows: from oak wood (C1), from orange wood (C2), from Valonia oak wood (C3), from Marabu wood (C4), extruded charcoal from beech wood (C5), from coconut shells (C6), and from hazelnut shells (C7). The grilling times for each charcoal type were 6 min for the beef patties and 7 min for the beef steaks, until the internal temperature reached at least 74°C. The total concentration of 16 PAHs (PAH16) in beef steaks grilled with C1 (35.75 µg/kg) and C7 (36.39 µg/kg) was higher than that of C3 (23.80 µg/kg) and C6 (24.48 µg/kg; $p < 0.05$). The highest amounts of PAH16 (216.40 µg/kg) were determined in the beef patty samples grilled using C5 ($p < 0.05$). The summation of benzo[a]anthracene, chrysene, benzo[b]fluoranthene and benzo[a]pyrene, referred to as PAH4, was not detected in any of the beef steaks, whereas it was determined in the beef patties grilled with C2 (7.72 µg/kg) and C5 (22.95 µg/kg; $p < 0.05$). The PAH16 concentrations of the beef patty samples in each charcoal group were significantly higher compared to the beef steaks ($p < 0.05$). To avoid the formation of high PAH levels, the use of extruded charcoal and hazelnut shell charcoal should therefore be avoided when charcoal grilling beef steaks and beef patties, and low-fat meat products should be preferred.

Keywords charcoal-grilling, contaminant, meat products, polycyclic aromatic hydrocarbon

Introduction

Meat and meat products are generally consumed after a cooking process. Cooking processes improve digestibility, reduce exposure to microorganisms and give meat and meat products unique sensory and nutritional properties (Cordeiro et al., 2020; Nuray and Oz, 2019; Sobral et al., 2018). However, cooking processes can cause carcinogenic compounds such as polycyclic aromatic hydrocarbons (PAHs; Bouvard et al., 2015;

Sun et al., 2019).

PAHs are a type of hydrocarbon molecule containing two or more benzene rings. Naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, and anthracene are categorised as light PAHs consisting of two to three rings. Fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene, dibenzo[a,h]anthracene, and benzo[g,h,i]perylene are categorised as heavy PAHs because they consist of four or more rings (Hamidi et al., 2016). Light PAHs have very low toxicity because they are more volatile, while heavy PAHs have higher toxicity because they are more stable (Ewa and Danuta, 2017).

The incomplete combustion or pyrolysis of organic material leads to the formation of PAHs (Lee et al., 2019; Sun et al., 2019). Currently, more than 10,000 types of PAHs are known, of which about 660 different PAH compounds have been identified so far (Hwang et al., 2019; Masuda et al., 2019). The US Environmental Protection Agency has listed 16 PAH compounds [light PAHs (6)+heavy PAHs (10) as mentioned above] commonly found in samples that exhibit high toxicity in humans (Zelinkova and Wenzl, 2015). One of the main ways to come into contact with PAHs is through eating food, especially grilled and smoked meat products (Domingo and Nadal, 2015).

PAHs in grilled and/or barbecued meat and meat products can be formed in three ways. One way is pyrolysis of organic material directly on the surface of the food. Another way is smoke contamination from the heat source. Another way is contact of dripping fat with charcoals that produce smoke, including PAHs. The most important factors affecting level of PAHs during grilling are the distance from the heat source, the fat level of the raw product and the grilling time (Cordeiro et al., 2020; Rose et al., 2015; Viegas et al., 2012). In addition, the different chemical characteristics of the various charcoals could affect the formation of PAHs during charcoal grilling (Kim et al., 2021).

One of the most popular cooking methods for meat and meat products is grilling, which gives them a unique aroma and flavour (Oz and Kaya, 2019; Viegas et al., 2012). Many types of charcoal, such as wood charcoal and/or charcoal from organic waste material (e.g., coconut shell charcoal, hazelnut shell charcoal), are used as a heat source in grilling (Kim et al., 2021). Oak wood charcoal is produced at about 600°C–650°C (Pehlivan and Kahraman, 2011) and is generally used for cooking on grills. Another charcoal that is often used for grilling in Türkiye is orange wood (*Citrus sinensis*) charcoal, which is easy to ignite. The Valonia oak (*Quercus ithaburensis* subsp. *macrolepis*) is a deciduous tree endemic to the eastern Mediterranean region, growing naturally in western and southern Anatolia (Türkiye), Greece and southern Albania (Pantera et al., 2008). It was reported that the wood of the Valonia oak was used extensively for the production of charcoal (Giannakopoulou, 2002). Marabu is known as *Dichrostachys cinerea* and originates from Africa, but was transplanted to Cuba because of its attractive flowers. Cuba produces charcoal from the marabu wood, which is used for grilling and generates considerable income (Idowu and Fletcher, 2020). Extruded charcoal is made by pressing organic waste with kindling such as barium nitrate (Sepetdjian et al., 2010). The chemicals in the extruded charcoals could cause hazardous substances when burned for cooking (Kim et al., 2021). Coconut waste accumulates everywhere in the form of shells, husks and coconut fibres from the coconut milk and oil processing industries (Ahmad et al., 2020). Coconut shells are a good source of charcoal due to their high lignin content (Yerima and Grema, 2018). Hazelnut shells are a potentially important source of energy for Türkiye. The use of agricultural residues to produce charcoal is an alternative process to the other energy sources (Demirbaş, 1999).

There are limited studies in the literature investigating the effects of various types of charcoal on the formation of PAH in meat products (Kim et al., 2021; Oz, 2021; Viegas et al., 2012). Viegas et al. (2012) investigated the effects of wood and coconut charcoal on PAHs in samples of beef and salmon. Kim et al. (2021) found the impacts of three different charcoals on the occurrence of PAH in grilled beef, pork and chicken meat samples. Oz (2021) determined the effects of two charcoals

(briquette and wood charcoal) and fat content on the PAH content of five grilled fish species. However, as far as we know, no study has yet examined the effects of many charcoals such as orange wood charcoal, marabu wood charcoal, hazelnut shell charcoal, and Valonia oak wood charcoal used in grilling on the formation of PAHs in meat and meat products. Therefore, due to the lack of a comprehensive study on the effects of various charcoals on the occurrence of PAH compounds in meat products, the current study aimed to determine the effects of various charcoals on the formation of PAH16 in grilled beef steaks and patties. In addition, the effects of fresh and processed meat products on the occurrence of PAHs during grilling were also determined. Another goal of this study was to suggest a suitable charcoal for grilling meat products to limit exposure to PAHs.

Materials and Methods

Chemicals

All chemicals and solvents used in the current study were suitable for high-performance liquid chromatography and were of analytical grade. The standards for 16 PAHs (naphthalene, acenaphthylene, acenaphthylene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, indeno[1,2,3-*cd*]pyrene, dibenzo[*a,h*]anthracene and benzo[*ghi*]perylene) were purchased from Supelco (Oakville, ON, Canada).

Materials

The beef steaks used in this study were obtained from the *Longissimus dorsi* of beef carcasses. The muscle was purchased from a butcher in Konya, Türkiye. For the preparation of the beef patty samples, the beef (*Biceps femoris*) and beef fat were obtained from a butcher in Konya, Türkiye. The salt (Salina, Ankara, Türkiye) used in the production of beef patty samples was purchased from a market in Konya.

Seven different types of charcoal were obtained: charcoal from oak wood (C1; Konya, Türkiye), charcoal from orange wood (C2; Konya, Türkiye), charcoal from Valonia oak wood (C3; Isparta, Türkiye), charcoal from marabou wood (C4; Cuba), extruded charcoal (from beech wood, starch and barium nitrate; C5; Russia), charcoal from coconut shells (C6; Indonesia), and charcoal from hazelnut shells (C7; Giresun, Türkiye). The details of the charcoals used are given in Table 1. The photographs of the different charcoals used in this study are shown in Fig. 1.

Table 1. Charcoals

| Charcoal code | Charcoal types | Latin names of the coal origin | Caloric value of charcoals (kcal) |
|---------------|--|--|-----------------------------------|
| C1 | Oak wood charcoal | <i>Quercus robur</i> | 5,930±100 |
| C2 | Orange wood charcoal | <i>Citrus sinensis</i> | 7,020±100 |
| C3 | Valonia oak wood charcoal | <i>Quercus ithaburensis</i> subsp. <i>macrolepis</i> | 7,460±100 |
| C4 | Marabu wood charcoal | <i>Dichrostachys cinerea</i> | 7,270±100 |
| C5 | Extruded charcoal (from beech wood, starch and barium nitrate) | <i>Fagus sylvatica</i> L. | 7,840±100 |
| C6 | Coconut shell charcoal | <i>Cocos nucifera</i> L. | 7,060±100 |
| C7 | Hazelnut shell charcoal | <i>Corylus avellana</i> | 7,070±100 |



Fig. 1. Photographs of the different charcoal types. C1: Oak wood charcoal, C2: Orange wood charcoal, C3: Valonia oak wood charcoal, C4: Marabu wood charcoal, C5: Extruded charcoal from beech wood, C6: Coconut shell charcoal, C7: Hazelnut shell charcoal.

Sample preparation

The *Longissimus dorsi* was cut into steaks of approximate dimensions 4 cm width×13 cm length×0.75 cm thickness, corresponding to a weight of 66.0 ± 3.5 g. A total of 42 beef steaks were obtained.

The beef (*B. femoris*) and beef fat were minced twice using a 3-mm plate grinder. The amount of beef fat used was adjusted to 14% fat in the minced meat. The other ingredients (1.0% salt and 4.0% water) were added and then mixed for 5 min. This patty dough was stored for 3 hours at 4°C and formed into patties in a petri dish (40 g per patty) to obtain an average size (55 mm diameter and 10 mm thickness). A total of 42 beef patties were prepared. The prepared beef steaks and beef patties were randomly divided into seven groups for grilling with different charcoals.

Charcoal grilling procedure

A grill (25 cm width, 40 cm length, and 9 cm height; Konya, Türkiye) was used to cook the samples. About 0.75 kg of each charcoal (C1, C2, C3, C4, C5, C6, and C7) was spread on the grill for balanced heating. After the charcoals had turned to embers, the samples were grilled. The distance between the samples and the heat source was 7 cm. The samples were turned once during grilling halfway through the total cooking time. The internal temperature reached at least 74°C in all groups. The internal temperature of the samples was measured with a thermometer (Digitale Bratengabel, TCM, Hamburg, Germany). The grilling times with C1, C2, C3, C4, C5, C6, and C7 were 6 min for the beef patties and 7 min for the beef steaks until well cooked. The samples were not treated with oil before or after grilling. Samples cooled to room temperature were ground with a blender before analysis.

Determination of proximate composition

The water, total fat, total protein, and total ash content of beef steak and beef patty samples were determined according to AOAC (2000). The water content was determined by oven drying at 105°C. The total fat content of the samples was quantified with a Soxhlet extraction and petroleum ether was used for extraction. The total protein content of the samples was determined using the Kjeldahl method, in which the determination of the total amount of nitrogen (N) is followed by a conversion to the protein content using the conversion factor (6.25). The total ash content of the samples was determined after combustion in a furnace at 550°C. The water, total fat, total protein, and total ash content of the samples was expressed as a percentage (%).

Polycyclic aromatic hydrocarbon (PAH) analysis

Polycyclic aromatic hydrocarbon (PAH) extraction and gas chromatography/mass spectrometry (GC/MS) analysis

Extraction and purification procedures were carried out based on the modified method reported by Kılıç Büyükkurt et al. (2020). A homogenised sample of 25 g was weighed into a flask. For saponification, 12 g of potassium hydroxide and 100 mL of ethanol were added to the flask. Then 5 mL of the internal standard anthracene-D10 (20.5 ppm) and 1 mL PAH mix (500 ng/kg) were added. The mixture was kept at 40°C for 2 hours under a reflux condenser. After filtration of the solution, it was cooled to room temperature. It was then transferred to a separating funnel where 100 mL of water and 100 mL of cyclohexane were added. The ethanol/water phase was discarded. The cyclohexane phase was rinsed with the mixture of 50 mL N, N-Dimethylformamide and water (9:1). Then 50 mL NaCl solution (1%) was added and rinsed again. After the phase separation was formed in the solution, the cyclohexane phase (5 mL) was transferred into the quick, easy, cheap, effective, rugged, and safe (QuEChERS) dispersive solid phase extraction (dSPE) tubes with magnesium sulphate and primary secondary amine as sorbent, and the purification procedure was started. The tubes (QuEChERS dSPE) were shaken manually for 1 min and then it was centrifuged (at 2,750×g for 5 min). After centrifugation, the upper phase (1 mL) was transferred to vials.

For the determination of PAHs in the samples, the method developed by Sik et al. (2016) for the analysis of GC-MS was used. Samples prepared in vials were injected into the GC system (Trace GC Ultra, Thermo Scientific, Waltham, MA, USA) equipped with an ISQ single-quadrupole mass spectrometer (Thermo Scientific). PAHs were separated with a Thermo Scientific HP-5MS column (15 m×0.25 mm×0.25 µm). The carrier gas (helium) had a constant flow rate of 1 mL/min. The oven temperature was first set to 50°C and held for 0.1 min. Then the temperature was increased to 180°C at 20°C/min and held for 1 min. Then the temperature was raised to 250°C at 20°C/min and held for 1 min, and finally it was raised to 300°C at 20°C/min, which was held for 3.5 min. The temperatures of the injector and detector were set to 280°C. The injection volume was 1 µL and the samples were injected in splitless mode.

Validation of polycyclic aromatic hydrocarbon (PAH) analysis

The PAH concentration in the samples was calculated using a calibration curve method with different concentrations of standards (Taverniers et al., 2004). The PAH analytical method was validated with respect to the limits of detection (LOD), the limits of quantification (LOQ), the linear equation, the coefficient of the regression line (r^2) and the recovery rate. The LODs, LOQs, linear equations, regression line coefficient (r^2) and recoveries of PAH standards are given in Table 2. Five replicates of the spiked samples were prepared for the determination of recovery rates. The contents of the PAH compounds

Table 2. Limits of detection (LOD), limits of quantification (LOQ), linear equations, regression coefficients (r^2) and recovery rates of PAHs determined with GC-MS

| PAH compounds | LOD ($\mu\text{g}/\text{kg}$) | LOQ ($\mu\text{g}/\text{kg}$) | Linear equation ($y = a + bx$) | r^2 | Recovery (%) |
|---------------------------------|---------------------------------|---------------------------------|-------------------------------------|--------|--------------|
| Naphthalene | 1.984 | 6.613 | $y = 10.383 + 0.680x$ | 0.9948 | 114.86 |
| Acenaphthylene | 0.651 | 2.170 | $y = -0.570 + 0.807x$ | 0.9987 | 100.10 |
| Acenaphthene | 0.991 | 3.303 | $y = 0.001 + 0.337x$ | 0.9988 | 99.04 |
| Fluorene | 0.329 | 1.097 | $y = -0.064 + 0.384x$ | 0.9991 | 96.22 |
| Phenanthrene | 0.332 | 1.107 | $y = 0.100 + 0.392x$ | 0.9987 | 91.66 |
| Anthracene | 0.658 | 2.193 | $y = -0.290 + 0.372x$ | 0.9982 | 93.50 |
| Fluoranthene | 0.495 | 1.650 | $y = -0.259 + 0.318x$ | 0.9978 | 97.92 |
| Pyrene | 0.503 | 1.677 | $y = -0.306 + 0.360x$ | 0.9977 | 97.74 |
| Benzo[<i>a</i>]anthracene | 1.347 | 4.490 | $y = -0.858 + 0.353x$ | 0.9908 | 112.86 |
| Chrysene | 1.650 | 5.500 | $y = -0.343 + 0.431x$ | 0.9961 | 107.62 |
| Benzo[<i>b</i>]fluoranthene | 0.990 | 3.300 | $y = -1.220 + 0.465x$ | 0.9959 | 102.46 |
| Benzo[<i>k</i>]fluoranthene | 1.623 | 5.410 | $y = -1.668 + 0.631x$ | 0.9912 | 104.48 |
| Benzo[<i>a</i>]pyrene | 1.155 | 3.850 | $y = -1.786 + 0.562x$ | 0.9916 | 108.44 |
| Indeno[1,2,3- <i>cd</i>]pyrene | 1.287 | 4.290 | $y = -0.891 + 0.241x$ | 0.9930 | 102.96 |
| Dibenzo[<i>a,h</i>]anthracene | 1.169 | 3.897 | $y = -0.987 + 0.237x$ | 0.9936 | 99.84 |
| Benzo[<i>ghi</i>]perylene | 0.821 | 2.737 | $y = -0.692 + 0.257x$ | 0.9938 | 104.84 |

PAHs, polycyclic aromatic hydrocarbons; GC-MS, gas chromatography–mass spectrometry.

in the samples were given as $\mu\text{g}/\text{kg}$ wet weight of sample.

Statistical analysis

Two independent replicates were conducted using a completely randomised factorial design with seven treatments (C1, C2, C3, C4, C5, C6, and C7) and two meat types (beef steak and beef patty). For the statistical analysis of the levels of PAHs, an analysis of variance was performed with the general linear model using Minitab 16.0 with one-way assignment. The different charcoal and meat types were analysed as a fixed factor, while the replicate was considered as a random factor. Significant differences between groups were further analysed using Tukey's Multiple Range Test at 5% significance level.

After the Kaiser-Meyer-Olkin (KMO) value was determined using SPSS version 29 (IBM, Armonk, NY, USA), a principal component analysis (PCA) was performed to show and clarify the relationship between the charcoal types, meat products and analytical parameters (PAHs) using JMP software (JMP Pro 16.0, JMP, Cary, NC, USA).

Results and Discussion

Proximate composition

The water, total protein, total fat, and total ash contents of the beef steak and beef patty samples are given in Table 3. The beef steak had the highest water and total protein content ($p < 0.05$), while the beef patty had the highest total fat content ($p < 0.05$). This is due to the fact that the beef fat was added to the formulation in the production of beef patty. The difference

Table 3. Proximate compositions of raw beef steaks and beef patties

| Meat types | Water (%) | Total protein (%) | Total fat (%) | Total ash (%) |
|------------|-------------------------|-------------------------|-------------------------|------------------------|
| Beef steak | 64.09±0.09 ^A | 19.93±0.08 ^A | 13.48±0.16 ^B | 2.20±0.02 ^A |
| Beef patty | 62.15±0.22 ^B | 17.57±0.17 ^B | 17.82±0.42 ^A | 2.27±0.03 ^A |

Mean±SE.

^{A,B} Means within a column with different uppercase superscript letters indicate significant differences ($p < 0.05$).

between the total ash content of beef steak and beef patty was insignificant ($p > 0.05$). Nutrients in meat products such as fats and proteins are cracked under high-temperature conditions of 200°C and above to generate light rings with low molecular weight and intermediate free radicals with high reactivity. The two are then cyclised, leading to the formation of PAHs (Singh et al., 2020). On the other hand, PAHs have also been reported to be produced by the smoke formed when the fats in meat products drip onto the heat source (Alomirah et al., 2011).

Polycyclic aromatic hydrocarbon (PAH) levels

Thermal processing is the most important factor influencing the formation of PAHs in meat and meat products. Conventional meat processing methods that promote PAHs include heating, frying, toasting, grilling, baking, smoking, drying, and roasting (Iwegbue et al., 2019). Grilling involves placing an intermediate product over a heat source, usually charcoal, and exposing it to a high temperature (Park et al., 2017). The PAH content of grilled meat and meat products depends on several processing parameters, such as the construction of the grill, the type of heat source, the temperature and the type of meat (Duedahl-Olesen and Ionas, 2022).

PAHs in beef steaks grilled with different charcoals are given Table 4. Naphthalene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene, and dibenzo[a,h]anthracene were not detected in all beef steak samples. Different charcoals did not affect the fluoranthene and benzo[ghi]perylene levels of beef steaks ($p > 0.05$). Acenaphthylene (4.08 µg/kg) was only determined in beef steaks grilled with C2 (orange wood charcoal) and anthracene (2.25 µg/kg) only in beef steaks grilled with C7 (hazelnut shell charcoal; $p < 0.05$). Beef steaks grilled with hazelnut shell charcoal (C7; 5.15 µg/kg) had a higher acenaphthene content than samples grilled with C3 (3.57 µg/kg) and C6 (3.65 µg/kg; $p < 0.05$). C1 and C7 had the highest content of phenanthrene in beef steaks ($p < 0.05$). The sum of PAH4 was not determined in any of the grilled beef steaks due to absence of benzo[a]anthracene, chrysene, benzo[b]fluoranthene and benzo[a]pyrene. The total concentration of the 16 PAHs (PAH16) in beef steaks grilled using oak wood charcoal (C1; 35.75 µg/kg) and hazelnut shell charcoal (C7; 36.39 µg/kg) was higher than that of C3 (23.80 µg/kg) and C6 (24.48 µg/kg; $p < 0.05$). It has been reported that the type and shape of the heat source when grilling with charcoal has an influence on the PAH content in grilled foods (Oz and Yuzer, 2016). The seven different charcoals used in our study were obtained from different sources (e.g., wood, organic material) and had different manufacturing processes. Especially in the production of charcoal, the carbonization temperature has an important influence on the volatile components of the charcoal. When the carbonization temperature is high, the volatile content of the charcoal is low. If the carbonization temperature is low, the amount of volatile compounds in the charcoal is high. High levels of the volatile compounds can produce various PAHs during grilling (Prathomtong et al., 2016). This could be related to the fact that the C1 (oak wood charcoal) and C7 (hazelnut shell charcoal) in our study caused a higher PAH content in the beef steaks. Güler and Aydın Temel (2023) reported that hazelnut shell charcoal had higher volatile matter than wood waste charcoal.

The amounts of PAH components formed in the beef patties grilled with different coals are listed in Table 4. Of the 16

Table 4. Effects of different charcoals and types of meat products on the PAH content in grilled samples

| Type of meat product | PAH compounds (µg/kg wet weight) | Different charcoals | | | | | | |
|----------------------|----------------------------------|------------------------------|-------------------------------|------------------------------|-------------------------------|-------------------------------|------------------------------|-------------------------------|
| | | C1 | C2 | C3 | C4 | C5 | C6 | C7 |
| Beef steak samples | Na | ND ^{Aa} | ND ^{Aa} | ND ^{Aa} | ND ^{Aa} | ND ^{Ab} | ND ^{Aa} | ND ^{Aa} |
| | Ac | ND ^{Bb} | 4.08±0.80 ^{Aa} | ND ^{Bb} | ND ^{Bb} | ND ^{Bb} | ND ^{Bb} | ND ^{Bb} |
| | Ace | 5.00 ±0.16 ^{ABb} | 4.34 ±0.25 ^{ABb} | 3.57 ±0.30 ^{Bb} | 3.66 ±0.31 ^{ABb} | 3.77 ±0.19 ^{ABb} | 3.65 ±0.33 ^{Bb} | 5.15 ±0.63 ^{Ab} |
| | F | 2.30 ±0.31 ^{ABb} | 2.72 ±0.29 ^{Ab} | 1.38 ±0.10 ^{Bb} | 1.65 ±0.17 ^{ABb} | 1.45 ±0.08 ^{Bb} | 1.52 ±0.11 ^{Bb} | 2.29 ±0.28 ^{ABb} |
| | Pa | 19.99 ±1.02 ^{Ab} | 10.72 ±0.73 ^{Cb} | 11.91 ±0.82 ^{Cb} | 12.43 ±0.84 ^{Cb} | 13.29 ±1.04 ^{BCb} | 12.02 ±0.71 ^{Cb} | 18.46 ±1.47 ^{ABb} |
| | A | ND ^{Bb} | ND ^{Bb} | ND ^{Bb} | ND ^{Bb} | ND ^{Bb} | ND ^{Bb} | 2.25 ±0.25 ^{Ab} |
| | Fl | 3.17 ±0.68 ^{Ab} | 1.83 ±0.22 ^{Ab} | 2.18 ±0.53 ^{Ab} | 2.78 ±0.31 ^{Ab} | 2.67 ±0.17 ^{Ab} | 2.30 ±0.40 ^{Ab} | 2.87 ±0.37 ^{Ab} |
| | P | 2.43 ±0.23 ^{ABb} | 1.73 ±0.13 ^{Bb} | 2.00 ±0.03 ^{ABb} | 2.76 ±0.21 ^{Ab} | 2.02 ±0.15 ^{ABb} | 1.94 ±0.17 ^{ABb} | 2.53 ±0.15 ^{ABb} |
| | BaA | ND ^{Aa} | ND ^{Aa} | ND ^{Aa} | ND ^{Aa} | ND ^{Ab} | ND ^{Aa} | ND ^{Aa} |
| | Ch | ND ^{Aa} | ND ^{Aa} | ND ^{Aa} | ND ^{Aa} | ND ^{Ab} | ND ^{Aa} | ND ^{Aa} |
| | BbF | ND ^{Aa} | ND ^{Ab} | ND ^{Aa} | ND ^{Aa} | ND ^{Ab} | ND ^{Aa} | ND ^{Aa} |
| | BkF | ND ^{Aa} | ND ^{Aa} | ND ^{Aa} | ND ^{Aa} | ND ^{Ab} | ND ^{Aa} | ND ^{Aa} |
| | BaP | ND ^{Aa} | ND ^{Aa} | ND ^{Aa} | ND ^{Aa} | ND ^{Ab} | ND ^{Aa} | ND ^{Aa} |
| | IP | ND ^{Aa} | ND ^{Ab} | ND ^{Aa} | ND ^{Aa} | ND ^{Ab} | ND ^{Aa} | ND ^{Aa} |
| | DhA | ND ^{Aa} | ND ^{Aa} | ND ^{Aa} | ND ^{Aa} | ND ^{Aa} | ND ^{Aa} | ND ^{Aa} |
| | BgP | 2.87 ±0.10 ^{Aa} | 2.76 ±0.01 ^{Ab} | 2.77 ±0.01 ^{Aa} | 3.04 ±0.19 ^{Aa} | 2.75 ±0.01 ^{Ab} | 3.06 ±0.07 ^{Aa} | 2.86 ±0.05 ^{Aa} |
| | PAH4 | ND ^{Aa} | ND ^{Ab} | ND ^{Aa} | ND ^{Aa} | ND ^{Ab} | ND ^{Aa} | ND ^{Aa} |
| | PAH16 | 35.75 ±2.49 ^{Ab} | 27.50 ±2.21 ^{ABb} | 23.80 ±1.73 ^{Bb} | 26.31 ±1.95 ^{ABb} | 25.93 ±1.56 ^{ABb} | 24.48 ±0.81 ^{Bb} | 36.39 ±3.00 ^{Ab} |
| | Beef patty samples | Na | ND ^{Ba} | ND ^{Ba} | ND ^{Ba} | ND ^{Ba} | 6.36 ±0.35 ^{Aa} | ND ^{Ba} |
| Ac | | 2.14 ±0.17 ^{Ba} | 4.86 ±1.47 ^{Ba} | 3.35 ±0.85 ^{Ba} | 2.81 ±0.35 ^{Ba} | 14.48 ±3.20 ^{Aa} | 2.40 ±0.16 ^{Ba} | 2.81 ±0.41 ^{Ba} |
| Ace | | 6.46 ±0.22 ^{CDa} | 7.04 ±0.39 ^{CDa} | 8.37 ±0.53 ^{BCa} | 5.75 ±0.58 ^{Da} | 10.57 ±0.09 ^{Aa} | 7.96 ±0.38 ^{BCa} | 9.92 ±0.19 ^{ABa} |
| F | | 4.29 ±0.42 ^{Ba} | 4.52 ±0.43 ^{Ba} | 4.75 ±0.01 ^{Ba} | 5.47 ±0.01 ^{Ba} | 11.49 ±1.31 ^{Aa} | 5.33 ±0.21 ^{Ba} | 7.68 ±1.29 ^{ABa} |
| Pa | | 31.44 ±5.68 ^{Ba} | 34.76 ±5.56 ^{Ba} | 36.40 ±0.79 ^{Ba} | 29.57 ±4.64 ^{Ba} | 77.58 ±7.39 ^{Aa} | 32.80 ±0.96 ^{Ba} | 42.15 ±7.63 ^{Ba} |
| A | | 4.03 ±0.41 ^{Ba} | 5.10 ±1.01 ^{Ba} | 4.66 ±0.02 ^{Ba} | 4.29 ±0.20 ^{Ba} | 11.90 ±1.17 ^{Aa} | 4.01 ±0.01 ^{Ba} | 6.07 ±0.97 ^{Ba} |
| Fl | | 5.73 ±1.08 ^{Ba} | 8.15 ±1.79 ^{Ba} | 6.48 ±0.01 ^{Ba} | 5.13 ±1.09 ^{Ba} | 20.42 ±2.61 ^{Aa} | 5.69 ±0.45 ^{Ba} | 5.61 ±0.84 ^{Ba} |
| P | | 5.47 ±0.46 ^{Ba} | 9.56 ±2.07 ^{Ba} | 6.82 ±0.09 ^{Ba} | 5.64 ±0.95 ^{Ba} | 22.19 ±5.08 ^{Aa} | 7.13 ±0.95 ^{Ba} | 7.62 ±1.16 ^{Ba} |
| BaA | | ND ^{Ba} | ND ^{Ba} | ND ^{Ba} | ND ^{Ba} | 6.60 ±1.12 ^{Aa} | ND ^{Ba} | ND ^{Ba} |

Table 4. Effects of different charcoals and types of meat products on the PAH content in grilled samples (continued)

| Type of meat product | PAH compounds (µg/kg wet weight) | Different charcoals | | | | | | |
|----------------------|----------------------------------|------------------------------|-------------------------------|------------------------------|------------------------------|--------------------------------|------------------------------|------------------------------|
| | | C1 | C2 | C3 | C4 | C5 | C6 | C7 |
| Beef patty samples | Ch | ND ^{Ba} | ND ^{Ba} | ND ^{Ba} | ND ^{Ba} | 5.40 ±0.39 ^{Aa} | ND ^{Ba} | ND ^{Ba} |
| | BbF | ND ^{Ca} | 3.83 ±0.54 ^{Ba} | ND ^{Ca} | ND ^{Ca} | 5.79 ±1.63 ^{Aa} | ND ^{Ca} | ND ^{Ca} |
| | BkF | ND ^{Ba} | ND ^{Ba} | ND ^{Ba} | ND ^{Ba} | 5.59 ±0.67 ^{Aa} | ND ^{Ba} | ND ^{Ba} |
| | BaP | ND ^{Ba} | 3.89 ±0.39 ^{ABa} | ND ^{Ba} | ND ^{Ba} | 5.17 ±1.10 ^{Aa} | ND ^{Ba} | ND ^{Ba} |
| | IP | ND ^{Ca} | 4.62 ±0.52 ^{Ba} | ND ^{Ca} | ND ^{Ca} | 6.35 ±0.64 ^{Aa} | ND ^{Ca} | ND ^{Ca} |
| | DhA | ND ^{Aa} | ND ^{Aa} | ND ^{Aa} | ND ^{Aa} | ND ^{Aa} | ND ^{Aa} | ND ^{Aa} |
| | BgP | 2.99 ±0.11 ^{Ba} | 3.86 ±0.72 ^{ABa} | 2.91 ±0.11 ^{Ba} | 3.11 ±0.03 ^{Ba} | 6.54 ±1.28 ^{Aa} | 3.06 ±0.06 ^{Ba} | 2.87 ±0.03 ^{Ba} |
| | PAH4 | ND ^{Ca} | 7.72 ±0.93 ^{Ba} | ND ^{Ca} | ND ^{Ca} | 22.95 ±3.25 ^{Aa} | ND ^{Ca} | ND ^{Ca} |
| | PAH16 | 62.89 ±6.34 ^{Ba} | 90.18 ±15.12 ^{Ba} | 74.74 ±0.31 ^{Ba} | 61.75 ±7.07 ^{Ba} | 216.40 ±28.85 ^{Aa} | 69.38 ±2.45 ^{Ba} | 85.74 ±9.56 ^{Ba} |

C1: Oak wood charcoal, C2: Orange wood charcoal, C3: Valonia oak wood charcoal, C4: Marabu wood charcoal, C5: Extruded charcoal from beech wood, C6: Coconut shell.

^{A-D} Different superscript capital letters within the different charcoals in the same type of meat products indicate significant differences ($p < 0.05$).

^{a,b} Different superscript lowercase letters within the type of meat products in the same type of charcoal indicate significant differences ($p < 0.05$).

PAH, polycyclic aromatic hydrocarbon; Na, naphthalene; ND, not detected; Ac, acenaphthylene; Ace, acenaphthylene; F, fluorene; Pa, phenanthrene; A, anthracene; Fl, fluoranthene; P, pyrene; BaA, benzo[*a*]anthracene; Ch, chrysene; BbF, benzo[*b*]fluoranthene; BkF, benzo[*k*]fluoranthene; BaP, benzo[*a*]pyrene; IP, indeno[1,2,3-*cd*]pyrene; DhA, dibenzo[*a,h*]anthracene; BgP, benzo[*ghi*]perylene; PAH4, BaA+Ch+BbF+BaP; PAH16, total 16 PAHs.

PAH compounds, only dibenzo[*a,h*]anthracene was not detected in any of the beef patty samples. Naphthalene (6.36 µg/kg), benzo[*a*]anthracene (6.60 µg/kg), chrysene (5.40 µg/kg), and benzo[*k*]fluoranthene (5.59 µg/kg) were only determined in beef patties grilled with extruded charcoal (C5; $p < 0.05$). The beef patties grilled with charcoals C5 (10.57 µg/kg) and C7 (9.92 µg/kg) had the highest acenaphthene content ($p < 0.05$). With regard to acenaphthylene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[*b*]fluoranthene, benzo[*a*]pyrene, and indeno[1,2,3-*cd*]pyrene, the beef patties grilled with extruded charcoal (C5) had higher levels than the other beef patty groups ($p < 0.05$). Therefore, the highest amounts of PAH4 (22.95 µg/kg) and PAH16 (216.40 µg/kg) were determined in the beef patty samples grilled with C5 ($p < 0.05$). The differences in the sum of PAH16 levels for groups other than C5 were insignificant ($p > 0.05$). It has been reported that extruded charcoal is carbonized at a lower temperature (600°C–800°C) and contains ignition chemicals such as barium nitrate (Prathomtong et al., 2016). Therefore, this could be the reason why our PAH16 content was highest in the beef patties grilled with extruded charcoal. Similar results were observed in grilled samples of pork belly, beef loin and chicken thighs with extruded charcoal, which contained more PAH compounds than those from black and white charcoal (Kim et al., 2021).

The effects of the type of meat products on the PAH content in the samples of grilled beef steak and beef patties are shown in Table 4. The sum of PAH4 was not detected in any of the beef steaks, whereas it was determined in the beef patties grilled with C2 (7.72 µg/kg) and C5 (22.95 µg/kg; $p < 0.05$). The PAH16 concentrations of the beef patty samples in each charcoal group were significantly higher compared to beef steaks ($p < 0.05$). It is assumed that the higher fat content (Table 3) of beef patties is the reason for the higher PAH content of beef patties compared to beef steaks. Saito et al. (2014) and Wang et al.

(2018) reported that differences in PAH levels formed when grilling meat products over charcoal could be due to the amount of fat contained in the meat product. This is because the fat in the food drips onto the heat source during grilling and burns, producing smoke. The PAHs are deposited on the surface of the meats by the smoke (Kim et al., 2021; Saito et al., 2014; Wang et al., 2018). Similarly, Kim et al. (2021) determined that pork belly, which had a higher fat content compared to beef loin and chicken thigh, was the group with the highest 16 PAH level as a result of grilling with extruded charcoal. Viegas et al. (2012) also attributed the higher PAH contents of the salmon samples grilled with coconut and wood charcoal to the higher fat content of the salmon samples. In addition, it has been noted that a high water content in meat and meat products can reduce the formation of PAH by inhibiting the temperature rise during cooking (Onopiuk et al., 2021). The fact that the water content of beef steaks in the present study is higher than that of beef patties (Table 3) supports the lower PAH content of beef steaks compared to beef patties. On the other hand, in this study C1 and C7 resulted in higher PAH content in beef steaks ($p < 0.05$), while C5 resulted in the highest PAH content in grilled beef patties ($p < 0.05$). This contradictory situation may be attributed to the type of the meat product and, in particular, to the complex formed by the dripping of the fat contained in the meat onto the charcoal. It has been pointed out that charcoal can produce light PAHs when meat products are grilled with charcoal. During grilling, the melted fat of the meat product dripping onto the charcoal can produce heavy PAHs (García-Lomillo et al., 2017). In our study, it was determined that more heavy PAHs were formed in the beef patty samples grilled with C5 than in the other groups of beef patties (Table 4) and beef steaks grilled with seven different charcoals (Table 4).

Principal component analysis (PCA) results

The KMO is a statistic that measures the adequacy of samples. It has been reported that a KMO value of more than 0.5 can be used for factor analysis, while a KMO value of more than 0.8 is very appropriate for factor analysis (Li et al., 2020). In the present study, the KMO value was calculated as 0.828, and the significance level was determined as $p < 0.001$.

The PCA results are given in Table 5 and the PCA plots of the different charcoals (C1, C2, C3, C4, C5, C6, and C7) and meat products (B and M) are shown in Fig. 2. The first principal component (PC1) and the second principal component (PC2) were 86.04% and 8.81% respectively. The fluoranthene (0.991), pyrene (0.983), PAH16 (0.980), benzo[ghi]perylene (0.976) and acenaphthylene (0.968) were the most contributed factors for PC1. PC2 was identified with acenaphthene (0.656; Table 5). The beef steak samples (C1-B, C2-B, C3-B, C4-B, C5-B, C6-B, and C7-B) were located in the negative area of PC1 and PC2. The samples of C2-M, C5-M, and C7-M were located in the positive area of PC1 and C1-M, C3-M, C4-M, and C6-M were in the negative area of PC1. Additionally, C1-M, C3-M, C4-M, C6-M, and C7-M were in the positive area of PC2, while C2-M and C5-M were in the negative area of PC2 (Fig. 2). As shown in Fig. 2, the C5-M group contains most of the 16 PAH compounds.

Conclusion

The present study investigated the effects of seven different charcoals on the formation of PAHs in grilled beef steaks and beef patties. The levels of the PAHs were influenced by the type of charcoal and type of meat product. Oak wood charcoal (C1) and hazelnut shell charcoal (C7) caused a higher PAH16 content in the beef steaks. The highest levels of PAH4 and PAH16 were determined in the beef patty samples grilled with extruded charcoal (C5). The PAH16 concentrations of the beef patty samples in each charcoal group were very high compared to beef steaks, which can be attributed to the higher fat content of the beef patties. It could be suggested to choose meat products with a low-fat content and to avoid the use of extruded charcoal and hazelnut shell charcoal to reduce the formation of PAHs during grilling.

Table 5. PCA results in relation to different charcoals and different meat products by evaluation of PAHs

| Parameters | PC1 | PC2 |
|------------------------|-------|--------|
| Eigenvalue | 14.63 | 1.50 |
| Variability (%) | 86.04 | 8.81 |
| Cumulative (%) | 49.23 | 87.89 |
| Correlation | | |
| Naphthalene | 0.942 | -0.202 |
| Acenaphthylene | 0.968 | -0.015 |
| Acenaphthene | 0.734 | 0.656 |
| Fluorene | 0.876 | 0.451 |
| Phenanthrene | 0.928 | 0.361 |
| Anthracene | 0.920 | 0.370 |
| Fluoranthene | 0.991 | 0.077 |
| Pyrene | 0.983 | 0.153 |
| Benzo[a]anthracene | 0.942 | -0.202 |
| Chrysene | 0.942 | -0.202 |
| Benzo[b]fluoranthene | 0.836 | -0.374 |
| Benzo[k]fluoranthene | 0.942 | -0.202 |
| Benzo[a]pyrene | 0.905 | -0.233 |
| Indeno[1,2,3-cd]pyrene | 0.911 | -0.234 |
| Dibenzo[a,h]anthracene | 0.528 | 0.218 |
| Benzo[ghi]perylene | 0.976 | -0.199 |
| PAH4 | 0.959 | -0.276 |
| PAH16 | 0.980 | 0.196 |

PCA, principal component analysis; PAHs, polycyclic aromatic hydrocarbons.

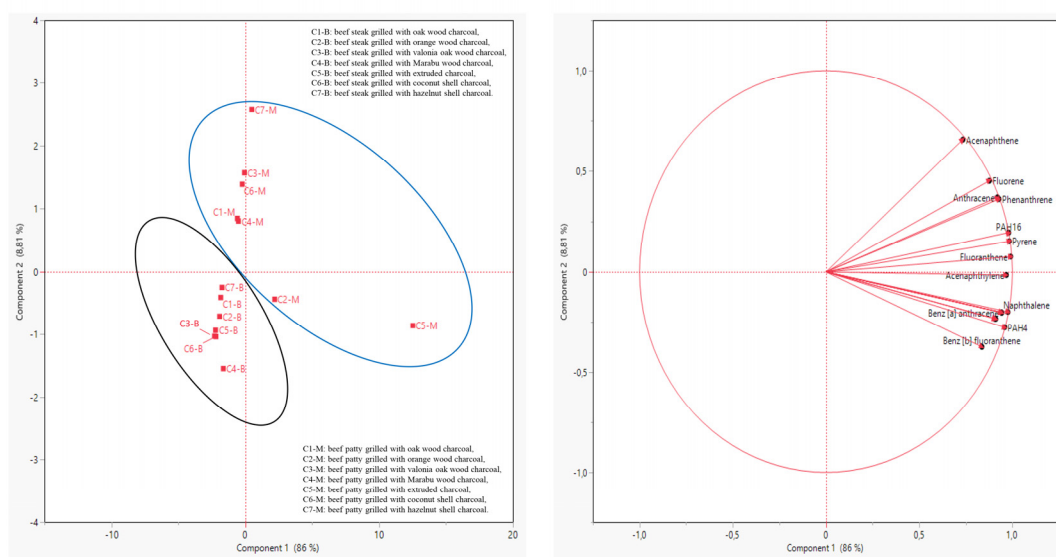


Fig. 2. PCA plots of the concentrations of 16 PAHs showing the relationships between charcoal types and type of meat products. PCA, principal component analysis; PAHs, polycyclic aromatic hydrocarbons.

Conflicts of Interest

The authors declare no potential conflicts of interest.

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Author Contributions

The article is prepared by a single author.

Ethics Approval

This article does not require IRB/IACUC approval because there are no human and animal participants.

References

- Ahmad RK, Sulaiman SA, Yusuf SB, Dol SS, Umar HA, Inayat M. 2020. The influence of pyrolysis process conditions on the quality of coconut shells charcoal. *Platf J Eng* 4:73-81.
- Alomirah H, Al-Zenki S, Al-Hooti S, Zaghoul S, Sawaya W, Ahmed N, Kannan K. 2011. Concentrations and dietary exposure to polycyclic aromatic hydrocarbons (PAHs) from grilled and smoked foods. *Food Control* 22:2028-2035.
- AOAC. 2000. Official methods of analysis. 17th ed. AOAC International, Washington, DC, USA.
- Bouvard V, Loomis D, Guyton KZ, Grosse Y, El Ghissassi F, Benbrahim-Tallaa L, Guha N, Mattock H, Straif K. 2015. Carcinogenicity of consumption of red and processed meat. *Lancet Oncol* 16:1599-1600.
- Cordeiro T, Viegas O, Silva M, Martins ZE, Fernandes I, Ferreira IMLPVO, Pinho O, Mateus N, Calhau C. 2020. Inhibitory effect of vinegars on the formation of polycyclic aromatic hydrocarbons in charcoal-grilled pork. *Meat Sci* 167:108083.
- Demirbaş A. 1999. Properties of charcoal derived from hazelnut shell and the production of briquettes using pyrolytic oil. *Energy* 24:141-150.
- Domingo JL, Nadal M. 2015. Human dietary exposure to polycyclic aromatic hydrocarbons: A review of the scientific literature. *Food Chem Toxicol* 86:144-153.
- Duedahl-Olesen L, Ionas AC. 2022. Formation and mitigation of PAHs in barbecued meat: A review. *Crit Rev Food Sci Nutr* 62:3553-3568.
- Ewa B, Danuta MŠ. 2017. Polycyclic aromatic hydrocarbons and PAH-related DNA adducts. *J Appl Genet* 58:321-330.
- García-Lomillo J, Viegas O, Gonzalez-Sanjose ML, Ferreira IMLPVO. 2017. Influence of red wine pomace seasoning and high-oxygen atmosphere storage on carcinogens formation in barbecued beef patties. *Meat Sci* 125:10-15.
- Giannakopoulou E. 2002. Valonia oak forests (17th–19th century): Economic factor and competition challenge. *Valanidia Forest: Past, Present, Central Amphitheater TEI, Messologi, Greece*. pp 67-96.
- Güler B, Aydın Temel F. 2023. Investigation of value-added charcoal production from full-scale hydrothermal carbonization

- of hazelnut husk and wood waste. *Biomass Convers Biorefin* (in press). doi: 10.1007/s13399-023-04191-z.
- Hamidi EN, Hajeb P, Selamat J, Razis AFA. 2016. Polycyclic aromatic hydrocarbons (PAHs) and their bioaccessibility in meat: A tool for assessing human cancer risk. *Asian Pac J Cancer Prev* 17:15-23.
- Hwang S, Kim C, Lee J, Park H, Lee G, Lee KG, Shin H, Kwon H. 2019. Carcinogenic risk associated with popular Korean dishes: An approach of combined risk assessments using oral slope factor and BMDL₁₀ values. *Food Res Int* 125:108530.
- Idowu GA, Fletcher AJ. 2020. The manufacture and characterisation of rosid angiosperm-derived biochars applied to water treatment. *Bioenergy Res* 13:387-396.
- Iwegbue CMA, Ogbuta AA, Otutu JO, Obi G, Egobueze FE, Martincigh BS. 2019. Evaluation of human exposure to polycyclic aromatic hydrocarbons from some edible oils and shea butter in Nigeria. *Polycycl Aromat Compd* 41:109-123.
- Kılıç Büyükkurt Ö, Aykın Dinçer E, Burak Çam İ, Candal C, Erbaş M. 2020. The influence of cooking methods and some marinades on polycyclic aromatic hydrocarbon formation in beef meat. *Polycycl Aromat Compd* 40:195-205.
- Kim HJ, Cho J, Jang A. 2021. Effect of charcoal type on the formation of polycyclic aromatic hydrocarbons in grilled meats. *Food Chem* 343:128453.
- Lee JG, Suh JH, Yoon HJ. 2019. Occurrence and risk characterization of polycyclic aromatic hydrocarbons of edible oils by the margin of exposure (MOE) approach. *Appl Biol Chem* 62:51.
- Li N, Huang J, Feng Y. 2020. Construction and confirmatory factor analysis of the core cognitive ability index system of ship C² system operators. *PLOS ONE* 15:e0237339.
- Masuda M, Wang Q, Tokumura M, Miyake Y, Amagai T. 2019. Simultaneous determination of polycyclic aromatic hydrocarbons and their chlorinated derivatives in grilled foods. *Ecotoxicol Environ Saf* 178:188-194.
- Nuray M, Oz F. 2019. The effect of using different types and rates of onion-water extract in meatball production on the formation of heterocyclic aromatic amines. *J Sci Food Agric* 99:3538-3547.
- Onopiuk A, Kołodziejczak K, Szpicer A, Wojtasik-Kalinowska I, Wierzbicka A, Półtorak A. 2021. Analysis of factors that influence the PAH profile and amount in meat products subjected to thermal processing. *Trends Food Sci Technol* 115:366-379.
- Oz E. 2021. The impact of fat content and charcoal types on quality and the development of carcinogenic polycyclic aromatic hydrocarbons and heterocyclic aromatic amines formation of barbecued fish. *Int J Food Sci Technol* 56:954-964.
- Oz E, Kaya M. 2019. The proteolytic changes in two different types of pastırma during the production. *J Food Process Preserv* 43:e14042.
- Oz F, Yuzer MO. 2016. The effects of cooking on wire and stone barbecue at different cooking levels on the formation of heterocyclic aromatic amines and polycyclic aromatic hydrocarbons in beef steak. *Food Chem* 203:59-66.
- Pantera A, Papadopoulos AM, Fotiadis G, Papanastasis VP. 2008. Distribution and phytogeographical analysis of *Quercus ithaburensis* ssp. macrolepis in Greece. *Ecol Mediter* 34:73-82.
- Park KC, Pyo H, Kim W, Yoon KS. 2017. Effects of cooking methods and tea marinades on the formation of benzo[a]pyrene in grilled pork belly (samgyeopsal). *Meat Sci* 129:1-8.
- Pehlivan E, Kahraman H, Pehlivan E. 2011. Sorption equilibrium of Cr(VI) ions on oak wood charcoal (*Carbo Ligni*) and charcoal ash as low-cost adsorbents. *Fuel Process Technol* 92:65-70.
- Prathomtong P, Panchatee C, Kunanopparat T, Srichumpuang W, Nopharatana M. 2016. Effects of charcoal composition and oil droplet combustion on the polycyclic aromatic hydrocarbon content of smoke during the grilling process. *Int Food Res J* 23:1372-1378.

- Rose M, Holland J, Dowding A, Petch S, White S, Fernandes A, Mortimer D. 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 Chem Toxicol* 78:1-9.
- Saito E, Tanaka N, Miyazaki A, Tsuzaki M. 2014. Concentration and particle size distribution of polycyclic aromatic hydrocarbons formed by thermal cooking. *Food Chem* 153:285-291.
- Sepetdjian E, Saliba N, Shihadeh A. 2010. Carcinogenic PAH in waterpipe charcoal products. *Food Chem Toxicol* 48:3242-3245.
- Sik B, Tongur T, ErKaymaz T, Yatmaz HA. 2016. Development and validation of a rapid and environmentally friendly analysis method for determination of polycyclic aromatic hydrocarbons in water by modification of QuEChERS extraction method. *J Environ Prot Ecol* 17:435-444.
- Singh L, Agarwal T, Simal-Gandara J. 2020. PAHs, diet and cancer prevention: Cooking process driven-strategies. *Trends Food Sci Technol* 99:487-506.
- Sobral MMC, Cunha SC, Faria MA, Ferreira IMPLVO. 2018. Domestic cooking of muscle foods: Impact on composition of nutrients and contaminants. *Compr Rev Food Sci Food Saf* 17:309-333.
- Sun Y, Wu S, Gong G. 2019. Trends of research on polycyclic aromatic hydrocarbons in food: A 20-year perspective from 1997 to 2017. *Trends Food Sci Technol* 83:86-98.
- Taverniers I, De Loose M, Van Bockstaele E. 2004. Trends in quality in the analytical laboratory. II. Analytical method validation and quality assurance. *TrAC Trends Anal Chem* 23:535-552.
- Viegas O, Novo P, Pinto E, Pinho O, Ferreira IMPLVO. 2012. Effect of charcoal types and grilling conditions on formation of heterocyclic aromatic amines (HAs) and polycyclic aromatic hydrocarbons (PAHs) in grilled muscle foods. *Food Chem Toxicol* 50:2128-2134.
- Wang C, Xie Y, Qi J, Yu Y, Bai Y, Dai C, Li C, Xu X, Zhou G. 2018. Effect of tea marinades on the formation of polycyclic aromatic hydrocarbons in charcoal-grilled chicken wings. *Food Control* 93:325-333.
- Yerima I, Grema MZ. 2018. The potential of coconut shell as biofuel. *J Middle East North Afr Sci* 4:11-15.
- Zelinkova Z, Wenzl T. 2015. The occurrence of 16 EPA PAHs in food: A review. *Polycycl Aromat Compd* 35:248-284.