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EFFICACY OF ANTIOXIDANT MARINATION IN THERMAL RISK MODULATION: COMPARATIVE PAH CONTAMINATION DYNAMICS IN BEEF BRISKET AND SHOULDER MUSCLES SUBJECTED TO GRILLING, FRYING, AND PRESSURE COOKING

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Abstract

The Polycyclic Aromatic Hydrocarbons (PAH) are carcinogenic and mutagenic contaminants generated by thermal processes mainly during high temperature cooking, via lipid degradation and pyrolytic reactions. In this study, the effect of thermal processing and marination on the occurrence of PAHs in beef brisket and shoulder muscles cooked by charcoal grilling (170°C), pan frying (114°C) and electric pot cooking (90 °C) was investigated. Significant differences between treatments were observed for Σ15 PAHs, PAH4, benzo[a]pyrene (BaP) and marination uptake values as a function of cooking method and muscle type. Samples of charcoal-grilled brisket (B-MC) had the highest concentration of Σ15 PAHs (22.50 ± 0.48 µg/kg) and PAH4 (0.86 ± 0.09 µg/kg). On the electric pot cooking at 90°C resulted in a lower concentration of PAH4 especially in B-ME (0.25 ± 0.01 µg/kg) and S-ME (0.22 ± 0.01 µg/kg), which reflects the mitigative effect of moderate thermal processing conditions.Two-way ANOVA showed significant interaction of muscle type and cooking treatment ($p < 0.001$). There was a significant negative correlation between the cooking loss and BaP concentration ($r = -0.714$, $p < 0.05$). Uptake of marination differed among treatments but the effect of marination on the occurrence of PAHs was treatment-dependent. In general, the main factors affecting the occurrence of PAHs in beef products were cooking temperature, smoke exposure and physicochemical alterations during cooking.

Key words*: beef brisket, beef shoulder, electric pressure cooking, marination, PAH.*

INTRODUCTION

Beef is a food with superior nutritional value (Zhang et al., 2025), but it requires cooking processes that can lead to the formation of potentially toxic compounds, such as polycyclic aromatic hydrocarbons (Adeyeye, 2020; Sahin et al., 2020). Thermal processes, such as frying in a pan, grilling, baking, and smoking, not only modify the sensory properties of the meat but also the levels of hydrocarbons generated. Consumers appreciate the particular taste of roasted beef acquired during thermal processing, for flavor creation at high temperatures (Kathuria et al., 2023). The Maillard reaction results from the interaction of amino compounds and reducing sugars, which provides food-rich flavors and sensory qualities (Chu et al., 2025). Different studies have shown that polycyclic aromatic hydrocarbons (PAH) (Chiang et al., 2020) are toxic chemicals formed during the incomplete combustion (Kalteh et al., 2024) of organic materials by smoking, roasting, grilling, barbecuing (Coroian et al., 2020; Coroian et al., 2022; Mastan et al., 2023; Șoimușan et al., 2024). Marinating, beside adding extra flavor to the prepared meat (Onopiuk et al., 2022; Oformata et al., 2024), also plays a role in inhibiting the formation of polycyclic aromatic compounds (Sampaio et al., 2021). Ciecierska et al. (2025) shown using electric grilling equipment or aluminium trays and marinating the meat, reduce the effects of PAH compounds.Research has demonstrated that choosing appropriate cooking methods by avoiding contact with flames, dripping fat into the heat source (Zahidah et al., 2024; Kwon et

al., 2025), and controlling the temperature substantially reduces the formation of PAHs (Duedahl-Olesen and Ionas, 2022). This study investigates the mitigation of 15 PAHs in beef meat (brisket and shoulder) through an antioxidant fruit-based marinade (sea buckthorn, cranberries, plums, sour cherries, and spices) across four thermal treatments.

MATERIALS AND METHODS

Certified organic beef from the Romanian cattle breed *Bălțată Românească*, sourced from the Silvania Bio farm (Sălaj County, Romania) was used as the raw material in this study.

Two muscle types with distinct anatomical and compositional characteristics were selected: brisket (*Musculus pectoralis)* and shoulder (*Musculus supraspinatus*). Prior to the experiment, visible connective tissue and excess surface fat were removed. Meat was portioned into samples weighing 60 g, with a uniform thickness of 1 cm to ensure comparable heat transfer during thermal processing. The experimental design consisted of two muscle types × four cooking treatments × five replicates, resulting in a total of 40 samples. The treatments were defined as follows:each treatment was performed in five independent replicates (n = 5). The sample codes presented in the Table 1 have been defined according to the experimental design. The first letter indicates the type of beef muscle (B = brisket, S = shoulder), the second letter indicates the marination state (C = control, M = marinated), and the last letter indicates the cooking method (C = charcoal grill, P = pan, E = electric pot).

Table 1. Experimental desing and sample coding

Sample code	Type Muscle	Treatment description
BC	Brisket control	Charcoal grill
BMC	Brisket marinated	Charcoal grill
BMP	Brisket marinated	Frying pan
BME	Brisket marinated	Electric pot
SC	Shoulder control	Charcoal grill
SCM	Shoulder marinated	Charcoal grill
SMP	Shoulder marinated	Frying pan
SME	Shoulder marinated	Electric pot

For treatments involving marination (MC, MP and ME), meat samples were subjected to an antioxidant fruit-based marinade designed to potentially reduce the formation of polycyclic aromatic hydrocarbons (PAHs) during thermal processing. The marinade formulation contained the following ingredients (g/1000 g of meat): 2.5% sea buckthorn (*Hippophae rhamnoides*), 2.5% cranberries (*Vaccinium macrocarpon*), 2.5% plums (*Prunus domestica*), 2.5% sour cherries (*Prunus cerasus*), 0.38% oregano (*Origanum vulgare*), 0.38% black pepper (*Piper nigrum*), 0.7% bay leaves (*Laurus nobilis*), salt 5%. The marination mixture was prepared using fruit-based ingredients with different processing conditions. Organic sea buckthorn berries and organic cranberries were used in frozen form ("quick frozen and optically selected"), supplied by Terarium Monte (Romania), no added sugar. According to the product specifications, the sea buckthorn berries contained 0.98% protein, 9.7% carbohydrates, 2.5% sugars, and 1.78% lipids per 100 g, with an energy value of 59 kcal. The cranberries contained 0.47% protein, 13.14% carbohydrates, 4.20% sugars, and 0.17% lipids per 100 g, with an energy value of 56 kcal. According to the manufacturer's specifications, the product was quick-frozen and stored at −18°C until use. Frozen sour cherries without pits supplied by FrescoVerde (Romania) were incorporated into the marinade formulation. According to the manufacturer's specifications, the product was quick-frozen and stored at −18°C until use. The nutritional composition per 100 g included 12.2 g carbohydrates, 8.5 g sugars, 1 g protein, and 0.3 g lipids, with an energy value of 50 kcal. The product originated from the European Union and was distributed by FrescoVerde SRL, Bihor County, Romania.Dried plums without pits (Prunus domestica) were used in dehydrated form and supplied by Fruit2Go Monicol (Republic of Moldova). According to the manufacturer's label, the dried plums contained protein 2.5 g, 49.0 g carbohydrates, 36.0 g sugars, 9.6 g fiber, and 0.9 g lipids per 100 g, with an energy value of 237 kcal. All fruit ingredients were commercially available food-grade products selected to reflect ingredients that are easily accessible to household consumers for domestic marination practices. The fruits were also chosen due to their natural content of organic acids, sugars, fibers, and bioactive

compounds potentially associated with physicochemical changes occurring during thermal processing. The ingredients were homogenized, by blender, to obtain a uniform mixture rich in natural antioxidant compounds such as polyphenols and organic acids. For each meat samples was aplicated 6 g of the marinade composition, with a brush and stored for 12 h at 4°C under refrigerated conditions. After the marination, 2 g of duck fat was applied with a brush to each sample prior to thermal processing. The fat layer was evenly distributed on the meat surface to simulate typical culinary conditions and to promote lipid interaction during cooking. Samples were subjected to three different cooking methods. Thermal processing was conducted using three cooking methods representing different heat transfer mechanisms and temperature regimes: charcoal grilling, frying pan cooking on a gas stove, and electric pot cooking. For charcoal grilling treatments (BC/SC and BMC/SMC), meat samples were cooked on a grill fueled with hexagonal coconut-shell bio charcoal briquettes, which provided a stable heat source and combustion environment. The samples were placed on the grill side away from the flame for indirect heat transfer at an average surface temperature of approximately 170 °C.For the frying pan treatment (BMP/SMP), samples were cooked in a ceramic frying pan placed on a gas-flame stove, with the pan surface temperature maintained at approximately 114°C.

The electric pot treatment (BME/SME) represented a lower-temperature cooking environment, where samples were prepared in an electric cooking pot, Tefal One Pot CY505E30, by setting the DIY Chef program that corresponds to level 3 at 90°C, without pressure and with the lid slightly open. During cooking, the internal temperature of each meat sample was continuously monitored using a calibrated digital thermocouple probe inserted into the geometric center of the sample. The endpoint temperature was standardized at 72°C, corresponding to the recommended safe internal temperature for well-done beef products. Temperature monitoring allowed accurate control of the cooking process and ensured comparability among the experimental treatments. For all cooking methods, the endpoint temperature was measured at the geometric center of each sample using a calibrated digital thermoprobe upon reaching the target temperature. Cooking was stopped immediately once this internal temperature was reached to ensure comparable thermal exposure across treatments, they were immediately taken from the grill, cooled to room temperature and re-weighed.To assess the weight loss, each sample was weighed before and after the respective treatment (grill, frying pan, electric pot). The formula for evaluating the cooking loss of the heat-treated meats is as follows:

Weight loss (%) = $M-M_1/M *100$

M - the weight of the unprocessed beef meat.

M_1 - the weight of the each treatment beef meat.

All chemicals and solvents used in the analysis were of HPLC grade and purchased from VWR International (Germany). Ultra-pure water was obtained using an ULTRACLEAR UV UF EVOQUA purification system (USA).

Antioxidant capacity was determined using the ACL assay kit (Analytik Jena, Germany). Analytical standards for polycyclic aromatic hydrocarbons and Trolox were obtained from Sigma-Aldrich (Darmstadt, Germany).

The analyzed samples consisted of beef brisket and beef shoulder muscles. Prior to analysis, the samples were homogenized to ensure sample uniformity. All measurements were performed in quintuplicate (n = 5). Polycyclic Aromatic Hydrocarbons (PAHs) Analysis were determined using a saponification followed by solid-phase purification procedure. Briefly, 10 g of homogenized beef brisket or shoulder sample were treated with 50 mL of 0.4 M KOH solution prepared in an ethanol-water mixture (9:1, v/v). The saponification process was performed in an ultrasonic bath at 60°C for 30 min. Following saponification, the mixture was filtered through filter paper, and the resulting extract was subjected to liquid-liquid extraction twice with 15 mL of cyclohexane. The combined organic phases were subsequently purified using a Florisil column. The purified extract was evaporated to dryness under a gentle stream of nitrogen and reconstituted in 1 mL of acetonitrile. Prior to chromatographic analysis, samples were filtered through 0.45 µm syringe filters. Analysis of PAHs by Chromatographic analysis was carried out

using a Waters Arc HPLC system (Waters Corporation, Milford, MA, USA) equipped with a fluorescence detector (FLD). Separation of 15 PAHs was achieved on an Inertsil ODS-P column (5 µm, 4.6 × 150 mm) maintained at 24°C. The injection volume was 10 µL. The mobile phase consisted of a water-acetonitrile gradient system. Detection was performed using a time-programmed fluorescence detection method optimized for each PAH compound.The PAHs analyzed were selected according to priority toxicological indicators defined by European food safety regulations. Benzo[a]pyrene (BaP) was evaluated separately as an important carcinogenic marker used in food safety assessment of thermally processed meat products. The analyzed PAHs compounds included naphthalene, acenaphthene, fluorene, pyrene, phenanthrene,anthracene,fluoranthene, benzo[a]anthracene(BaA),benzo[b]fluoranthen e (BbF) chrysene(CHR), benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, benzo[g,h,i]perylene, indeno[1,2,3-cd]pyrene. PAH4 concentrations were calculated as the sum of BaA, CHR, BbF, and BaP, while Σ15 PAHs represented the sum of all quantified compounds. All concentrations were expressed as µg/kg of sample. According to Commission Regulation (EU) No. 915/2023 regulates the sets the maximum Benzo[a]pyrene (BaP) concentration at 2 µg /kg and the content of the four heavy PAHs at 12 µg/kg in food products. All statistical analysis was performed using RStudio software (R version 4.5.2). All experiments were carried out in five independent replicates (n = 5) for each treatment and results are shown as mean values ± standard deviation (SD). The effect of cooking treatments on the concentrations of polycyclic aromatic hydrocarbons (PAHs) was evaluated by one-way analysis of variance (ANOVA). For significant differences among treatments ($p < 0.05$), pairwise comparisons among treatment means were done by the Tukey's honestly significant difference (HSD) post hoc test. Statistical analyses were performed separately for each individual PAH compound as well as for the calculated indicators Σ15 PAHs, PAH4 and benzo[a]pyrene (BaP). Statistical significance was accepted at $p < 0.05$ for differences among treatments. Different lower case letters above bars in graphs indicate statistically significant differences based on Tukey's HSD test, and treatments with at least one common letter were not significantly different.

RESULTS AND DISCUSSIONS

The comparison of experimental values demonstrates that the cooking method is the determining factor in the formation of polycyclic aromatic hydrocarbons in beef.The physicochemical composition of raw beef muscles as shown in Table 2.

Table 2. Physicochemical properties

Parameter	Brisket	Shoulder
Moisture (%)	71.68 ± 0.42	72.18 ± 0.37
Protein (%)	26.72 ± 0.56	27.11 ± 0.97
Fat (%)	0.42 ± 0.54	0.51 ± 0.05
Minerals (%)	0.93 ± 0.08	0.93 ± 0.08

Values are expressed as mean ± standard deviation (n = 5). Moisture, protein, fat, and mineral contents were determined prior to thermal processing according to standard AOAC methods. The initial composition of brisket and shoulder was similar as indicated by the moisture, protein, lipid and mineral contents. The observed differences in PAH occurrence between treatments are likely related not only to compositional factors, but also to treatment specific thermal conditions, cooking loss, marination uptake and physicochemical changes during cooking. Lipids are recognized precursors in PAH formation through thermal degradation and pyrolytic reactions; the relatively small differences in lipid content observed between the two muscles suggest that other factors played a more important role. The differences in PAH concentrations between brisket and shoulder samples cannot be explained by chemical composition alone. Other factors such as muscle microstructure, connective tissue content, water holding capacity, cooking loss and heat and smoke exposure may also have contributed to the treatment dependent variability observed in this study.This would indicate that physicochemical changes taking place during cooking may be more important determinants of PAH occurrence than initial compositional differences found between the two muscle types.The limits set by the European Food Safety Authority (EFSA, 2008)

indicate that all analyzed samples have concentrations of benzo(a)pyrene and PAH4 significantly below the maximum permissible thresholds for meat products. The concentrations of benzo[a]pyrene varied between 0.01 and 0.23 µg/kg, depending on the marination treatment (Figure 1).

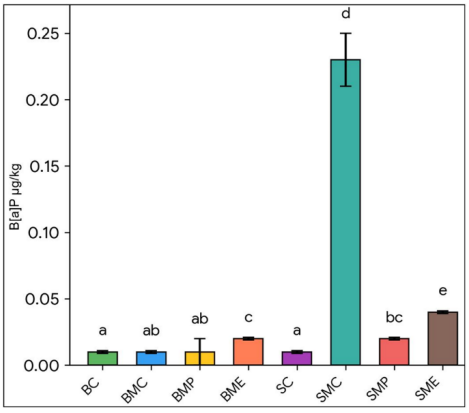

Figure 1. Benzo[a]pyrene (B[a]P) concentrations

The highest BaP value observed in the study (0.23 µg/kg, SMC treatment) remains nearly ten times lower than the European limit of 2 µg/kg. Different letters (a-e) above the bars indicate statistically significant differences ($p < 0.05$) according to Tukey's test.The linear scale emphasizes the critical accumulation of B[a]P in the SMC group compared to other treatments. Considered a carcinogenic marker, B[a]P is used as a classic indicator of PAH contamination. These results suggest that, under the evaluated experimental conditions, the preparation methods used do not generate PAH levels considered concerning from a toxicological standpoint. However, the differences observed between treatments indicate that cooking methods characterized by higher temperatures and direct contact with the heat source, such as charcoal grilling and gas frying, may promote a relatively higher accumulation of PAHs compared to methods based on indirect heat transfer.The formation of toxic compounds benzo[a]pyrene and other PAH4 compounds during thermal treatments is due to the pyrolysis of lipids in meat and incomplete combustion reactions at high temperatures. The dripping onto hot surfaces or charcoal can produce smoke rich in PAH precursors, which will condense on the surface of the meat (Singh et al., 2016). Using indirect heat transfer at lower temperatures plays an essential role in reducing the formation of compounds such as benzo[a]pyrene and PAHs (Sumer & Oz, 2023). The calculated 4 PAHs (Figure 2) values for all treatments (0.22–0.86 µg/kg) are well below the 12 µg/kg limit set for meat products subjected to thermal processing.

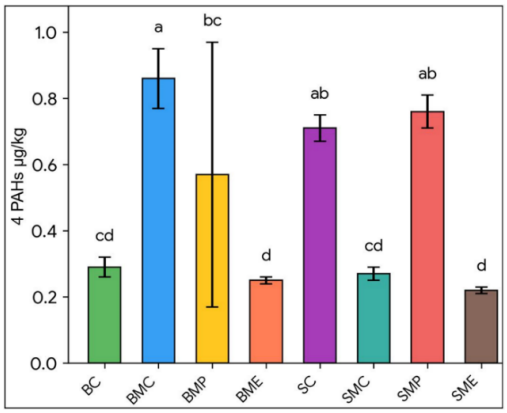

Figure 2. 4 PAHs concentrations

The maximum value was observed for BMC = 0.86 µg/kg followed by SMP = 0.76 µg/kg, SC = 0.71 µg/kg. Different letters above the bars indicate statistically significant differences between treatments ($p < 0.05$).

The toxicological indicator PAH4, which includes the compounds: Benzo(a)pyrene, Benzo(a)anthracene, Benzo(b)fluoranthene and Chrysene, is recommended by the European Food Safety Authority for contamination assessment (EFSA, 2008). All values are well below the European limit of 12 µg/kg, indicating a low toxicological risk for the analyzed samples. These results indicate that cooking on charcoal at 170°C promotes the formation of PAHs, especially in the case of brisket muscle, where the highest total value was recorded. The high temperature and contact with smoke generated by fat pyrolysis promote the formation of aromatic radicals (Lee et al., 2016). These proceses by condensation reactions, and the deposition of PAHs on the meat surface from direct contact with the heat source (Anjum et al., 2019). Abdel Monem et al., (2024) suggested that marinating beef steaks before charcoal grilling,

by using ingredients with antioxidant properties, to reduce PAHs formation and minimize risks to human health. In Figure 3, the highest total PAH concentration (Σ15 PAHs) was observed in the BMC samples (22.50 µg/kg), SMP (11.76 µg/kg), and SME (11.75 µg/kg).

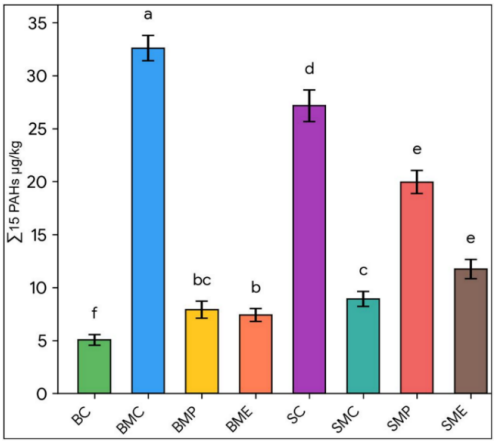

Figure 3. Total PAHs (Σ15 HAPs) concentrations

For the Brisket charcoal grill, the control sample BC = 5.05 µg/kg, and for the Shoulder, the results were for the control sample SC (17.76 µg/kg). The influence of marinating produced different effects depending on the cooking method. In the Brisket, the marinated sample BMC = 22.50 µg/kg, respectively the marinated Shoulder SMC = 8.91 µg/kg. Marination efficiency was strongly dependent on the meat cut. In the shoulder samples, total 15 PAHs levels were significantly reduced following marination, with reductions of 50% for SMC, 34% for SME, and 34% for SMP compared with the control. A similar trend was observed for total 4 PAHs, which decreased by 62% for SMC and 69% for SME and 14% BME. Marination of the brisket samples resulted in increased PAH concentrations relative to the control.Antioxidant compounds present in marinades can suppress PAH formation during cooking, by inhibition of lipid oxidation, but it can increase PAH if it contains sugars that promote Maillard reactions, due to marinading based on natural fruits (sea buckthorn, cranberries, plums, sour cherries).
Differences are observed between the types of muscle Brisket vs Shoulder, due to muscle structure, fat distribution, and water retention capacity. As shown in Table 3, both cooking temperature and marination uptake had a significant effect on the occurrence of PAHs in beef muscles.

Table 3. Effect of thermal processing and marination uptake on PAH levels in beef muscles

Sample code	∑15 HAPs	4 PAHs	B[a]P	Marinade uptake (%)
BMC	22.50 ± 0.48 ^{a}	0.86 ± 0.09 ^{a}	0.01 ± 0.00 ^{ab}	3,67 ± 1,39 ^{ab}
BMP	7.92 ± 0.48 ^{bc}	0.57 ± 0.40 ^{bc}	0.01 ± 0.01 ^{ab}	5,00 ± 1,67 ^{ab}
BME	7.40 ± 0.58 ^{b}	0.25 ± 0.01 ^{d}	0.02 ± 0.00 ^{c}	3,00 ± 13,9 ^{a}
SMC	8.91 ± 0.37 ^{c}	0.27 ± 0.02 ^{cd}	0.23 ± 0.02 ^{d}	6,67 ± 1,18 ^{b}
SMP	11.76 ± 0.40 ^{e}	0.76 ± 0.05 ^{ab}	0.02 ± 0.00 ^{bc}	4,33 ± 1,90 ^{ab}
SME	11.75 ± 0.43 ^{f}	0.22 ± 0.01 ^{d}	0.04 ± 0.00 ^{e}	2,67 ± 1,90 ^{a}

Two-way ANOVA analysis confirmed a significant interaction between muscle type and cooking treatment ($p < 0.001$), indicating that PAH occurrence is influenced simultaneously by muscle characteristics and thermal processing parameters. The different thermal treatments studied here, such as charcoal grilling (170°C), pan frying (114°C) and electric pot cooking (90°C), resulted in different contamination profiles for Σ15 PAHs, PAH4 and benzo[a]pyrene concentrations. Differences in the values for marinade uptake were also statistically significant. The highest marinade uptake was noted in SMC (6.67 ± 1.18) and the lowest value was in SME (2.67 ± 1.90). These differences may be due to differences in muscle structure, connective tissue content and water holding capacity between the brisket and shoulder muscles. The association between marinade uptake and PAH occurrence was not consistent across all treatments. Despite the high marinade uptake of SMC, high concentrations of benzo[a]pyrene (0.23 ± 0.02 µg/kg) were still detected. In contrast, BMC showed very high Σ15 PAHs concentrations despite comparatively low

values of marinade uptake. These results indicate that the formation of PAHs was mainly dependent on the intensity of the thermal exposure, physicochemical changes, smoke contact, cooking temperature, and during the thermal processing rather than the uptake of marinade alone. Analysis of the correlation between weight loss (%) and Benzo[a]pyrene concentration (µg/kg). The points represent the analyzed experimental groups, and the dotted line represents the linear regression trend in Figure 4.

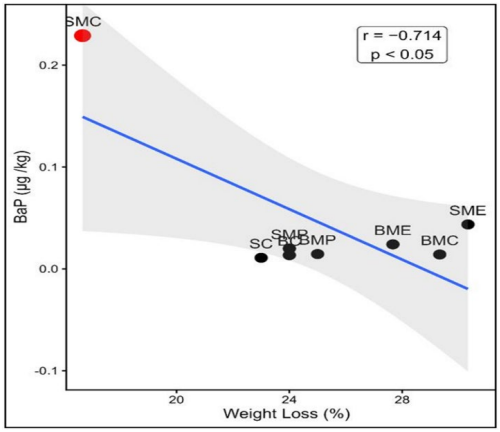

Figure 4. Linear regression analysis between weight loss (%) and Benzo[a]pyrene (B[a]P)concentration

A significant negative correlation ($r = -0.71$, $p < 0.05$) was observed between weight loss and Benzo[a]pyrene concentration, indicating that greater losses during cooking were associated with lower levels of BaP. Fat loss during cooking is another important factor that influences PAH formation. The weight loss values recorded in this study ranged from approximately 16% to 30%, depending on the treatment. Higher fat losses generally correspond to greater melting and release of lipids during cooking, may promote the formation of polycyclic aromatic hydrocarbons (PAH) as a result of pyrolytic reactions. In treatments where fat loss was high, such as BMC (29.33%) and SME (30.33%), high concentrations of several PAHs were observed. This relationship can be explained by the increased availability of lipid substrates undergoing thermal degradation. During thermal processing, meat loses moisture, leading to changes in texture and a reduction in cooking yield (Purslow et al., 2016). When the melted fat reaches hot surfaces or heating elements, it undergoes rapid pyrolysis, producing volatile compounds that participate in the formation of PAHs. In contrast, lower weight loss values can reduce the intensity of these reactions. For example, the SMC treatment, which showed the lowest fat loss (16.67%), generally exhibited reduced PAH concentrations compared to other treatments. This observation suggests, during cooking, that limiting fat dripping and subsequent pyrolysis could represent an effective strategy for reducing PAH contamination. Differences between brisket and shoulder samples may be partially related to differences in muscle structure, moisture retention, lipid distribution and thermal behavior during cooking, all of which can have an impact on PAH formation during thermal processing. However, the detailed physicochemical characterization of the muscle matrices studied was beyond the primary focus of the present study and will be reported separately in future work on marinade delivery efficiency and physicochemical properties of cooked beef products.

CONCLUSIONS

Results showed that the presence of PAHs in beef samples was significantly affected by the cooking conditions, thermal exposure and the type of muscles. The greatest concentrations of PAHs were found after the charcoal grilling (170°C), particularly in brisket. The lower cooking temperatures were associated with reduced PAH concentrations in the analysed samples, particularly during pan frying (114 °C) and electric pot cooking (90°C). Differences between brisket and shoulder muscles could be related to muscle structure, lipid distribution, water retention capacity and thermal behaviour during cooking. Shoulder samples showed higher values of marinade uptake and the effect of marination on the occurrence of PAHs was dependent on the treatment and not uniform in all cooking conditions. Correlation and regression analysis also indicated that the physicochemical changes in thermal processing could be a factor that contribute to the variability of PAHs in cooked beef products. The main factors

associated with the total occurrence of PAHs reported were cooking temperature, exposure to smoke and the intensity of heat. There were statistically significant differences among treatments. The concentrations of benzo[a]pyrene (BaP) and PAH4 were significantly lower than the maximum levels, 2 µg/kg for BaP and 12 µg/kg for PAH4, established by Commission Regulation (EU) No. 915/2023. It is recommended to minimize the amount of fat that drips onto the heat source, maintain controlled cooking temperatures and reduce exposure to smoke. The concentrations of PAHs in beef products can be reduced by these practices.

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