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Different Marinades and Types of Grills and Their Impact on Grilled Pork Neck Loins Contamination with Polycyclic Aromatic Hydrocarbons

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Abstract: Meat processing methods affect its quality and, most importantly, its safety. As a kind of green processing technology, the effects of various marinades, including universal, pork, and honey mustard, and the most popular grilling tools on PAH contamination in pork neck loins, the most frequently grilled pork meat, were investigated. PAH analysis was performed using the QuEChERS–HPLC–FLD/DAD method and confirmed by GC/MS method. Weight loss and changes in individual color parameters after grilling were also analyzed. Grilling on a charcoal grill without an aluminum tray caused statistically the greatest PAH contamination. Some of these samples, according to the requirements of Commission Regulation (EU) No. 915/2023, should not be consumed by humans due to the high content of B[a]P (5.26 – 6.51 μg/kg). Studies have also shown that universal and pork marinade can reduce PAH contamination, whereas honey mustard marinade increases their accumulation in grilled products. Carefully choosing grilling equipment, such as using electric grills instead of charcoal or using aluminum trays when grilling with charcoal and marinating meat before cooking, is essential for consumers and food producers. These practices can significantly reduce the harmful health effects of PAHs, making them vital steps toward safer food preparation.

Keywords: PAHs; pork neck loins; grilling; marinades; QuEChERS; HPLC–FLD/DAD; GC/MS

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1. Introduction

Polycyclic aromatic hydrocarbons (PAHs, polyarenes) are a diverse group of colorless, white, pale yellow, or green solids belonging to organic compounds, consisting of carbon and hydrogen atoms joined by benzene ring systems. Depending on the number of benzene rings in their structure, polyarenes are classified as light PAHs (2–3 or 2–4 rings, depending on categorization) and heavy PAHs (4–5 to several rings). In addition to carbon and hydrogen, polyarenes can contain nitrogen, sulfur, or oxygen atoms attached to benzene rings [1,2].

Over the years, many organizations have studied the effects of polyarenes on animal and human organisms to monitor the harmful effects of polycyclic aromatic hydrocarbons [3]. The origins date back to 1976 when the United States Environmental Protection Agency [4] established a list of 16 PAHs, including the most known benzo[a]pyrene (B[a]P), as indicator compounds with strong and low toxicity to humans. In December 2002, the European Union Scientific Committee on Food [5] identified 15 heavy PAHs as the most dangerous for humans. Three years later, in 2005, the FAO WHO Expert Committee on Food Additives [6] classified 13 of these as mutagenic and carcinogenic compounds. The Commission Recommendation (EC) No. 108/2005 and the EFSA Panel on Contaminants in the Food Chain (CONTAM) stated that B[a]P is not a suitable indicator for other PAHs in food products [7]. Consequently, the sum of 4 heavy PAHs - benzo[a]pyrene,

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benzo[a]anthracene (B[a]A), benzo[b]fluoranthene (B[b]F), and chrysene (Chr) - has been recognized as the most reliable marker for PAH presence in foodstuffs [8].

Apart from the natural environment, the main route of exposure to polyarenes is the consumption of food, in particular processed food. Meat and animal products are the second largest food product consumption group, and they pose a risk of polyarenes dietary intake. The type of meat, the content of fat in which contaminants accumulate, and the food processing method greatly impact PAH contamination. In particular, heat–treated products are exposed to PAH formation. The kind of meat, fat content, method and time of heat processing, or heat source significantly affects PAH concentration in such products [9–11].

Due to the high risk of PAH contamination in processed food products, selecting the appropriate raw material and the heat treatment method can effectively minimize the danger of these compounds [12]. In the case of grilling, which is a popular method of food preparation, especially in the summer season, two methods of meat preparation, direct and indirect grilling, can be distinguished. In the case of both methods, the raw material is placed over the heat source, with the difference being that in the indirect method of grilling, the meat is not placed directly over the heat source [13,14]. Preparing food using a tray or aluminum foil minimizes contact of the melting fat with the heat source, and less PAH is produced [15]. Electric and ceramic grills, used in homes and restaurants, are also becoming increasingly popular. It is also important to properly prepare the raw material before it is cooked. Marinade food not only allows you to give dishes a unique taste and smell, but many studies have shown that it has beneficial health effects, including inhibiting the formation of harmful substances such as PAHs [16,17].

Commission Regulation (EU) No. 915/2023 regulates the permissible amount of polyarenes in food products subjected to grilling. It sets the maximum B[a]P concentration at 5 μg/kg and 4 heavy PAHs at 30 μg/kg [18].

Studies analyzing the formation and occurrence of PAHs in grilled meat products, as well as developing strategies to prevent and reduce PAH contamination, are still crucial. Although numerous researchers focus on minimizing PAH levels in heat-treated meats, they typically emphasize the 16 EPA-listed PAHs. However, based on the SCF and EFSA recommendations discussed earlier, scientists should target the more toxic PAHs identified by the SCF. Considering those mentioned above, this study examined PAH contamination of pork neck loins prepared in different marinades, commonly used in Poland, and grilled using charcoal and electric grills. The research aimed to select the marination and grilling tool whose use minimizes PAH contamination in pork neck loins and enhances the safety of such products. Our previous study examined poultry meat, another frequently grilled meat in Poland. PAH analysis was devoted to 19 polyarenes, including 15 heavy SCF PAHs, B[a]P and 4 heavy marker PAHs, and 4 light EPA PAHs, which are generally the most prevalent in PAH contamination profiles. The determination of PAHs was conducted using the QuEChERS extraction method with liquid chromatography and fluorescence and diode array detectors (QuEChERS-HPLC–FLD/DAD) and confirmed by gas chromatography coupled with mass spectrometry (GC/MS). The findings of this research will offer crucial insights into how grilling methods and marination influence PAH contamination levels in grilled pork meat. Furthermore, they suggest effective strategies for reducing these harmful compounds to minimize their intake via diet and promote safer food consumption.

2. Materials and Methods

2.1. Research Material, Its Preparation for Grilling, and Experimental Design

The research material was raw pork neck loins from a local Warsaw market in Poland. The samples were cut into 100 g portions and subsequently marinated using three preselected marinades commonly used in Poland for pork neck marination treatment: universal, pork, and honey mustard marinades. These marinades were deliberately chosen due to their widespread consumer preference.

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Each marinade was prepared strictly as stated in the manufacturer's instructions, with their exact formulations detailed in Table 1.

Table 1. Composition of marinades.

Marinade Treatment	Ingredients [g/100 g of Meat]	Code
Universal marinade	5 g universal seasoning (composition: salt, rosemary (10.3%), basil, sugar, onion, paprika, oregano, marjoram, ground mustard, coriander, sunflower oil, thyme, lemon juice, turmeric, cayenne pepper), 5 g refined rapeseed oil	MU
Pork marinade	5 g pork seasoning (composition: salt, garlic (22.0%), red pepper (14.0%), coriander, rosemary, black pepper, citric acid, parsley (2.2%), bay leaves), 5 g refined rapeseed oil	MK
Honey mustard marinade	5 g spicy mustard (water, white mustard, spirit vinegar, black mustard, sugar, salt, aroma, turmeric extract), 3.5 g lime honey, 1 g refined rapeseed oil, 0.5 g freshly squeezed lemon juice	MM

The unmarinated pork neck loins and the samples after marination treatment were placed in a refrigerator (4 ± 1°C) in glass food containers until the next morning. Each sample was done in triplicate, and the grilling process was conducted in two batches (n = 6). Therefore, six samples of each product type were subjected to analysis. The next day, prior to grilling, all pork neck loins were brought to room temperature until their internal temperature reached 15–20°C, as verified using a HANNA Instrument HI 98804 thermometer (Woonsocket, RI, USA).

The experimental procedure was designed encompassing marination with preselected marinades, heat treatment on various grill types, measurement of weight loss post-grilling, assessment of color parameters in both raw and grilled samples, preparation for determination of PAHs via the QuEChERS method, and comprehensive qualitative-quantitative PAH analysis using high-performance liquid chromatography with fluorescence and diode-array detectors (HPLC–FLD/DAD) and results confirmation by gas chromatography coupled with mass spectrometry (GC/MS).

2.2. Grilling Tools and Cooking Procedure

To evaluate the influence of grilling methods and grill types on PAH formation, the following grills were utilized: a charcoal grill without a tray (Weber Original Kettle E-4710, Weber-Stephen Deutschland GmbH, Ingelheim am Rhein, Germany) and the same charcoal grill with an aluminum tray, an electric cast iron contact grill with corrugated surfaces both top and bottom (Combi Grill, GR-1000 model, Optimum, Mińsk Mazowiecki, Poland), and an electric ceramic contact grill with corrugated and smooth surface for top and bottom, respectively (SpidoCook, XP010PR model, UNOX, Cadoneghe, Italy).

Before heat treatment, all grills were preheated for a minimum of 10 min. During this period, the initial weights of the pork neck loins were noted. Once the preheating was complete, the grilling process started. The specific grilling parameters for each grill are detailed in Table 2. Upon reaching the target medium level of meat doneness and the required internal temperature at the geometric center of the meat (>80°C), the samples were immediately taken from the grill, cooled and re-weighed, and subsequently subjected to PAH determination.

Table 2. Grilling parameters.

Type of Grill	Grill Temperature [°C]	Grilling Time [min]	Temperature at the End of the Grilling Process in the Product Geometric Center [°C]
W1	240 – 300	12	94.5
W2	170 – 220	17	90.2
E1	180 – 200	15	93.2
E2	200 – 220	15	94.9

W1 – charcoal grill without a tray, W2 – charcoal grill with an aluminum tray, E1 – electric ceramic contact grill, E2 – electric cast iron contact grill.

2.3. Weight Loss

To assess the weight loss, the pork neck loin samples were weighed in triplicate both before and after grilling. The formula for calculating cooking loss in grilled meat samples is as follows:

$$\boldsymbol{Weight\ loss} = \frac{\boldsymbol{M - M_g}}{\boldsymbol{M}} \cdot \mathbf{100\%}$$

M – weight of raw pork neck loin sample

M_g – weight of grilled pork neck loin sample

2.4. Color Measurement

CIE L*a*b* color parameters of both raw and grilled meat samples were measured using a Minolta Colorimeter (Chroma Meter CR–200, Konica Minolta Corp., Japan), calibrated with a white reference plate (L* = 97.83, a* = -0.45, b* = +1.88). The color differences between samples before and after grilling (ΔE) were determined based on the measured lightness (L*), redness (a*), and yellowness (b*) values.

$$\mathbf{\Delta E=[(\Delta L\text{\textasciicircum}*)+(\Delta a\text{\textasciicircum}*)+(\Delta b\text{\textasciicircum}*)]^{0.5}}$$

2.5. Chemicals and Materials

Acetonitrile (HPLC gradient grade), sodium chloride, and anhydrous magnesium sulfate (analytical purity >99.0%) were procured from Avantor Performance Materials Poland S.A. (Gliwice, Poland). Sorbents utilized in the QuEChERS method included primary secondary amine (PSA), silica gel modified with C18 groups, and graphitized carbon black (GCB), specifically Sepra PSA Bulk Packing, Sepra C18–E Bulk Packing, and Sepra GCB Bulk Packing, obtained from Phenomenex (Warsaw, Poland). Standard mixtures of 15 SCF PAHs (PAH–Mix 183, Dr. Ehrenstorfer) and 16 PAHs from the US EPA list (PAH–Mix 9, Dr. Ehrenstorfer) were obtained from Witko (Łódź, Poland), together with a deuterated PAH mixture (PAH-Mix 918, Dr. Ehrenstorfer) used for quantitative confirmation of results by GC/MS method. The 15 SCF PAH mixture consisted of benzo[a]pyrene, benzo[a]anthracene, benzo[b]fluoranthene, chrysene, 5–methylchrysene (5-MChr), cyclopenta[c,d]pyrene (C[cd]P), benzo[j]fluoranthene (B[j]F), benzo[k]fluoranthene (B[k]F), benzo[g,h,i]perylene (B[ghi]P), indeno[cd]pyrene (I[cd]P), dibenzo[a,h]anthracene (D[ah]A), dibenzo[a,l]pyrene (D[al]P), dibenzo[a,e]pyrene (D[ae]P), dibenzo[a,h]pyrene (D[ah]P), dibenzo[a,i]pyrene (D[ai]P). The second PAH standard mixture was exclusively used for the quantification of four light PAHs: phenanthrene (Phen), anthracene (Anthr), fluoranthene (F), and pyrene (Pyr). Deionized water was obtained from a Millipore Milli-Q water purification system. Polytetrafluoroethylene (PTFE) syringe filters (25 mm i.d., 1 µm pore size) and Falcon centrifuge tubes (PTFE) were sourced from BioAnalytic (Gdańsk, Poland).

2.6. Determination of PAHs Using the QuEChERS–HPLC–FLD/DAD Method

The preparation of samples and qualitative-quantitative PAH determination was conducted using the methodology outlined by Ciecierska and Komorowska [14] in previous scientific work on other research material and Ciecierska et al. [19]. The methodology consisted of PAH extraction and

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sample purification with the QuEChERS method, followed by subsequent chromatographic analysis using the HPLC–FLD/DAD and results confirmation by GC/MS technique.

To enhance sample representativeness and maximize the contact area with the reagent, grilled pork neck loin samples were homogenized using an IKA A11 basic laboratory grinder (IKA Poland Sp. z o.o., Warsaw, Poland) before the process of extraction and purification.

To extract fat and PAHs, 5 g of homogenized meat sample was weighed into a 50 mL Falcon centrifuge tube, followed by 10 mL of acetonitrile added. The mixture was vortexed for 1 min. Subsequently, 4 g of magnesium sulfate and 1 g of sodium chloride were added, and the tube was vortexed for 3 min before centrifugation at 3400 rpm for 3 min using a laboratory centrifuge (MPW—352R, Warsaw, Poland). Following phase separation, 4 mL of the upper layer was applied into a 15 mL centrifuge tube containing purification sorbents: 900 mg of $MgSO_4$, 300 mg of PSA, 150 mg of C18, and 10–20 mg of GCB, then vortexed for 3 min and centrifuged again at 3400 rpm for 3 min. The obtained supernatant after filtration (PTFE filter, 0.2 μm of pore diameter) into a chromatography vial was subsequently analyzed using an HPLC–FLD/DAD system and after changing the solvent to cyclohexane, analyzed by GC/MS to confirm the results.

The determinations of 4 light and 15 heavy-marker SCF PAHs in grilled pork neck loins were conducted following the methodology described by Ciecierska and Komorowska [14]. A Nexera Shimadzu HPLC system (LC–40DXR, Kyoto, Japan) equipped with an RF–20XL fluorescence detector and an SPD–M10AVP diode-array detector was utilized. Chromatographic separations were carried out on a Kinetex–PAH column (150 mm, 4.6 mm, 3.5 μm; Phenomenex, Warsaw, Poland) at 30 °C with specific gradient elution conditions, employing a mobile phase composed of acetonitrile (A) and water (B) (70:30, v/v) with 1.5 mL/min flow rate. Fluorescence and diode-array detection parameters varied by compound, and they were presented in the abovementioned methodology

2.7. Quantification and Validation of the QuEChERS–HPLC–FLD/DAD Method

Quantification and validation of PAH determination using the QuEChERS–HPLC–FLD/DAD method in grilled meat samples were characterized in the previous study conducted by Ciecierska and Komorowska [14]. The validation parameters for 4 heavy-marker PAHs analysis, including the limit of detection (LOD: 0.06–0.08), limit of quantification (LOQ: 0.12–0.16), recovery rates (%) with relative standard deviations (84.8±7.7 % – 89.2±7.7 %, and $HORRAT_R$ values (0.6-0.7), confirmed compliance with Commission Regulation (EU) No. 836/2011 [20]. Additionally, the method exhibited satisfactory performance for both other PAHs from the SCF list and the 4 light PAHs from the US EPA list [14]. Figure 1 presents the chromatograms of heavy polyarenes detected in pork neck loin samples, both after different marination treatments and without it, grilled on the charcoal grill without a tray.

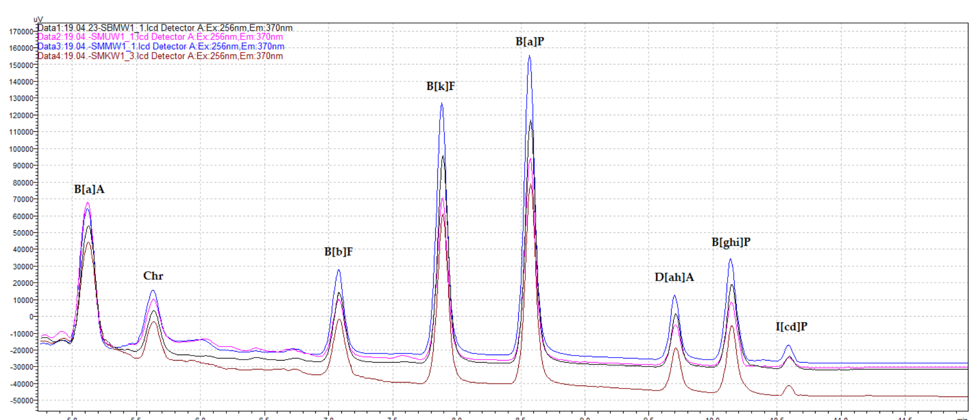

Figure 1. The HPLC–FLD chromatograms of heavy SCF PAHs of pork neck loin samples marinated in universal marinade (pink), honey–mustard marinade (blue), pork marinade (brown), and without marination (black), grilled on the charcoal grill without a tray.

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2.8. Confirmation of Results by GC/MS Method

The samples were also analyzed by gas chromatography with mass spectrometry (GC/MS) using an Agilent 7890A/ 5975C VL MSD Gas Chromatograph-Mass Spectrometer (Agilent Technologies Inc., Santa Clara, CA, USA) to confirm the results obtained by the HPLC-FLD/DAD method. This confirmation was performed using the deuterated PAH standard mixture mentioned before. The following conditions were utilized: Agilent J&W Select PAH GC Column, 30 m, 0.25 mm, 0.15 μm (Agilent Technologies Inc., Santa Clara, CA, USA), a helium carrier gas flow of 1.2 mL min−1, inlet temperature 300 °C, injection volume 1 μL with splitless injection mode; the MS Quad and MS source temperature: 200 and 300 °C, respectively. The temperature program was as follows: 40 °C (2 min), 40–180 °C (15 °C/min), 180 °C (1 min), 180–200 °C (2 °C/min), 200 °C (1 min), 200–320 °C (3 °C/min) and 320 °C (15 min). The ion abundance within the m/z range of 100–400 was measured using a detector voltage of 1.5 kV and electron ionization at 70 eV. Selected ion monitoring (SIM) mode was employed for each PAH, measuring the two most abundant and characteristic ions. Identification of PAHs was based on comparing particular analyte retention times in real samples with those of PAH standard solutions, along with the characteristic ions monitored for each compound. For tested samples of grilled pork neck loins, the data obtained from the GC/MS and HPLC–FLD/DAD methods demonstrated statistically insignificant differences, confirming the reliability of the applied analytical approach.

2.8. Statistical Analysis

Statistical analysis was conducted using Statistica v.10-PL (StatSoft, Inc., Tulsa, OK, USA). The normality of data distribution was assessed by comparing p-values, with the null hypothesis rejected at $p \leq 0.05$. To evaluate the significance of differences in the mean PAH contents across different samples' variants of grilled pork neck regarding the type of grill and marinades used, a two-way analysis of variance (ANOVA) and Tukey's test were applied at a significance level of $\alpha = 0.05$. Furthermore, the Pearson correlation was analyzed to examine relationships between weight loss, color components, and the concentrations of 15 SCF PAHs and all 19 PAHs.

3. Results and Discussion

3.1. Analysis of the Meat Weight Loss After the Grilling Process

The grilling process melts fat, evaporates water, or leaks cell juices from the raw material, thus reducing the weight after heat treatment [21]. The weight loss was within the 30.0 – 54.8% range (Table 3), depending on the grilling method and tool chosen and sample type. The highest cooking loss was found on the charcoal grill without a tray and for the cast iron electric contact grill (46.6 – 54.8%), and the lowest was found on the ceramic contact grill (30.0 – 43.4%). The Pearson analysis showed no correlation between weight loss and 15 heavy PAH contamination ($p = 0.119$). The lack of linear correlation may be because the weight loss of individual samples was influenced by factors such as different types of grills, temperature, and time of grilling samples. This greatly impacted the slope of the regression line and, thus, the correlation rate. Furthermore, comparing the results of the correlation of weight loss and the sum of 19 PAHs, only a weak positive correlation was shown ($p = 0.027$), which may result from the formation of a relatively large amount of light PAHs in the process of fat pyrolysis.

Table 3. Weight loss in various marinated and unmarinated grilled pork neck loins samples.

Universal Marinade		Pork Marinade		Honey Mustard Marinade		Without Marinade	
Sample Code	Weight Loss [%]	Sample Code	Weight Loss [%]	Sample Code	Weight Loss [%]	Sample Code	Weight Loss [%]
SMUW1	41.1±1.63^{c*}	SMKW1	54.7±0.57^{d}	SMMW1	45.4±0.32^{b}	SBMW1	50.3±0.23^{c}
SMUW2	30.0±0.00^{a}	SMKW2	32.0±0.50^{a}	SMMW2	43.4±0.61^{a}	SBMW2	34.5±0.62^{a}

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SMUE1	37.2±0.05[b]	SMKE1	38.0±0.12[b]	SMME1	52.0±0.08[c]	SBME1	45.9±0.09[b]
SMUE2	46.4±0.05[d]	SMKE2	43.4±0.13[c]	SMME2	53.5±0.15[d]	SBME2	54.8±0.00[d]

n = 6 (six samples of every kind of product were analyzed). * Different values in the same marination treatment followed by the same lowercase letter (a–d), meaning one analyzed comparison, are not significantly different at α=0.05 level. S – pork neck loins, MU – universal marinade, MK – pork marinade, MM – honey mustard marinade, BM – without marinade.

W1 – charcoal grill without a tray, W2 – charcoal grill with an aluminum tray, E1 – ceramic contact grill, E2 – cast iron contact grill.Grilling on a charcoal grill without a tray, with the highest grilling temperature, caused one of the greatest weight loss. The aluminum tray reduced the direct contact of the raw material with the heat source and significantly reduced the weight loss. The difference was also observed for electric grills, where a cast iron grill showed a much greater weight loss than a ceramic grill. The results agreed with the conclusion formulated by Purslow, Oiseth, Hughes, and Warner [22] that during cooking, water expellees from the meat, which causes its weight to decrease. The same researchers concluded that the higher the grilling temperature, the greater the weight loss. Latoch, Głuchowski, and Czarniecka–Skubina [23], Alugwu, Okonkwo, and Ngadi [24], and War Nur Zahidah et al. [25] also showed that with higher temperatures of cooking as well as longer cooking time weight loss was significantly greater.

3.2. Color Analysis

Table 4 depicts the effect of different marination treatments and grilling tools on the color of pork neck loins. The obtained results indicated that the color parameters of the samples were affected by the different marinades and cooking methods. By analyzing the parameter L*, it was noted that the sample marinated in honey mustard marinade grilled on a charcoal grill without a tray was significantly brighter (48.46), and the unmarinated sample was significantly darker (55.88) than the other samples. Compared to the electric grills on both cast iron and ceramic grills, significantly higher values of parameter L* were obtained for both samples: marinated in honey mustard marinade and unmarinated. For the a* color component, the highest redness value was shown in the unmarinated charcoal grill sample without a tray (12.21) and the charcoal grill with an aluminum tray in the honey mustard marinade sample (11.01). However, there were no statistically significant differences between the different types of marinade for electric grills. In the case of parameter b*, the value of the yellow component depends on the tested variant. The highest proportion of yellow color was noted in the case of pork neck grilled on charcoal grills marinated in universal marinade (SMUW1 – 14.01, SMUW2 – 14.45), while the lowest for samples without marinade prepared on electric grills (SBME2 – 11.76, SBME1 – 13.51).

Following comparing the color changes before and after grilling, the ΔE parameter was determined (Table 5). For some samples (SMKW1, SMKE1, SMME1, SMUE2), it was noted that the color differences before and after the grilling process were invisible to an inexperienced person. A slight color change in the universal and the pork marinade could be explained by the intense color of the spice and, in the case of the honey mustard marinade, by the lowest grilling temperature and the low pressure of the heating plates on the product in samples from ceramic contact grill.

Pearson correlation of color components and PAHs in pork neck loins was carried out. The analysis showed a weak, negative correlation between the L* parameter and 19 PAHs ($p = 0.008$) and a weak, positive correlation between a* and 15 PAHs ($p = 0.021$) and 19 PAHs ($p = 0.019$). There was no significant correlation between lightness and the sum of 15 PAHs and yellowness and the sum of 15 and 19 PAHs.

Based on the study results, marination treatments and grilling techniques significantly impact the color parameters of analyzed samples. Furthermore, Pearson's correlation proves that a lower proportion of redness (a*) corresponds to a higher PAH contamination of the samples. In contrast, lighter-colored samples exhibit lower light and heavy PAH content levels. The findings agreed with the studies conducted by other researchers. Assogba et al. [26] in their research proved that pork, after grilling, becomes darker due to such factors as the Maillard reaction, pyrolysis of fat,

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carbonization of proteins, caramelization of sugars, or denaturation of hemoglobin and myoglobin. Moreover, Cho et al. [27], in their studies on barbecued pork patties, confirmed that marination influenced the color parameters. Silva, Hamer, and Guénard [28] reported that the proportion of redness and yellowness increases due to the grilling process. In addition, the research conducted by Wang, Dong, Zhang, Yu, and Wang [29] confirms that high temperature influences the color change of heat-treated products.

Table 4. Color of marinated and unmarinated pork neck loins before and after grilling on different grills.

Cooking Method	Sample	L*	a*	b*
Before grilling	SMU	33.66±0.75^{a*A**}	10.52±0.92^{aB}	12.66±1.77^{bA}
	SMK	37.16±0.28^{aA}	10.99±0.83^{aB}	5.38±0.61^{abA}
	SMM	52.19±0.56^{bB}	11.66±1.55^{aA}	8.01±0.86^{bA}
	SBM	448.70±2.90^{bB}	13.16±1.05^{aC}	4.24±0.42^{aA}
Charcoal grill without a tray	SMUW1	38.38±2.43^{aA}	6.54±0.27^{aA}	14.01±1.09^{bB}
	SMKW1	39.18±4.29^{aA}	9.00±2.15^{aB}	7.46±0.77^{aA}
	SMMW1	48.46±1.67^{bAB}	9.25±0.87^{aA}	10.55±0.61^{abA}
	SBMW1	44.70±3.80^{aA}	12.21±2.16^{bBC}	7.91±3.04^{aAB}
Charcoal grill with an aluminum tray	SMUW2	40.89±2.11^{aA}	6.32±1.25^{aA}	14.45±1.00^{bB}
	SMKW2	46.42±1.22^{aB}	5.25±0.63^{aA}	12.38±1.25^{abB}
	SMMW2	44.30±3.96^{aA}	11.01±0.31^{bA}	10.45±0.26^{aA}
	SBMW2	55.88±2.20^{bC}	7.82±0.67^{aA}	11.31±1.32^{abBC}
Ceramic contact grill	SMUE1	40.59±4.92^{aA}	6.86±0.85^{aA}	8.59±1.22^{aA}
	SMKE1	40.26±1.40^{aAB}	8.03±1.00^{aAB}	7.52±1.73^{aA}
	SMME1	49.45±2.73^{bAB}	8.71±0.65^{aA}	9.84±0.76^{abA}
	SBME1	47.28±2.49^{bB}	8.91±0.94^{aA}	13.51±1.74^{bC}
Cast iron contact grill	SMUE2	35.02±1.26^{aA}	7.69±0.80^{aA}	10.82±0.92^{abAB}
	SMKE2	40.24±1.62^{aAB}	8.42±2.18^{aB}	7.71±1.59^{aA}
	SMME2	46.87±0.68^{bAB}	8.25±0.25^{aA}	9.39±0.33^{abA}
	SBME2	46.96±4.86^{bB}	9.85±1.89^{aAB}	11.76±3.40^{bC}

n = 6 (six samples of every kind of product were analyzed). * Different values in the same method of grilling followed by the same lowercase letter (a–d) are not significantly different at α=0.05 level. ** Different values in the same marination treatment followed by the same capital letter (A–D) are not significantly different at α=0.05 level.

Table 5. The difference in color of pork neck loins before and after grilling.

Charcoal Grill Without a Tray		Charcoal Grill With an Aluminum Tray		Ceramic Contact Grill		Cast Iron Contact Grill	
Sample	ΔE	Sample	ΔE	Sample	ΔE	Sample	ΔE
SMUW1	6.32±2.59^{a*A**}	SMUW2	8.55±2.66a^{bA}	SMUE1	14.96±4.45^{bB}	SMUE2	4.53±0.85^{aA}
SMKW1	3.52±1.22^{aA}	SMKW2	12.95±1.54^{bB}	SMKE1	4.64±1.74^{aA}	SMKE2	5.54±1.08^{aA}
SMMW1	5.12±1.81^{aA}	SMMW2	8.29±3.07^{aA}	SMME1	4.42±1.95^{aA}	SMME2	6.46±1.05^{aA}
SBMW1	14.51±2.36^{bA}	SBMW2	11.40±3.18^{bA}	SBME1	10.30±1.37^{bA}	SBME2	12.51±1.95^{bA}

* Different values in the same method of grilling followed by the same lowercase letter (a–b) are not significantly different al α=0.05 level. ** Different values in the same marination treatment followed by the same capital letters (A–B) are not significantly different at α=0.05 level.

3.3. Analysis of PAH Contamination in Grilled Pork Neck Loins

3.3.1. Effects of Various Grilling Tools on the Formation of B[a]P, 4 Heavy–Marker PAHs, and 15 PAHs

Figure 2 presents the PAH content in pork neck samples prepared using four grilling tools. Results include the sum of 19 analyzed PAHs, including 15 heavy PAHs, 4 heavy and marker SCF PAHs, and B[a]P. The study's findings proved the statistical differences in PAH content among various grilling tools analyzed. Significantly, the highest total contents of 15 PAHs, 4 marker-heavy PAHs, and B[a]P were noted in the pork neck grilled on a charcoal grill without a tray (22.97 – 34.56

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μg/kg, 13.10 – 19.62 μg/kg, 4.33 – 6.51, respectively). The use of an aluminum tray when charcoal grilling significantly reduced the PAHs contamination (11.06 – 18.59 μg/kg, 5.86 – 10.30 μg/kg, 2.06 – 3.57 μg/kg, respectively) by preventing direct contact with the flames with the raw meat. Melting fat from pork neck loins, characterized by a high fat content, reacted with fire to form PAHs, which, along with the smoke, were deposited on the surface of the meat. Studies have also shown that samples grilled on electric grills were significantly less contaminated in each variant than charcoal grills without a tray and in most variants from charcoal grills using an aluminum tray. Furthermore, it was proved that neck loins grilled on a ceramic electric grill had significantly lower levels of 15 PAHs and 4 heavy PAHs (5.89 – 10.69 μg/kg, 3.27 – 5.83 μg/kg, respectively) than in the samples prepared on an electric iron grill (7.70 – 12.69 μg/kg, 4.20 – 6.78 μg/kg, respectively). In addition, the B[a]P level for neck loins marinated in universal marinade also decreased significantly for ceramic contact grills. The differences observed for electric grills are mainly due to the heat treatment temperature and equipment design. The higher cooking temperature contributed to a greater accumulation of harmful compounds. In addition, in the case of a cast-iron grill, the lower heating surface was constructed of a corrugated heating plate, in which the fat accumulated in the grooves recesses and promotes a higher concentration of polyarenes. In the case of a ceramic grill, the bottom heating surface was flat, and the melting fat did not accumulate in the cavities; therefore, there was no greater accumulation of PAHs.

The obtained results of grilled neck loin samples contamination by PAHs showed that two of the analyzed samples grilled on a charcoal grill without a tray exceeded the limit for B[a]P (SMMW1: 6.51 μg/kg, SBMW1: 5.26 μg/kg), however did not exceed the limit for 4 heavy PAHs set in the Commission Regulation (EU) No. 915/2023 of 25 April 2023 for grilled meat products (respectively, 5 μg/kg and 30 μg/kg). With these results, it should be recalled that the sum of the 4 heavy-marker PAHs is considered to be a better indicator of the occurrence of PAHs in food than B[a]P alone.

There are many studies aimed at reducing PAH formation in food. One way constantly being researched is to change the grilling method to reduce PAH contamination. In research conducted by Lee et al. [30], it was observed that when grilling beef loin samples on a charcoal grill with the prevention of fat dripping into the heat source, the amount of harmful B[a]P was significantly lower (0.78 μg/kg) than when fat dripping onto a fire (3.23 μg/kg). In addition, thanks to the use of a modified grill, contamination with 4 heavy PAHs decreased by 85%. Moreover, the contamination of 4 PAHs was 48% lower for pork, which contained less fat. Therefore, this study also confirmed that avoiding direct contact with the fat at the high temperature of the heated flames reduces the formation of PAHs [30]. Fat does not drop into the fire, so PAH formation and deposition on food surfaces are limited. In addition, preparing meat at high temperatures or long cooking times also leads to greater formation of PAHs [31]. In the research conducted by Anjum, Shehzad, Rahat, and Khan [32], the type of grilling equipment used significantly affects the safety of grilled products. The study showed that charcoal-grilled meat was more contaminated with PAH than electric or gas-grilled meat. It was also confirmed that factors that can prevent the formation of harmful PAHs are lower cooking temperatures and the protection of food from direct contact with the heat source. Eldaly et al. [33], in a study on the effect of product wrapping in aluminum foil on the reduction of PAH formation, showed

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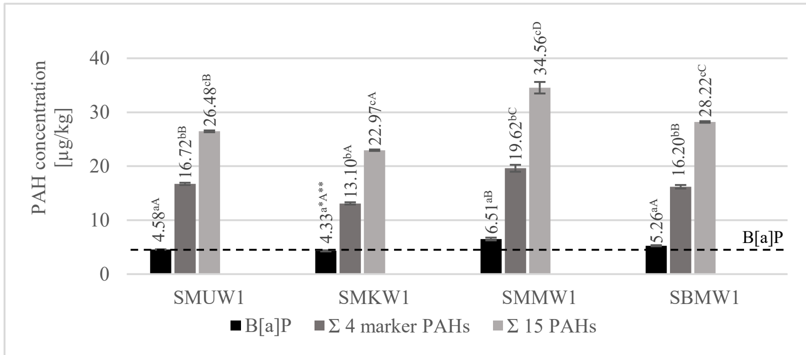

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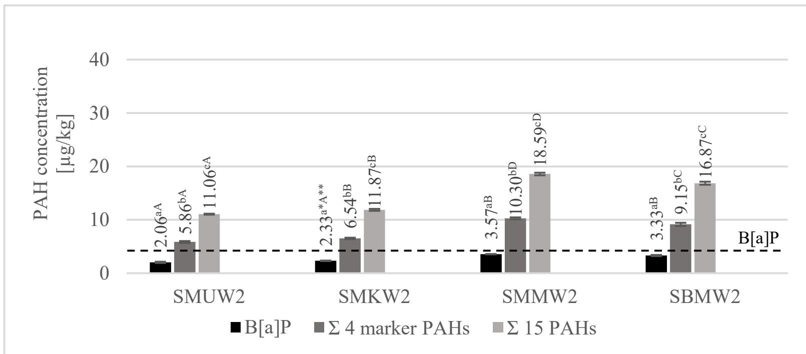

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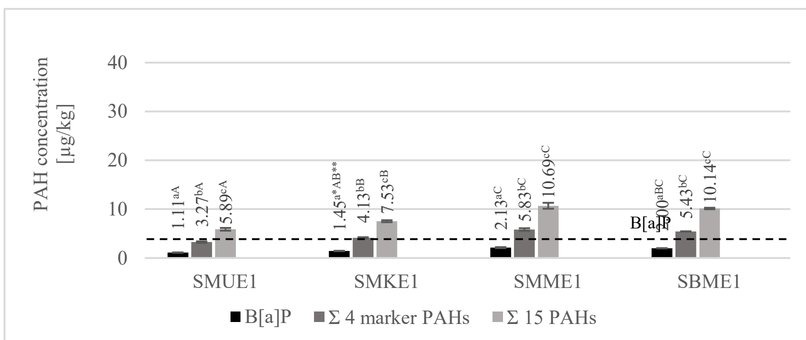

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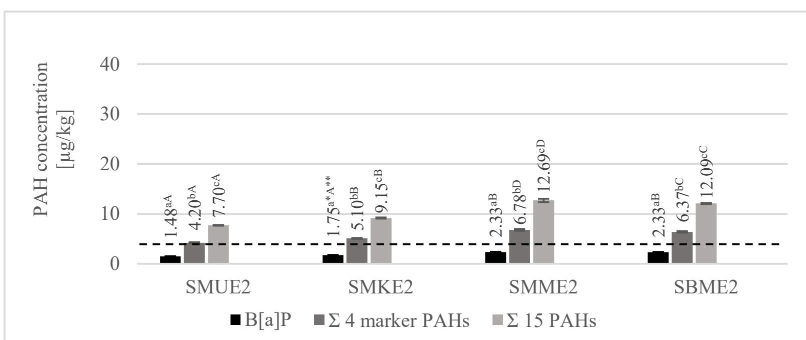

c.

Figure 2. Comparison of mean content of B[a]P, 4 marker–SCF PAHs, and total 15 PAHs for pork neck loin samples grilled on the charcoal grill without a tray (W1), charcoal grill with an aluminum tray (W2), ceramic contact grill (E1) and cast iron grill (E2) marinated in various marinades a) universal marinade (SMU), b) pork marinade (SMK), c) honey–mustard marinade (SMM), d) without marination (SBM). * Different values for various grills in the same method of grilling followed by different lowercase letters (a–c) are significantly different at α=0.05 level. ** Different values for various grilling in the same method of grilling followed by different capital letters (A–D) are significantly different at α=0.05 level that regardless of the meat type,

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aluminum foil creates a protective barrier between the product and the grill, as a result of which the PAH concentration is significantly lower (beef 1.27 μg/kg, mutton 6.28 μg/kg) compared to the control sample without wrapping (beef 6.83 μg/kg, mutton 26.82 μg/kg). Park, Pyo, Kim, and Yoon [16] found that for traditional grilling, increasing the distance of the product from the heat source and using a barrier to allow air circulation during grilling lower PAH contamination. Applying these changes reduced B[a]P concentration by more than 6 times.

3.3.2. Effect of Various Marinades on PAH Formation of B[a]P, 4 Heavy–Marker PAHs, and 15 PAHs

In preventing PAH contamination as a kind of green processing technology, three different marinades were used to reduce PAH levels in grilled pork neck loins. Figure 3 depicts the data of the mean PAH content, including B[a]P, the sum of 4 heavy–marker SCF PAHs and 15 SCF PAHs in pork neck loins treated with three different marinades and samples without marination.

The study on whether marination affects PAH contamination in analyzed products showed that marinades significantly reduce it but, in some cases, can even increase PAH levels in grilled pork on different grilling tools. For the charcoal grill without a tray, the contents of 15 PAHs varied for each of the samples, with significantly the lowest concentration stated in the pork marinade sample (6.31 μg/kg) and the highest noted in the honey mustard sample (8.84 μg/kg). The contents of 4 heavy PAHs and B[a]P were significantly lower in the universal and pork marinade than in the honey mustard marinade and samples without marination. On the charcoal grill with a tray, the levels for the sum of 15 PAHs were also significantly different for each of the variants, with the lowest content for pork in the universal marinade (3.31 μg/kg) and the highest for honey mustard marinade (5.29 μg/kg). Significantly, the lowest total concentration for 4 heavy-marker PAHs was observed in samples treated with universal marinade (1.53 μg/kg) but the highest with honey mustard marinade (2.8 μg/kg). Regarding the B[a]P content, significantly, the lowest level of content was stated for the universal marinade, whereas in the other marination treatments, it did not differ significantly. Considering electric grilling, for a ceramic grill with a lower grilling temperature, significantly the lowest values for the sum of 15 PAHs and 4 marker PAHs were observed in samples with the pork marinade (7.53 μg/kg, 4.13 μg/kg, respectively), and the highest in the honey mustard marinade (10.69 μg/kg, 5.83 μg/kg, respectively). In the case of the cast iron grill, statistically, the highest total contents of 15 PAHs and 4 heavy-marker PAHs were found for honey mustard marinade (12.69 μg/kg, 6.78 μg/kg, respectively), and samples without marination treatment (12.09 μg/kg, 6.37 μg/kg, respectively). Statistically, the lowest contamination levels were noted for pork marinade samples (9.15 μg/kg, 5.10 μg/kg, respectively) and universal marinade (7.70 μg/kg, 4.20 μg/kg). For B[a]P, there were no significant statistical differences between the analyzed samples.

The results above confirm that marinating products before their heat treatment can effectively reduce the formation of undesirable contaminants such as PAHs (4 PAHS: 24–29%, 15 PAHs: 31–32%. However, comparing the results to honey–mustard marinated products, it can be concluded that using certain ingredients in marinated products, such as honey, may contribute to increased pollution by PAHs (4 PAHS: 13%, 15 PAHs: 12% more so compared to unmarinated necks).

Other studies also confirm that marinating food before heat treatment can significantly inhibit PAH formation. Cordeiro et al. [34], when preparing pork on a wood grill, showed that marinating in black bean vinegar inhibited the formation of PAHs (82%), as did white wine vinegar (79%). Red wine and apple cider vinegar reduced the formation of PAHs by 66%, and the weakest inhibitory effect was shown for fruit vinegar with raspberry juice. Bulanda and Janoszka [35] tested pork tenderloins, which they stuffed with dried fruit and baked in a baking bag. The studies showed that dried fruits significantly reduced contamination with PAHs by 35 – 58%. Hussein, Edris, and Kirella [36] also noticed that marinating effectively reduced contamination by these carcinogenic compounds. Adding thyme oil in 0.5 – 1.5% significantly reduced the PAH amount in samples (39 – 74%). Similar results were obtained by Yu et al. [37] in duck wings baked over an open fire and marinated with the addition of coriander. Marination treatment reduced PAH formation from 65.0%

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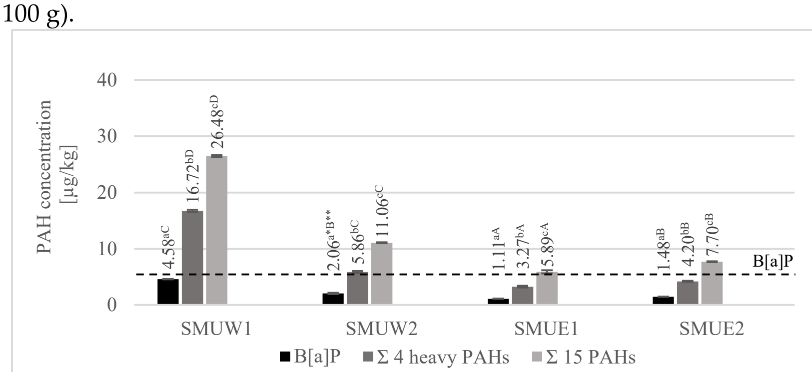
to 87.4%. Furthermore, Kim et al. [38], studying the influence of pork belly marination in Korean Gochujang paste, showed polyarene reduction by 63.06%.

The results obtained in this work have also shown that marination can even contribute to a greater accumulation of polycyclic aromatic hydrocarbons. Comparing the data obtained with the requirements established in Regulation (EU) No. 915/2023 of 25 April 2023 [18] for grilled meat products, it was observed that for two samples: unmarinated pork neck loins and marinated in honey mustard marinade grilled on a charcoal grill without a tray, the B[a]P content was exceeded (5.26 μg/kg, 6.51 μg/kg, respectively). Moreover, in this case, the B[a]P concentration increase due to marinating with this marinade was about 24% compared to the unmarinated pork neck loins.

Honey used for marinating is an ingredient susceptible to pollution by polyarenes. Bees taking long flights visit many areas contaminated by harmful elements from soil, air, and other environmental pollutants such as PAHs [21]. Kazazic, Djapo-Lavic, Mehic, and Jesenkovic-Habul [39] studied PAH contamination in honey from urbanized and non-urbanized areas. The level of total PAH concentration in honey taken from industrial areas was almost 5 times higher (12.58 μg/kg) than from hives located far from cities and factories (2.68 – 4.76 μg/kg). Significant differences in PAH contamination levels in honey depending on the region of origin are also concluded in the research by Surma, Sadowska-Rociek, and Draszanowska [40]. In addition, the harmful effect of honey on PAH formation is also influenced by the high temperature during the grilling process, which changes associated with the Maillard reaction cause the formation of PAHs. The studies on the harmful effects of honey added to marinades were also investigated by Nor Hasyimah et al. [41] by grilling marinated beef on fire. The studies reported that the samples previously marinated in honey-spices marinade and then prepared on the grill had a significantly higher concentration of PAHs (B[a]P 4.49 – 32.60 μg/kg; PAH8 78.47 – 164.40 μg/kg) than unmarinated grilled samples (B[a]P 2.67 – 4.60 μg/kg; PAH8 34.59 – 89.52 μg/kg). The researchers formulated the conclusion that samples marinated in honey from *Trigona* sp. bees had higher PAH contamination (total PAHs: 97.05 – 350.38 μg/kg) than the samples marinated with honey from honeybees *Apis mellifera* (total PAHs: 168.04 – 296.03 μg/kg). These differences were due to the content of simple carbohydrates, which, under the influence of high temperature, degrade to chloropropanols, which are precursors in PAH formation. There was significantly less reducing sugars in *Apis mellifera* honey (1.54 ± 0.04 g/100 g) than in *Trigona* bee honey (2.42 ± 0.11 g/ 100 g).



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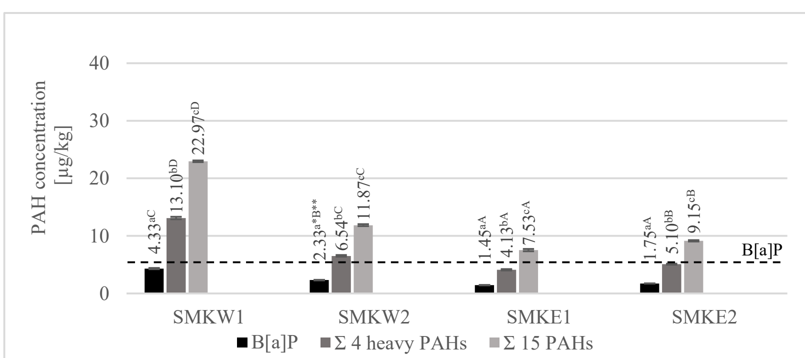

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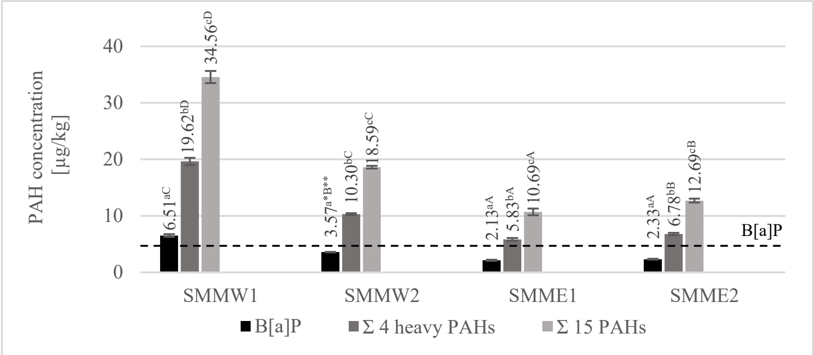

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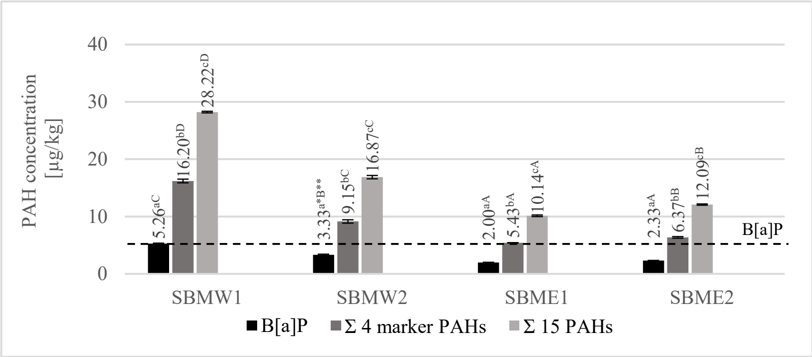

d.

Figure 3. Comparison of mean content of B[a]P, 4 marker–SCF PAHs, and total 15 PAHs for pork neck loin samples marinated in universal, pork, honey–mustard marinade and without marination (respectively SMU, SMK, SMM, and SBM) grilled on a) a charcoal grill without a tray (W1), b) a charcoal grill with an aluminum tray (W2), c) a ceramic contact grill (E1), d) a cast iron grill (E2). * Different values for various grills in the same method of marination treatment followed by different lowercase letters (a–c) are significantly different at α=0.05 level. ** Different values for various marination treatments in the same method of grilling followed by different capital letters (A–D) are significantly different at α=0.05 level. .

3.3.3. Analysis of PAH Qualitative Profiles of PAH Contamination

Pork neck samples' qualitative profiles of PAH contamination were analyzed with a percentage of 4 light and 15 heavy SCF PAHs (Figure 4, Table S1). For samples prepared on a charcoal grill without a tray, a higher level of light hydrocarbons (W1: 89 – 94%) was recorded than samples prepared on an aluminum tray (W2: 56 – 62%). The pyrolysis of the fats during the grilling of the samples was the main factor for which the values between these samples differed. The melting fat from pork neck loins and scavenging on the heated coals led to the formation of PAHs, which, together with the smoke, were lifted upwards and then deposited on the surface of the products. The aluminum tray effectively reduced flame-to-fat contact, resulting in a lower proportion of the formed light hydrocarbons. For electric grills (Figure 5, Table S2), the PAH contamination profiles did not reveal such a differentiation between the ceramic and cast iron grills (E1: 61 – 79%, E2: 68 – 76%). When analyzing the different variants of the samples on the grills applied, there were differences between light and heavy PAHs. The type of device, its design, the grilling parameters, and the previous preparation of the raw material are important factors affecting grilled meat products' contamination by PAHs [12].

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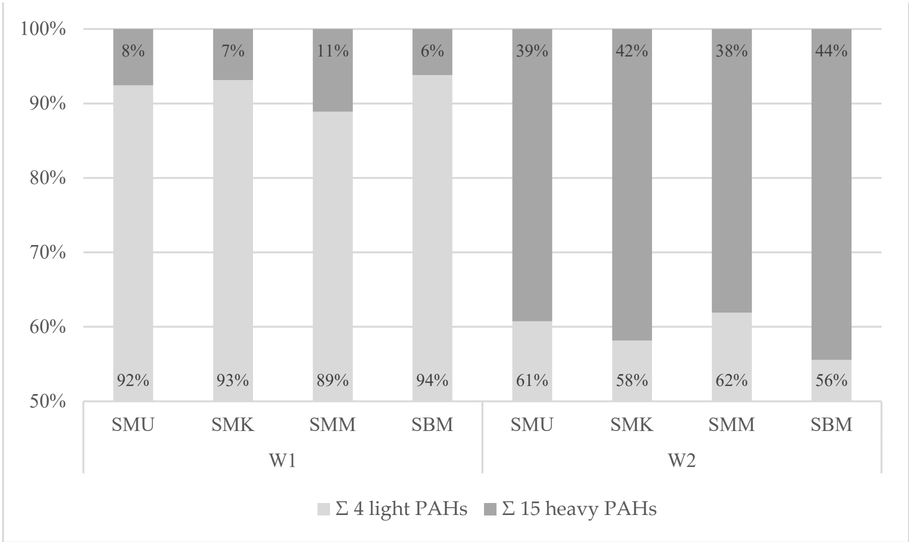

Figure 4. The qualitative profile of the 4 light and the 15 heavy PAHs in the 19 PAHs content in pork neck loins prepared on the charcoal grill without tray (W1) and the charcoal grill with an aluminum tray (W2).

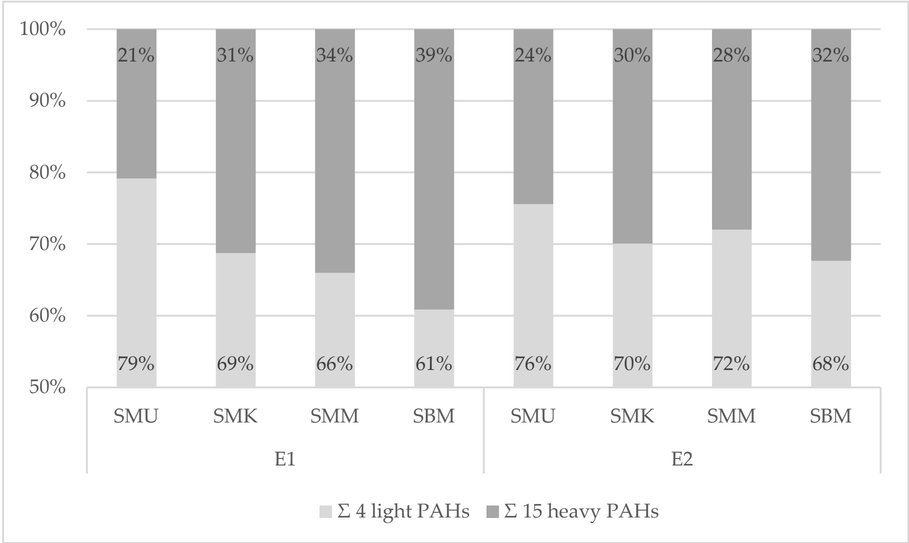

Figure 5. The qualitative profile of the 4 light and the 15 heavy PAHs in the 19 PAHs content in pork neck loins prepared on the electric ceramic contact grill (E1) and electric cast iron contact grill (E2).

4. Conclusions

Grilled meat product consumption can threaten human health and sometimes even exceed permitted daily intake norms. Subjecting pork neck loin to grilling on various grilling devices after their marinating proved that conscious and proper choice of marinades, e.g., universal or pork marinade, rich in phenolic compounds, as well as a selection of appropriate type of grilling device, e.g., electric grills instead of charcoal grills can significantly improve the food safety. Charcoal grills, through direct contact of the raw material with the heat source, cause the greatest levels of PAH contamination. An aluminum tray effectively reduces the amount of accumulated PAHs, as does using electric grills. In addition, marination also significantly affects the amount of PAHs. Therefore, key strategies for minimizing PAH concentrations in green processing technology involve marination before heat treatment. Marinades rich in phenolic compounds allow for the reduction of PAH amounts and ensure product safety. Nevertheless, the results obtained in this work pay attention to

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the ingredients used for marinades, as some can cause greater contamination by polyarenes. The honey used for marinades caused higher PAH concentrations and, in some samples, even exceeded the permitted B[a]P level. It is imperative to conduct further research investigating the impact of different marinades and grilling techniques and expand on the knowledge of reducing PAH contamination in meat-processed products to develop effective strategies for minimizing dietary intake of these heat-induced toxicants. These measures are necessary to increase food safety and safeguard public health.

Supplementary Materials: Table S1: Content of individual PAHs in pork neck loins in different marinades grilled on the charcoal grill without a tray (W1) and with an aluminum tray (W2); Table S2: Content of individual PAHs in pork neck loins in different marinades grilled on the electric ceramic contact grill (E1) and cast iron contact grill (E2).

Author Contributions: Conceptualization, M.C.; methodology, M.C., M.B., M.R.; software, M.C., M.B.; validation, M.C.; formal analysis, M.C.; investigation, M.C. and U.K.; data curation, M.C., M.B; writing—original draft preparation, M.C. and U.K.; writing—review and editing, M.C., U.K., M.R.; visualization, M.C. and U.K.; supervision, M.C.; project administration, M.C. All authors have read and agreed to the published version of the manuscript.

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