

GRILLED *VERSUS* FIRE-ROASTED SAUSAGE – THE CONTENT OF POLYCYCLIC AROMATIC HYDROCARBONS AND HEALTH SAFETY

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A b s t r a c t

The concentrations of polycyclic aromatic hydrocarbons (PAHs) were determined in grilled sausage and fire-roasted sausage, and the results were compared with statutory threshold levels. Sausages were grilled over charcoal and roasted over a campfire. Sausage was heated until the temperature in the geometric center of the sample reached 75°C. Nine PAH compounds were identified by high-performance liquid chromatography with fluorescence detection (HPLC-FLD) using an internal calibration standard. Thermal processing increased the content of PAHs in the analyzed samples, and significantly higher levels of contamination were noted in fire-roasted sausage than in grilled sausage. In grilled sausage, the content of BaP and Σ 4 PAHs (BaA, BaP, CHR, BbF) did not exceed the maximum permissible levels of 5 and 30 $\mu\text{g kg}^{-1}$, respectively, whereas in fire-roasted sausage, the concentrations of the above compounds exceeded maximum levels several fold and reached 18 and 73 $\mu\text{g kg}^{-1}$, respectively. The results of this study indicate that fire-roasted sausage poses a significant risk of exposure to PAHs.

KIELBASA GRILLOWANA *VERSUS* OPIEKANA W OGNIU – ZAWARTOŚĆ WIELOPIERŚCIENIOWYCH WĘGLOWODORÓW AROMATYCZNYCH A BEZPIECZEŃSTWO ZDROWOTNE

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A b s t r a k t

Celem pracy było określenie zanieczyszczenia związkami z grupy WWA grillowanej i opiekanej w ogniu kielbasy oraz ocena tego zanieczyszczenia w odniesieniu do obowiązujących regulacji prawnych. Obróbkę termiczną prowadzono na grillu węglowym oraz w ognisku. Surowce ogrzewano do uzyskania temperatury 75°C w centrum geometrycznym próbek. Oznaczenie 9 związków z grupy WWA przeprowadzono metodą wzorca wewnętrznego, techniką HPLC/FLD. Obróbka termiczna kielbasy skutkowałą zwiększeniem zawartości związków z grupy WWA w produktach, przy czym zanieczyszczenie kielbasy z ogniska było znacząco większe w porównaniu z produktami grillowanymi. W grillowanej kielbasie zawartość BaP i Σ 4 WWA (BaA, BaP, CHR, BbF) nie przekroczyła dopuszczalnych poziomów maksymalnych 5 i 30 $\mu\text{g kg}^{-1}$, natomiast w kielbasie opiekanej w ogniu zawartość tych związków wielokrotnie przekraczała dopuszczalne poziomy, osiągając wartości odpowiednio 18 i 73 $\mu\text{g kg}^{-1}$. Kielbasa opiekana w ogniu stanowi więc istotne źródło narażenia zdrowotnego na związki WWA.

Introduction

Processed meat products, including grilled meats, contain polycyclic aromatic hydrocarbons (PAHs), and their consumption could pose a health risk for humans. Polycyclic aromatic hydrocarbons with more than 4 aromatic rings are known as heavy polyarenes which are characterized by greater stability and higher toxicity than light polyarenes (JANOSZKA 2011, FARHADIAN et al. 2010). In vivo studies performed on laboratory animals revealed that PAHs exert genotoxic and mutagenic effects on somatic cells. The metabolic activation of PAHs to epoxide intermediates is responsible for the mutagenic and carcinogenic properties of those compounds. The resulting diol epoxides form covalent bonds with cellular macromolecules, including DNA, which could lead to DNA replication errors and mutations (BENFORD et al. 2010, BENIGNI and PASSERINI 2002, JANOSZKA 2011, JÄGERSTAD and SKOG 2005). The International Agency for Research on Cancer (IARC) has classified PAHs as substances probably carcinogenic for humans (group 2A) or possibly carcinogenic for humans (group 2B) (IARC 2010, JANOSZKA 2011). The maximum permissible levels of PAHs in selected foodstuffs, including smoked and grilled meat products, have been defined to guarantee consumer safety based on the frequency of consumption of different food products (Commission Regulation (EU) No 835/2011). Fire-roasted sausage is rarely sold in retail, and it is prepared individually by consumers in spring and summer.

In charcoal barbecues, food is heated by hot air with a temperature of 150–320°C (CIEMNIAK 2007, DYREMARK et al. 1995, ŠIMKO 2002). In grilled foods, smoke from incomplete fuel combustion and compounds produced during pyrolysis of organic substances are the main sources of contamination with PAHs (DYREMARK et al. 1995, EFSA 2008, FARHADIAN et al. 2011, JÄGERSTAD and SKOG 2005, REINIK et al. 2007). Sausages are also popularly roasted over a campfire. The wood burning process is relatively unstable, and wood is

thermally decomposed during open fire burning (with unlimited availability of atmospheric oxygen), thermolysis (under anaerobic conditions) or pyrolysis (with limited availability of atmospheric oxygen). At temperatures higher than 425–450°C during pyrolysis or 800°C during open fire burning, roasted products become highly contaminated with PAHs. The rate of combustion can be controlled only by modifying the availability of oxygen (DOLATOWSKI et al. 2014).

The aim of this study was to determine the concentrations of polycyclic aromatic hydrocarbons in grilled and fire-roasted medium-ground sausages, and to compare the results with the statutory maximum limits of PAHs.

Materials and Methods

Materials

The experiment was performed on cooked and smoked medium-ground sausages which were grilled on charcoal or roasted over a campfire. Sausages were purchased in a chain store based on the personnel's ranking of the most popular barbecue products in the summer season. The purchased products had the following composition (based on label data): sausage 1 (S_1) – pork (68%), water, beef (5%), potato starch, pork fat, salt, soybean protein, pork protein, spices and additives; sausage 2 (S_2) – pork (60%), water, salt, pork collagen, hydrolyzed corn protein, spices and additives. Sausage samples of approximately 100 g were grilled on charcoal and roasted over a campfire.

Thermal processing

Sausages were grilled on charcoal in the Outdoor 83521T barbecue. The cooking grid was suspended 0.2 m above the flames, and grid diameter was 0.5 m. Charcoal briquettes (3.5 kg) were used as fuel. The average grilling temperature was $247^\circ\text{C} \pm 16$. Fire-roasted sausages were prepared in pyramid-shape campfires when most wood had turned to charcoal. Campfire 1 (CF1) was built of beech wood, and campfire 2 (CF2) was made of beech and debarked birch wood. Sausages were roasted away from direct flame, at a distance where temperature did not exceed 350°C . Sausages were roasted until the temperature inside the geometric center of each sample reached 75°C , and the time required to achieve the above temperature was determined experimentally ($n = 9$). The average grilling time was 11 minutes, and the average fire-roasting time was 8 minutes. Temperature was measured with the Hanna

Instrument HI 92804 C thermometer and temperature probes (HI 766 B, HI 762 PWL). Samples in duplicate ($n = 2$) of S_1 and S_2 (raw material: sausages 1 and 2), GS1 and GS2 (grilled sausages 1 and 2), GST1 and GST2 (sausages 1 and 2 grilled on aluminum trays), CF11 and CF21 (sausage 1 roasted in campfire 1 and 2), CF12 and CF22 (sausage 2 roasted in campfire 1 and 2) were analyzed to determine the content of PAHs ($n = 3$).

Polycyclic aromatic hydrocarbons – analytical procedure

The following reagents and standards were used in the PAH analysis: acetonitrile (ACN), dichloromethane (DCM), chloroform, methanol (HPLC Labscan, Dublin, Ireland), fluoranthene, pyrene, benz[*a*]anthracene (BaA), chrysene (CHR), benzo[*b*]fluoranthene (BbF), benzo[*k*]fluoranthene (BkF), benzo[*a*]pyrene (BaP), dibenz[*a,h*]anthracene (DhA), benzo[*ghi*]perylene (BghiP) in acetonitrile – 10 ng μl^{-1} (Ehrenstorfer GmbH, Germany). The remaining reagents were of analytical grade.

The analyses were carried out based on the method described by WĘGRZYN et al. (2005) where the lipid fraction was extracted and PAHs were separated by preparative size-exclusion chromatography (SEC). Quantitative and qualitative analyses of PAHs were performed by reversed-phase high-performance liquid chromatography with fluorescence detection (HPLC-FLD; stationary phase – non-polar, mobile phase – water, acetonitrile).

Homogenized samples of 1 g were placed in glass test tubes, and the internal standard and 1.5 ml of methanol were added. Samples were shaken in a vortex mixer for 1 min, and they were combined with 3 ml of chloroform and 1.5 ml of water. The mixture was shaken for 3 min and centrifuged for 10 min at 10,000 rpm. The chloroform solution containing the PAH lipid fraction was passed through grade 4 Whatman filter paper into a 10 ml test tube. The samples were extracted with 3 ml of chloroform, shaken in a vortex mixer for 3 min and centrifuged for 10 min at 10,000 rpm. The chloroform fraction was passed through filter paper. Chloroform fractions were combined and evaporated to dryness under nitrogen in a water bath (40°C). The residue left after evaporation was dissolved in 4 ml of dichloromethane. The extracts were purified by SEC with the mobile phase (DCM) at a flow rate of 1 ml min^{-1} . Measurements in the UV detector were performed at $\lambda = 254$ nm. Dichloromethane extracts of 400 μl were purified. Eluent was directed to the fraction collector and it was evaporated until dryness under nitrogen in a water bath (40°C). Dry residues were dissolved in 200 μl of ACN. The samples were applied onto a chromatographic column in the HPLC-FLD system. Chromatographic separation was performed with the water/acetonitrile mobile

phase in the gradient elution mode. Samples were applied to columns with 50:50 water : acetonitrile. Acetonitrile concentration was increased to 100% within 20 min, it was maintained for 15 min and returned to the initial level. The mobile phase had a flow rate of 0.8 ml min⁻¹ and temperature of 25°C. The volume of the samples applied to the column was 20 µl. The column was heated at 25°C in a thermostat. The fluorescence detector was calibrated based on the retention times of benzo[*b*]chrysene and benzo[*a*]pyrene (KUBIAK et al. 2011, WĘGRZYN et al. 2005). The applied analytical procedure fulfilled the requirements of Commission Regulation (EC) No. 333/2007 of 28 March 2007. The limit of detection (LOD, µg kg⁻¹), limit of quantification (LOQ, µg kg⁻¹) and recovery [%] were determined at: 0.2/0.4/102 for fluoranthene, 0.2/0.4/96 for pyrene, 0.2/0.4/101 for BaA, 0.1/0.3/78 for CHR, 0.1/0.3/98 for BbF, 0.1/0.2/104 for BkF, 0.1/0.3/90 for DhA, 0.1/0.3/76 for BghiP, and 0.1/0.2/102 for BaP, respectively. The concentrations of PAHs in untreated sausages (raw material) and heat-treated sausages (final products) were used in a comparative analysis.

The results were expressed by means ± standard deviation, which were calculated in a spreadsheet (Excel). The significance of differences between mean PAH concentrations was determined by one-way ANOVA at $p \leq 0.05$.

Results and Discussion

The concentrations of the analyzed PAHs in untreated and thermally processed sausages are presented in Table 1 for sausage 1 and Table 2 for sausage 2. As expected, untreated sausage (cooked and smoked) was contaminated with PAHs from the smoking process. The concentrations of PAHs in sausage 1 (S_1) and sausage 2 (S_2) were comparable at 28.9 and 28.13 µg kg⁻¹, respectively. The quality of the examined sausages, relative to the PAH content of smoked meat products, evaluated based on the content of BaP and Σ 4 PAHs (BaA, BaP, CHR, BbF), fulfilled the requirements of Commission Regulation (EU) No. 835/2011 of 19 August 2011. The concentrations of four PAHs in the analyzed sausages did not exceed 12 µg kg⁻¹, whereas BaP content was below the method detection limit at 0.2 µg kg⁻¹.

The contamination levels of smoked sausage determined in this study are consistent with the results reported by other authors. KUBIAK et al. (2011) analyzed 466 samples of smoked meat products and observed that BaP concentrations in sausages characterized by various degrees of grinding did not exceed the maximum level of 5 µg kg⁻¹. In a study by CIECIERSKA et al. (2011), the average content of BaP in the outer and inner layers of smoked medium-ground sausages was determined at 0.89 and 0.32 µg kg⁻¹, respective-

ly. Total contamination, expressed by the concentrations of 15 PAHs which are classified as potentially carcinogenic by the Scientific Committee on Food of the European Food Safety Authority (SCF-EFSA), was determined at $38.27 \mu\text{g kg}^{-1}$ in outer layers. The concentrations of 4 PAHs (BaA, BaP, CHR, BbF) in the analyzed samples of smoked medium-ground sausage exceeded the maximum level of $12 \mu\text{g kg}^{-1}$ only in outer layers ($17.49 \mu\text{g kg}^{-1}$), whereas significantly lower levels ($2.84 \mu\text{g kg}^{-1}$) were found in inner layers (CIECIERSKA et al. 2011). According to the cited authors, the observed variations in the PAH content of smoked meat products can be attributed mainly to differences in the applied smoking methods.

Table 1

The content of PAHs in raw material and the final product – sausage 1

PAHs	Experimental material									
	S ₁		GS1		GST1		CF11		CF21	
	$\mu\text{g kg}^{-1}$	SD	$\mu\text{g kg}^{-1}$	SD	$\mu\text{g kg}^{-1}$	SD	$\mu\text{g kg}^{-1}$	SD	$\mu\text{g kg}^{-1}$	SD
Fluoranthene	12.5 ^A	0.29	14.19 ^A	1.32	10.73 ^B	0.44	58.22 ^C	2.35	36.77 ^D	2.42
Pyrene	15.07 ^A	0.39	17.26 ^A	1.77	13.14 ^B	0.38	68.56 ^C	1.78	40.35 ^D	2.09
Benz[<i>a</i>]anthracene	0.81 ^A	0.07	1.66 ^B	0.09	0.84 ^A	0.03	19.04 ^C	0.77	12.03 ^D	0.17
Chrysene	0.52 ^A	0.03	1.46 ^B	0.13	0.68 ^C	0.02	16.35 ^D	0.36	10.49 ^E	0.26
Benzo[<i>b</i>]fluoranthene	blm	–	1.49 ^A	0.18	0.7 ^B	0.02	19.36 ^C	0.09	15.71 ^D	0.12
Benzo[<i>k</i>]fluoranthene	blm	–	0.57 ^A	0.02	0.31 ^B	0.01	7.29 ^C	0.08	5.11 ^D	0.05
Benzo[<i>a</i>]pyrene	blm	–	1.51^A	0.07	0.99^B	0.02	18.72^C	0.9	12.6^D	0.13
Dibenz[<i>a,h</i>]anthracene	blm	–	blm	–	blm	–	0.81 ^A	0.04	0.63 ^B	0.04
Benzo[<i>g,h,i</i>]perylene	blm	–	1.00 ^A	0.11	0.62 ^B	0.02	11.69 ^C	0.27	8.31 ^D	0.46
Σ 4 PAHs	1.33^A	0.1	6.12^B	0.47	3.21^C	0.09	73.47^D	2.12	50.83^E	0.68
Σ PAHs	28.9^A	0.78	39.14^B	3.69	28.01^A	0.94	220.04^C	6.64	142^D	5.74

Explanations: S₁ – sausage 1, GS1 – grilled sausage 1, GST1 – sausage 1 grilled on aluminum trays, CF11 – sausage 1 roasted in campfire 1, CF21 – sausage 1 roasted in campfire 2;

Σ PAHs – the sum of fluoranthene, pyrene, benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, dibenz[*a,h*]anthracene, benzo[*g,h,i*]perylene;

Σ 4 PAHs – the sum of benz[*a*]anthracene, benzo[*a*]pyrene, chrysene and benzo[*b*]fluoranthene;

A, B... – means in rows denoted by different superscript letters differ significantly at $p \leq 0.05$;

SD – standard deviation ($n = 6$);

blm – below the quantification limit of the method.

The contamination of sausage 1 (S₁) increased after thermal processing and was determined at $39.14 \mu\text{g kg}^{-1}$ in grilled sausage (GS1), $220.04 \mu\text{g kg}^{-1}$ in sausage roasted in campfire 1 (CF11) and $142 \mu\text{g kg}^{-1}$ in sausage roasted in campfire 2 (CF12).

Grilling increased the concentrations of the analyzed PAHs by more than 35% relative to initial levels, but the content of BaP and Σ 4 PAHs did not

exceed the maximum levels for grilled meat products which are set at 5 and 30 $\mu\text{g kg}^{-1}$, respectively. Sausages grilled on aluminum trays were characterized by practically unchanged total PAH content and higher levels of BaA, BaP, CHR and BbF, but the concentrations of those 4 PAHs did not exceed maximum levels.

The contamination of grilled products with PAHs is determined by various factors, including the chemical composition of processed material, time and temperature of grilling, and the applied grilling method (CIEMNIAK 2007, WIEK et al. 2013). Regardless of the processed material and grilling method, the content of PAHs generally does not exceed 5 $\mu\text{g kg}^{-1}$ in sausage. In a study by LARSSON et al. (1983), BaP concentrations did not exceed 0.2–0.3 $\mu\text{g kg}^{-1}$ in Frankfurter sausages cooked on an electric grill, and they were below 1 $\mu\text{g kg}^{-1}$ in sausages grilled over charcoal. In this study, the BaP content of grilled sausage was determined at 1.51 $\mu\text{g kg}^{-1}$ for sausage 1 and 1.58 $\mu\text{g kg}^{-1}$ for sausage 2. Similar values were reported by MOTTIER et al. (2000) in whose study, BaP concentrations in lamb sausage reached 0.32–2.81 $\mu\text{g kg}^{-1}$. In the grilling process, not all fuel components are completely combusted during pyrolysis, and volatile products, including PAHs, are deposited on grilled food (DYREMARK et al. 1995, WIEK et al. 2013). According to researchers, the contamination of grilled foods with PAHs can be reduced through the use of physical barriers such as aluminum trays (CIEMNIAK 2007, TKACZ et al. 2012). TKACZ et al. (2012) reported that aluminum trays can reduce PAH contamination by more than 70% and BaP levels by up to 80%.

A dramatic increase in PAH levels was noted in fire-roasted sausage 1. Contamination levels increased nearly 8-fold in sausage roasted in campfire 1 and nearly 5-fold in sausage roasted in campfire 2. The BaP content of the above samples was determined at 18.72 and 12.6 $\mu\text{g kg}^{-1}$, respectively, and the content of Σ 4 PAHs – at 73.47 and 50.83 $\mu\text{g kg}^{-1}$, respectively. Similar changes were observed in sausage 2 (S_2) after fire roasting (Table 2). Thermal processing increased the concentrations of PAHs in the analyzed products, and the contamination levels noted in fire-roasted sausage were significantly higher than in grilled sausage.

The maximum levels of PAHs set by Commission Regulation (EC) No. 835/2011 of 19 August 2011 apply only to meat and meat products that have undergone a heat treatment process known to potentially result in the formation of PAHs, i.e. only grilling and barbecuing. The concentrations of BaP and Σ 4 PAHs, which are particularly hazardous for consumer health, in fire-roasted sausage significantly exceeded the maximum levels for grilled products.

There are scant published data on the contamination of fire-roasted sausage with PAHs. Most studies focus on the health risks associated with the

Table 2

The content of PAHs in raw material and the final product – sausage 2

PAHs	Experimental material									
	S ₂		GS1		GST1		CF11		CF21	
	µg kg ⁻¹	SD	µg kg ⁻¹	SD	µg kg ⁻¹	SD	µg kg ⁻¹	SD	µg kg ⁻¹	SD
Fluoranthene	12.21 ^A	0.21	15.51 ^B	0.94	11.17 ^C	0.31	55.88 ^D	1.66	39.19 ^E	1.72
Pyrene	14.69 ^A	0.27	19.03 ^B	1.18	13.78 ^A	0.27	66.78 ^C	1.26	42.44 ^D	1.48
Benz[<i>a</i>]anthracene	0.74 ^A	0.05	1.75 ^B	0.06	0.87 ^A	0.02	18.27 ^C	0.56	12.20 ^D	0.12
Chrysene	0.49 ^A	0.02	1.59 ^B	0.09	0.67 ^C	0.01	16.00 ^D	0.25	10.75 ^E	0.18
Benzo[<i>b</i>]fluoranthene	blm	–	1.67 ^A	0.12	0.68 ^B	0.01	19.45 ^C	0.06	15.83 ^D	0.09
Benzo[<i>k</i>]fluoranthene	blm	–	0.60 ^A	0.01	0.32 ^B	0.01	7.21 ^C	0.06	5.16 ^D	0.03
Benzo[<i>a</i>]pyrene	blm	–	1.58^A	0.05	0.98^B	0.01	17.81^C	0.64	12.73^D	0.09
Dibenz[<i>a,h</i>]anthracene	blm	–	blm	–	blm	–	0.85 ^A	0.03	0.67 ^B	0.02
Benzo[<i>g,h,i</i>]perylene	blm	–	1.10 ^A	0.07	0.63 ^B	0.01	11.96 ^C	0.19	7.86 ^D	0.32
Σ 4 PAHs	1.23^A	0.07	6.59^B	0.32	3.2^C	0.05	71.53^D	1.51	51.51^E	0.48
Σ PAHs	28.13^A	0.55	42.83^B	2.52	29.1^A	0.65	214.21^C	4.71	146.83^D	4.05

Explanations: S₂ – sausage 2, GS2 – grilled sausage 2, GST2 – sausage 2 grilled on aluminum trays, CF12 – sausage 2 roasted in campfire 1, CF22 – sausage 2 roasted in campfire 2;

Σ PAHs – the sum of fluoranthene, pyrene, benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, dibenz[*a,h*]anthracene, benzo[*g,h,i*]perylene;

Σ 4 PAHs – the sum of benz[*a*]anthracene, benzo[*a*]pyrene, chrysene and benzo[*b*]fluoranthene;

A, B... – means in rows denoted by different superscript letters differ significantly at $p \leq 0.05$;

SD – standard deviation ($n = 6$);

blm – below the quantification limit of the method.

consumption of smoked and grilled meat products which are generally characterized by high levels of PAHs. In this study, fire-roasted sausage was significantly more contaminated with PAHs than grilled sausage. Similar results were reported by LARSSON et al. (1983) who observed that roasting over an open fire can dramatically increase the BaP content of sausage to more than 50 µg kg⁻¹. In our study, BaP concentrations in fire-roasted sausage did not exceed 20 µg kg⁻¹, probably because the analyzed samples were roasted farther away from the flames. In meat products roasted over an open fire, high contamination with PAHs can be attributed to the high temperature of the wood burning process, which increases from an ignition temperature of 300–350°C to 800°C or even 1100°C (DOLATOWSKI et al. 2014).

The presence of dibenz[*a,h*]anthracene was also noted in fire-roasted sausage. This compound considered to be five times more toxic than BaP – the toxicity equivalency factor (TEF) is 1 for BaP and 5 for DhA. Dibenz[*a,h*]anthracene was not found in grilled samples.

According to the SCF-EFSA, the content of 15 PAHs in processed food should be monitored to minimize potential health hazards for consumers. The

above list does not include fluoranthene and pyrene which have been placed on the list of 16 PAHs classified as priority pollutants by the US Environmental Protection Agency (US EPA 1984). In the present study, the percentage of fluoranthene and pyrene in the total PAH content of grilled sausage was high in the range of 54–95%, but samples with a higher total PAH content were characterized by lower concentrations of fluoranthene and pyrene.

Conclusions

The following conclusions were formulated based on the results of this study:

- fire-roasted sausage is a significant source of potentially toxic PAHs;
- the PAH content of grilled sausage did not exceed the statutory maximum limits for meat products;
- the use of aluminum trays contributed to a significant decrease in the contamination of grilled sausage with PAHs;
- the contamination of fire-roasted sausage with PAHs varied significantly depending on the type of wood;
- the maximum permissible levels have not been set for contaminants in fire-roasted sausage, which is prepared individually by consumers; therefore, the potential health hazards associated with the consumption of fire-roasted sausage should be communicated to the public in scientific and popular science literature.

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