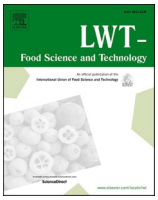
LWT - Food Science and Technology 184 (2023) 114971

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt



Formation of pyrolysis-affected PAHs, oxygenated PAHs and MCPDs in home smoked meat

Lisa Zastrow [a,1], Christopher Albert [a,1], Karl Speer [b], Karl-Heinz Schwind [a], Wolfgang Jira [a,*]

[a] Department of Safety and Quality of Meat, Max Rubner-Institut (MRI), E.-C.-Baumann-Straße 20, 95326, Kulmbach, Germany
[b] Faculty of Chemistry and Food Chemistry, Technical University of Dresden, Bergstraße 66, 01069, Dresden, Germany

ARTICLE INFO

Keywords:
OPAHs
PAHs
3-MCPD
Home smoking
Offset smoker
Kettle grill

ABSTRACT

Traditional American home smoking of meat is becoming more and more popular also in Europe due to the positive characteristics of home smoked meat such as flavor, taste and texture. However, the long and intensive smoke exposure leads to the formation of pyrolysis-affected contaminants. Here, the contents of eight oxygenated polycyclic aromatic hydrocarbons (OPAHs), six PAHs, and free 3- and 2-MCPD were investigated in home smoked meat. The meat was prepared under seven different conditions (four replicates, each) defined by smoking device (offset smoker or kettle grill), heating material (beech wood logs or charcoal), smoking material (logs or chips) and sample pretreatment (unsalted and salted). The highest median contents were observed for salted meat prepared on an offset smoker using logs (OPAH4: 31 μg/kg; PAH4: 68 μg/kg; 3-MCPD: 98 μg/kg; 2-MCPD: 7 μg/kg), exceeding the PAH4 EU maximum level for barbecued meat of 30 μg/kg. Salting of meat before smoking had a great effect on the 3- and 2-MCPD content, but not on the OPAHs and PAHs. 3- and 2-MCPD noticeably penetrated the smoked product in contrast to the PAHs and OPAHs. An approximate prediction of the OPAH4 content on the basis of the PAH4 content is possible.

1. Introduction

Smoking of meat and meat products has a long tradition. Besides industrial smoking, home smoking has become more and more popular in recent years (Jaffe, Wang, & Chambers, 2017) particularly due to the special flavor of the final product (Swaney-Stueve et al., 2019). Home smoking also includes a slow cooking process at low temperatures up to 140 °C. The pyrolysis of wood during smoking results in the formation of substances contributing to the typical aroma and color of the smoked products. These compounds particularly include phenols, heterocycles, and short-chain carboxylic acids (Kjallstrand & Petersson, 2001; Simon, de la Calle, Palme, Meier, & Anklam, 2005). However, in addition to these quality-enhancing compounds, undesirable by-products are formed by incomplete combustion. This is especially important when meat is smoked intensively and for a long time, since this usually takes place during home smoking using various recipes (e.g. for pulled pork).

Polycyclic aromatic hydrocarbons (PAHs) are among the best-researched heat-induced contaminants. It is well known that some PAH representatives, such as benzo[a]pyrene (BaP), have carcinogenic properties (Zhang, Chen, & Zhang, 2021). BaP as well as benzo[a]anthracene (BaA), benzo[b]fluoranthene (BbF), and chrysene (CHR) representing several hundred PAH substances, were summarized as PAH4 (European Food Safety Authority, 2008). Currently, the maximum levels for BaP and PAH4 in smoked meat and meat products are 2 and 12 μg/kg and in barbecued meat and meat products 5 and 30 μg/kg, respectively (European Commission, 2011). However, in a very limited number of studies also individual oxygenated PAHs (OPAHs) were detected in traditionally smoked meat (Chen et al., 2014) and industrially smoked sausages (Zastrow, Schwind, Schwägele, & Speer, 2019). These substances have a mutagenic and genotoxic potential, as they can directly attack the DNA and other macromolecules (Bolton, Trush, Penning, Dryhurst, & Monks, 2000; Lundstedt et al., 2007; Yu, 2002) and are generally thought to be more toxic than PAHs (Clergé, Le Goff, Lopez, Ledauphin, & Delépée, 2019; Ma & Wu, 2022). Nevertheless, no OPAH maximum levels in food have been established in Commission Regulation (EC) No 1881/2006 so far.

Furthermore, the formation of unesterified 3- and 2-monochloropropanediol (3-MCPD and 2-MCPD) during smoking as well as its presence in smoked products was reported in a limited number of studies (Kuntzer & Weisshaar, 2006; Ostermeyer, Merkle, Karl, & Fritsche, 2021). Here,

* Corresponding author.
E-mail address: wolfgang.jira@mri.bund.de (W. Jira).
[1] The respective authors contributed equally to the preparation of this manuscript.

https://doi.org/10.1016/j.lwt.2023.114971
Received 29 March 2023; Received in revised form 7 June 2023; Accepted 9 June 2023
Available online 10 June 2023

the formation mechanism is proposed to be based on the pyrolysis of wood (Kuntzer & Weisshaar, 2006) and differs from the well known mechanism (Collier, Cromie, & Davies, 1991; Velisek, Calta, Crews, Hasnip, & Dolezal, 2003) observed for the heating of fat-containing food in the presence of sodium chloride. The International Agency for Research on Cancer (IARC, 2012) classified unesterified 3-MCPD as a possible human carcinogen (group 2B) and a tolerable daily intake (TDI) of 2 μg/kg body weight was established by the EFSA (European Food Safety Authority, 2018). 2-MCPD has not yet been classified with regard to carcinogenicity due to insufficient toxicological data (European Food Safety Authority, 2016).

To the best of our knowledge, no studies have been conducted to date that investigated the formation of all mentioned heat-induced contaminants during home smoking of meat or meat products. So far, only several studies were performed investigating the factors influencing PAH formation during industrial smoking such as the choice of the smoke generator (Pöhlmann, Hitzel, Schwägele, Speer, & Jira, 2013a) and the smoke generation temperature (Pöhlmann, Hitzel, Schwägele, Speer, & Jira, 2012). The aim of the present study was to investigate the formation as well as possible correlations between PAHs and OPAHs as well as 3- and 2-MCPD during home smoking of meat, applying various common smoking conditions using a kettle grill and an outdoor offset smoker. In addition, the penetration depth of these three groups of processing contaminants and the influence of dry marinade on their formation were investigated. Therefore, the contents of eight OPAHs and six PAHs (including PAH4) were determined simultaneously applying a previously published GC-HRMS method (Zastrow, Speer, Schwind, & Jira, 2021) and the contents of 3- and 2-MCPD were measured by a modified GC-HRMS method (Schallschmidt et al., 2012).

2. Materials and methods

2.1. Reagents and materials

Acetone, acetonitrile (ACN), cyclohexane, ethyl acetate (EA), n-hexane, and 2,2,4-trimethylpentane (isooctane) were obtained from LGC Standards (picograde; Wesel, Germany). Diethyl ether was supplied by VWR (Ph. Eur; Leuven, Belgium). Methanol was purchased from Merck (Ph Eur; Darmstadt, Germany). N-dodecane (anhydrous, ≥99%), phenylboronic acid (≥97%), poly(acrylic acid), partial sodium salt-graft-poly(ethylene oxide), cross-linked, 90−850 μm, sea sand (50−70 mesh), and the Supelclean tubes EZ-POP NP (2.5 g, 12 mL) were obtained from Sigma Aldrich (Taufkirchen, Germany). Anhydrous sodium sulfate was purchased from T.H. Geyer (p.a.; Renningen, Germany). Water was received by a Milli Q water purification system from Merck (Darmstadt, Germany). The native OPAHs anthracene-9,10-dione (ATQ), benzo[a]anthracene-7,12-dione (BaAQ), 11H-benzo[b]fluorene-11-one (BbFLO), 6H-benzo[cd]pyren-6-one (BcdPO), 7H-benzo [de]anthracene-7-one (BZA), 9,10-dihydro-8H-benzo[a]pyren-7-one (BaPO), fluoren-9-one (9FLO), and naphthacene-5,12-dione (NAPHQ) were purchased from Chiron AS (Trondheim, Norway). Fluorene (FLU) was obtained from Sigma Aldrich (Munich, Germany). The PAH4, anthracene (ANT), and the deuterated ANT-d10 were purchased from Restek (Bad Homburg, Germany). The deuterated compounds ATQ-d8 and FLU-d10 were from Chiron AS (Trondheim, Norway) and the deuterated PAH4 (BaA-d12, CHR-d12, BbF-d12, and BaP-d12) were from CDN Isotopes (Augsburg, Germany). The deuterated 2-MCPD-d5 and 3-MCPD-d5 were obtained from Toronto Research Chemicals (Toronto, Canada). For the smoking setups, a kettle grill 'Master-Touch GBS E−5750' from Weber (Ingelheim, Germany) and an offset smoker 'Taino Yuma' from Clic-Trade (Köln, Germany) were used. The wood chips and logs of beech wood were obtained from J. Rettenmaier & Söhne (Rosenberg, Germany). The logs were cut into pieces of 4 cm × 4 cm x 30 cm. The charcoal briquettes were from proFagus (Bodenfeld, Germany), the barbecue lighting cubes from Boomex (Essen, Germany), the lighting chimney from Weber (Ingelheim, Germany), and the smoking box from Westline Angelgeräte (Waldsolms, Germany).

2.2. Home smoked meat samples

In seven experimental setups that were defined by combinations of smoking device, heating medium, smoking material, and sample pretreatment, smoked samples were prepared from pork with four replicates each (in total n = 28; Table 1). For each replicate, a piece of pork neck was cut to sizes (length/width/height/average weight: 27 cm/13 cm/8 cm/2.4 kg). Only the meat for setup Offset1 was rubbed with 100 g of a spice mixture of 43% cane sugar, 32% salt, 11% paprika, 6% garlic, 6% onion and 2% pepper the day before home smoking.

During the smoking process, the core temperature of the meat and the temperature inside the offset smoker or kettle grill were recorded. The ventilation flaps and slots of the devices were constantly adjusted to obtain a chamber temperature between 120 and 140 °C. The meat was smoked until a core temperature of 75 °C was reached. The trials were performed outdoors in a covered and wind-protected location to keep the environmental influences as low as possible. All replicates were performed on different days.

The Offset1+2 setups were performed in parallel with an offset smoker fired with logs. For this purpose, 15 logs were ignited in the firebox of the smoker with seven pieces of grill lighter. After 10 min, the flap of the firebox was closed, and the ventilation flaps slightly opened. After another 10 min, one seasoned and one unseasoned piece of meat were placed inside. To maintain the temperature, two logs were added every half hour throughout the smoking process. The average wood consumption was about 7.4 kg.

For the Offset3+4 setups, the offset smoker was heated with charcoal briquettes. For this purpose, 4 kg of briquettes were ignited in two lighting chimneys, each with five pieces of grill lighter. After 35 min, the thoroughly glowing briquettes were transferred to the firebox of the offset smoker. At the same time, a piece of meat was placed in the cooking chamber. After 15 min, a portion of wood chips (200 g dry wood chips soaked in 200 g water for 30 min) was added to the glowing briquettes. For setup Offset4, another portion of wood chips was added to the firebox after 30 min. The kettle grill was heated with charcoal briquettes for all setups (Kettle1–3). Therefore, 1.7 kg briquettes were ignited in a lighting chimney with five pieces of grill lighter. After 25 min, the completely glowing briquettes were placed on one side of the kettle grill. The meat was placed on the other side so that it was not directly over the briquettes. A barrier of aluminum foil prevented the dripping of meat juices into the embers. The lid of the grill was closed. After another 15 min, differently prepared wood chips were added depending on the setups. For setup Kettle1, 200 g wood chips soaked in 200 g water for 30 min were added, and for setup Kettle2, 200 g dry wood chips were added directly to the embers. The 200 g dry wood chips for setup Kettle3 were placed in a smoking box and then positioned on the embers.

The smoked meat pieces were cut into 2 cm thick slices. Every second slice was taken and combined as one sample. The remaining slices were used to determine the penetration depth in Offset1+2. For this purpose, the outer 5 mm of each slice were cut off and combined as one sample (layer I). This was repeated for sample "layer II". The remaining core pieces were combined as sample "layer III". All samples were homogenized using a Grindomix GM 200 (Retsch, Haan, Germany) and were stored in sterile side-seal vacuum bags at −18 °C.

2.3. Analysis of OPAHs and PAHs

The home smoked meat samples were analyzed for eight OPAHs und six PAHs. The exact procedure, validation and performance parameters of the analytical method used were described previously (Zastrow et al., 2021). The samples were prepared in three steps: (i) preparation of the extraction cell with homogenized sample, drying agent and internal standard; (ii) accelerated solvent extraction using an ACN/EA mixture

Table 1
Process parameters applied in seven experimental setups (with four replicates each, total n = 28).

Label	Smoking device	Heating medium	Smoking material	Meat pretreatment	Water addition to wood chips	Smoking box	Average smoking time (h)[b]
Offset1	Offset smoker	Beech wood logs 7.5 kg[a]	Beech wood logs	Seasoned	–	No	5:09
Offset2	Offset smoker	Beech wood logs 7.5 kg[a]	Beech wood logs	Unseasoned	–	No	5:09
Offset3	Offset smoker	Charcoal briquettes 4.0 kg	Beech wood chips 0.2 kg	Unseasoned	Yes 0.2 L	No	5:47
Offset4	Offset smoker	Charcoal briquettes 4.0 kg	Beech wood chips 0.4 kg	Unseasoned	Yes 0.4 L	No	5:57
Kettle1	Kettle grill	Charcoal briquettes 1.7 kg	Beech wood chips 0.2 kg	Unseasoned	Yes 0.2 L	No	4:19
Kettle2	Kettle grill	Charcoal briquettes 1.7 kg	Beech wood chips 0.2 kg	Unseasoned	No	No	3:56
Kettle3	Kettle grill	Charcoal briquettes 1.7 kg	Beech wood chips 0.2 kg	Unseasoned	No	Yes	4:09

[a] Total amount of heating medium and smoking material.
[b] Average smoking time until core temperature of 75 °C has been reached. The temperature in the smoking chamber was kept between 120 and 140 °C.

(1/3, v/v); (iii) solid phase extraction with an ACN/EA mixture (97/3, v/v). Gas chromatography was performed with a Trace Ultra gas chromatograph (Thermo Fisher Scientific, Dreieich, Germany). The injector was operated in the splitless mode at 280 °C. The chromatographic separation was performed with an Rxi®-PAH column (60 m × 0.25 mm × 0.10 µm) from Restek (Bad Homburg, Germany). The injection volume was 1.5 µL, and helium was used as carrier gas with a constant flow of 1 mL/min. The following temperature program was applied: isothermal at 50 °C for 0.1 min, at 30 °C/min to 175 °C, at 6 °C/min to 265 °C, at 4 °C/min to 290 °C, at 30 °C/min to 320 °C and isothermal at 320 °C for 10 min. The high-resolution mass spectrometry analysis with a magnetic sector mass spectrometer DFS (Thermo Fisher Scientific, Dreieich, Germany) was performed in the electron impact positive ion mode (EI pos). The electron energy was 40 eV, and the temperatures of the transfer line and the ion source were 270 °C and 260 °C, respectively. The resolution of the mass spectrometer was set to 8000 (10% valley definition).

2.4. Analysis of free 3-MCPD and 2-MCPD

The extraction was carried out by accelerated solvent extraction (Schallschmidt et al., 2012). Briefly, the homogenized sample was lyophilized, spiked with internal standard (3-MCPD-d5 and 2-MCPD-d5), extracted by an ASE 350 (Thermo Fisher Scientific, Dreieich, Germany) and set to a volume of 50 mL by addition of deionized water. Subsequent extraction and derivatization was carried out according to AOCS Official Method Cd 29b 13 (AOCS, 2017). Therefore, an aliquot of 8 mL was extracted with a mixture of diethyl ether/EA (3/2, v/v), shaken briefly and centrifuged. The organic upper layers were combined and dried with anhydrous Na_2SO_4. The extraction was repeated twice more and the organic phases were combined. Afterwards, phenylboronic acid and 50 µL n-dodecane were added, the mixture was evaporated almost to dryness and adjusted to a final volume of 500 µL by the addition of isooctane.

The GC-HRMS measurement was performed with a Trace Ultra gas chromatograph (Thermo Fisher Scientific, Dreieich, Germany) coupled to a magnetic sector mass spectrometer DFS (Thermo Fisher Scientific (Dreieich, Germany). The gas chromatograph was equipped with a programmable temperature vaporizing injector (PTV) and an Rxi-17Sil MS column (30 m × 0.25 mm x 0.25 µm) from Restek (Bad Homburg, Germany). The following PTV program in splitless mode was used at an injection volume of 1 mL: isothermal at 110 °C for 0.05 min, at 5 °C/s to 165 °C for 9.5 min, at 5 °C/s to 320 °C for 8 min. Separation of the analytes was achieved with a constant helium flow of 1.4 mL/min and the following oven program: 110 °C for 0.5 min, at 8 °C/min to 180 °C, at 25 °C/min to 330 °C, and isothermal at 330 °C for 5 min. The temperatures of the transfer line and the ion source were 300 °C and 260 °C, respectively. The ionization was executed in EI pos with an electron energy of 45 eV. The quantification was carried out by the use of deuterated internal standards. For quantitative analysis m/z = 147.06 ($C_8H_8BO_2^+$) and m/z = 150.08 ($C_8H_5D_3BO_2^+$) were used for 3-MCPD, whereas m/z = 196.05 ($C_9H_{10}BClO_2^+$) and m/z = 201.08 ($C_9H_5D_5BClO_2^+$) were used for 2-MCPD.

2.5. Statistical analysis

The statistical analysis and the calculations were performed with JMP 15 (SAS Institute Inc., Heidelberg, Germany) and Excel (Microsoft Office 2019). The results are based on four replicates. Non-parametric tests as well as medians were used for evaluation because not all data were distributed normally. The data were tested for significant differences with the Wilcoxon test at $P < 0.05$. Boxplots show median, upper, and lower quartiles, and whiskers are a maximum of 1.5 times the interquartile distance, with outliers displayed as dots. Regression models of the entire, unweighted dataset were constructed to assess the correlations between selected OPAHs and PAHs. Since the quadratic term was not significant, linear regression was used. The coefficients of the linear (X) term, the intercept, and the adjusted coefficient of determination (R^2_{adj}) were calculated. If the intercept was not statistically significant, a regression-without-intercept was used. To estimate the prediction quality, the absolute and relative root mean square errors (RMSE) were calculated.

3. Results and discussion

3.1. OPAH and PAH contents in home smoked meat

The pork was smoked within seven different setups (4 replicates each, n = 28) that differed in terms of smoking device, heat source, smoking material and sample pretreatment (Table 1). Seven out of eight OPAHs were detected in at least one of the smoked meat samples. BaPO was not detected in any of the samples, same as previously not found in barbecued products (Zastrow, Judas, Speer, Schwind, & Jira, 2022). BaAQ and NAPHQ were found above the LOQ in eight (setups Offset1+2) of 28 samples, and BcdPO was found in 22 samples. FLU, ANT, BaA, and CHR were detected in all samples. BbF was detected in only twelve and BaP in 16 samples.

The highest contents of both OPAHs and PAHs were measured in the samples prepared according to the setups Offset1+2 (Fig. 1; median OPAH8: 311 µg/kg; median PAH6: 235 µg/kg). The BaP and PAH4 contents (median 13 µg/kg and 67 µg/kg, respectively) exceeded the maximum levels for smoked meat and meat products of 2 and 12 µg/kg as well as the maximum levels for barbecued meat of 5 and 30 µg/kg set by Regulation (EU) No 835/2011 (European Commission, 2011). The maximum PAH4 content was 106 µg/kg and therefore noticeably higher than observed in traditionally smoked meat and meat products (39 and

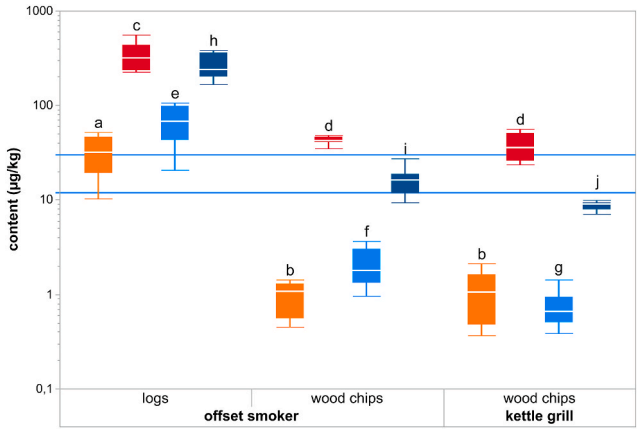


Fig. 1. Contents of OPAH4 (orange), OPAH8 (red), PAH4 (light blue), and PAH6 (dark blue) in meat smoked with different smoking devices and smoking materials (logarithmic scale of the y-axis). For detailed smoking parameters, see Table 1. The reference lines at 12 µg/kg and 30 µg/kg indicate the maximum level for PAH4 in smoked and barbecued meat, respectively, according to Regulation (EU) No 835/2011. Setups that did not differ significantly in their contents were combined (offset smoker/logs (n = 8): Offset1+2; offset smoker/wood chips (n = 8): Offset3+4; kettle grill/wood chips (n = 12): Kettle1–3). (OPAH4: sum of BaAQ, BbFLO, BcdPO, and NAPHQ; OPAH8: sum of OPAH4, ATQ, BaPO, BZA, and 9FLO; PAH4: sum of BaA, BaP, BbF, and CHR; PAH6: sum of PAH4, ANT, and FLU; different letters represent significant differences between experimental setups within OPAH or PAH group (Wilcoxon test, $P < 0.05$)). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

43 µg/kg, respectively) (Kafouris, Koukkidou, Christou, Hadjigeorgiou, & Yiannopoulos, 2020; Mastanjevic, Kartalovic, Vranesevic, Novakov, & Habschied, 2020).

No significant differences between the seasoned and unseasoned meat were detected in terms of OPAH4, OPAH8 as well as PAH4 and PAH6 contents. Therefore, the results of unseasoned and seasoned offset smoker logs were illustrated in combination in Fig. 1.

The setups with wood chips instead of logs showed 8-fold lower OPAH8 contents (median: 39 µg/kg) and 25-fold lower PAH6 contents (median: 10 µg/kg). The maximum PAH4 content of 3.6 µg/kg was comparable to industrially smoked sausages with maxima of 2.6 and 3.0 µg/kg (Pöhlmann et al., 2012; Pöhlmann, Hitzel, Schwägele, Speer, & Jira, 2013b). The use of charcoal briquettes instead of logs as a heating medium resulted in lower levels of pyrolysis-related contaminants in the meat samples, as the charcoal had already been pyrolyzed during charcoal manufacturing. Therefore, it is assumed that lower levels of pyrolysis-related by-products are released during its usage.

Doubling the amount of wood chips during home smoking with the offset smoker led to significantly higher levels only for BZA (Offset3: 0.4 µg/kg; Offset4: 0.8 µg/kg) and FLU (Offset3: 7.4 µg/kg; Offset4: 9.6 µg/kg). A heterogeneous distribution of wood chips and charcoal briquettes could explain the unobservable effect of an increase of smoking material on the contents of a majority of PAHs and OPAHs. Stacking wood chips on top of each other leads to a heterogeneous heat distribution, since some chips are not in direct contact with the coal, which affects the pyrolysis temperature and consequently the formation of PAHs (Pöhlmann et al., 2012).

In a direct comparison between the kettle grill and offset smoker experiments Kettle1 and Offset3, no difference was found in the contents of OPAH4 and OPAH6 in the corresponding smoked meat samples. However, 3-fold lower PAH4 contents (median: 0.7 µg/kg) and 2-fold lower PAH6 contents (median: 8.9 µg/kg) were detected in the kettle grill samples. The literature dealing with the reduction of smoke contaminants in industrial smoking recommends a separation of smoke production and smoking to reduce the PAH contents in food (Codex Alimentarius, 2009). Therefore, higher contamination levels should have been detected in the kettle grill samples. However, since a barrier made of aluminum foil was placed between the meat and the charcoal, which prevented fat and meat juices from dripping onto the heating medium, the formation of PAHs during home smoking in the kettle grill was kept low. Additionally, the shorter smoking time needed to reach the core temperature of the meat in the kettle grill experiments due to higher temperatures in the smoking chamber might have affected the PAH contents. Furthermore, in the offset smoker higher temperatures in the smoke formation chamber are necessary to obtain the required temperature in the smoking chamber compared to the kettle grill, due to the spatial separation. In consequence, the pyrolysis temperature might have been higher in the offset smoker compared to the kettle grill samples which might have further increased the formation of PAHs. Additional preparation of the wood chips (watered, not watered or in a smoking box) did not significantly affect the contents of the OPAHs and PAHs in the smoked meat samples. The fast evaporation of water, the good thermal conductivity of the smoking box and the long smoking time might be causal.

In all samples, the three aromatic ring compounds 9FLO (median: 63%), FLU (15%), ATQ (9%) and ANT (8%), had the largest proportions of the total OPAH8+PAH6 content (Fig. 2). The sum of these four compounds accounted for at least 70%. The proportion of OPAH8 (74%) was also noticeably higher than the proportion of PAH6 (26%) in all setups. These results are in good agreement with a previous study analyzing barbecued beef patties (Zastrow et al., 2022).

However, there were differences in the OPAH and PAH composition depending on the smoking material. For example, the proportion of PAH6 was greater in the setups with wood logs (44%) than with wood chips (23%). The proportion of heavier compounds and thus of toxicologically relevant OPAH4 was also higher using logs (6%) compared to woodchips (2%). In general, high-contaminated samples had higher percentages of the more toxicologically relevant OPAH4 and PAH4 than the low-contaminated samples, which is in agreement with a former study investigating PAH and OPAH contents for barbecueing (Zastrow et al., 2022).

In order to investigate the relations between OPAHs and PAHs, we examined the three pairs of individual OPAHs that had a corresponding PAH (9FLO/FLU, ATQ/ANT, and BaAQ/BaA). In addition, the sum

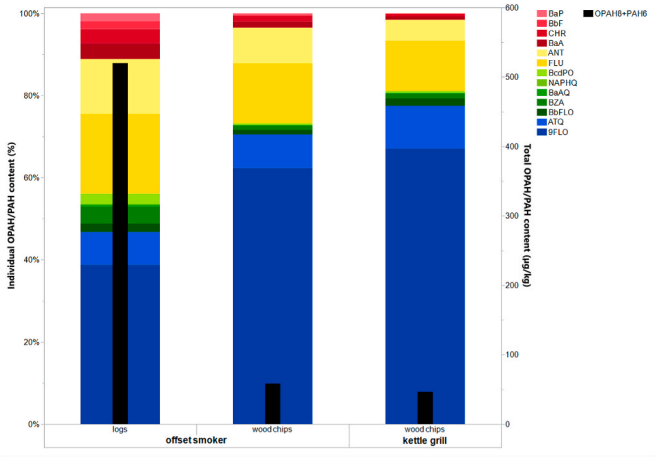


Fig. 2. Percentage composition (left scale of the y-axis) of the individual OPAHs and PAHs (coloured bars) and sum contents of OPAH8 and PAH6 (black narrow bars; right scale of the y-axis) in home smoked meat. The proportions of 3-ring-OPAHs and -PAHs are shown as blue and yellow bars. BaPO is not shown since it has not been detected. Setups that did not differ significantly in their content were combined (offset smoker/logs (n = 8): Offset1+2; offset smoker/wood chips (n = 8): Offset3+4; kettle grill/wood chips (n = 12): Kettle1–3). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

contents OPAH4 or OPAH8 were related to the PAH4 contents. For all correlations, linear regression models were calculated from the total unweighted dataset (Table 2). All individual and summed OPAHs increased with the corresponding PAHs with significant positive linear regression coefficients, ranging from 0.10 to 4.29. From the five models, 9FLO, ATQ and OPAH8 had significant positive intercepts, i.e. individual or summed OPAH contents could be expected at the intercept level even when no corresponding PAHs were detected. The OPAH/PAH pairs were strongly correlated with $R^2_{adj} > 0.9$, and prediction errors RMSE from rather high 34% down to moderate 11%. The strongest correlated pair was BaAQ/BaA with $R^2_{adj} = 0.99$ and RMSE = 11%. The correlations of the summed OPAH contents with PAH4 were also strong, with that of OPAH4 being higher than that of OPAH8 ($R^2_{adj} = 0.99$ vs. 0.94). The RMSEs were 17% and 30%, respectively. Although an RMSE of 17% is not exactly an accurate prediction, it is the best that is available to estimate OPAH4 contents retrospectively from data that measured PAH4, only.

3.2. MCPD contents in home smoked meat

In all home smoked meat samples free 3-MCPD could be detected (Fig. 3). Only in samples of Offset1+2, which showed the highest levels of free 3-MCPD, free 2-MCPD was also quantified with a median of 1 and 5 μg/kg, respectively. The observed concentration range of 3-MCPD (2–98 μg/kg) can be well classified in a series of commercially smoked ham (19–84 μg/kg) (Kuntzer & Weisshaar, 2006). Comparing the different smoking approaches Kettle1, Kettle2 and Kettle3, no significant differences in the content of free 3-MCPD ranging from 3.6 to 3.8 μg/kg were detected in the kettle grill samples. Watering the wood chips did not affect the amount of free 3-MCPD in the smoked meat samples of Kettle1+2, since the added water rapidly evaporated on the hot embers' surface. Thus, neither the induction period of the pyrolysis nor the cooking time of the meat was significantly prolonged. In consequence, the data of the setups Kettle1–3 were combined in Fig. 3.

In addition, low levels of free 3-MCPD were found in the samples prepared in both home smoking devices using the same amount of watered wood chips (median of 6.2 and 3.6 μg/kg, respectively). According to the Wilcoxon-test, these experimental setups (Kettle1, Offset3) were not significantly different. Thus, the geometry of the smoking chamber and the spatial separation between smoke formation and smoking do not seem to have a major influence on the content of free 3-MCPD in the corresponding smoked products. One possible reason for this might be the application of aluminum foil between the smoked food and the charcoal during the kettle grill experiments, which prevented fat and meat juices from dripping onto the charcoal. In consequence, no additional formation of 3-MCPD on the heating medium took place, which had been considered responsible for higher levels of free 3-MCPD in grilled meat prepared on gas and charcoal grills compared to electric grills (Schallschmidt et al., 2012). In the offset smoker, a slightly lower smoke density compared to the kettle grill due to a larger smoking chamber is postulated while using the same amount of wood. However, an increasing effect on the content of 3-MCPD compared to the kettle grill samples was not observed. The impact of the lower smoke density in Offset3 on the content of 3-MCPD might have been compensated by a slightly longer smoking time required to reach the specified cooking level of the meat samples.

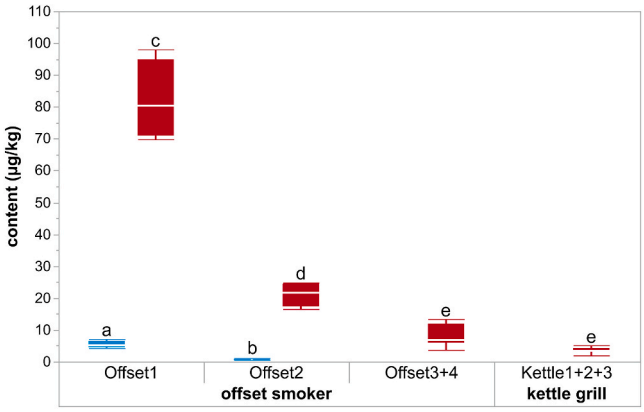


Fig. 3. Contents of detected 2-MCPD (blue) and 3-MCPD (red) in meat smoked with different smoking devices and smoking materials (Kettle1–3: n = 12; Offset1: n = 4; Offset2: n = 4; Offset3+4: n = 8). For detailed smoking parameters, see Table 1. Different letters represent significant differences between experimental setups within 2-MCPD or 3-MCPD (Wilcoxon test, $P < 0.05$). Setups using the same smoking device that did not differ significantly in their contents were combined. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Kuntzer and Weisshaar (2006) suggested a formation pathway of free 3-MCPD by pyrolysis of cellulose in presence of HCl via hydroxyacetone precursors and chloride under acidic conditions during smoke formation. Furthermore, they proved the presence of free 3-MCPD in the resulting smoke condensate. However, the use of twice the amount of smoked wood in Offset4 (median: 8.4 μg/kg) compared to Offset3 (median: 6.2 μg/kg) did not significantly affect the level of free 3-MCPD in smoked meat according to the Wilcoxon test ($P < 0.05$). Doubling the amount of wood chips did not necessarily result in a doubling of the smoke intensity and the MCPD content. Additionally, the pyrolysis conditions, which were considered as starting point for the formation of free MCPD, might have changed between both experimental setups. In Offset4, the amount of wood chips with direct contact to the charcoal surface was not raised in comparison to Offset3, due to the same amount of charcoal briquettes used in both setups. Furthermore, the air supply was not adapted to a larger quantity of wood chips. Both aspects could have affected the formation of MCPD or precursors and resulted in comparable contents of free MCPD in Offset3 and Offset4. In consequence, data of both setups were combined in Fig. 3.

In experiment Offset2, a median 3-MCPD content of 22 μg/kg was detected in the corresponding meat product, which was significantly higher than in Offset3 and Offset4, due to a much higher amount of pyrolyzed wood (7.4 kg logs in mean compared to 0.2 kg chips in Offset3 and 0.4 kg in Offset4). Since in contrast to the other experiments wood logs were used in Offset1 and Offset2 for smoking as well as for heating, higher amounts of cellulose were pyrolyzed, which resulted in higher contents of 3-MCPD in the corresponding samples. Assuming that wood chips and logs are comparable in the composition of macro ingredients, no linear correlation between the amount of wood and the content of 3-MCPD could be observed. One possible reason may be the lack of

Table 2
Regressions of individual or summed OPAHs on individual or summed PAHs (n = 28).

Y	X	Intercept (μg/kg)	Coefficient (X)	R^2_{adj}	RMSE	Mean Y (μg/kg)	RMSE (% Mean)
9FLO	FLU	23.00*	1.77*	0.90	31.35	91.07	34
ATQ	ANT	3.34*	0.54*	0.94	5.50	17.15	32
BaAQ	BaA	−0.06*	0.10*	1.00	0.07	0.64	11
OPAH4	PAH4	0	0.46*	0.99	1.68	9.81	17
OPAH8	PAH4	39.24*	4.29*	0.93	38.33	125.53	31

*$P < 0.05$.

comparability of the smoke generation conditions, since in the setups Offset1+2, logs represent both the heating medium and the smoking material and in the setups Offset3+4 and Kettle1–3 charcoal briquettes were added as heating source. Additionally, a varying smoking time required for the meat to reach the specified core temperature and a varying oxygen supply caused by a slightly different position of the ventilation flaps, may have influenced the 3-MCPD contents directly (due to a more complete combustion of the wood and in consequence a worse pyrolysis) and indirectly (due to higher temperatures in the smoking chamber and thus a shorter cooking and smoking time). These influencing factors cannot be completely leveled out by four repetitions per experimental setup and may also have contributed to the non-linear correlation between the amount of wood and the 3-MCPD content in the meat samples.

In the seasoned samples of Offset1, a median 3-MCPD content of 80 μg/kg was detected, which is almost four times as high as in the samples of Offset2 (22 μg/kg). Additionally, 2-MCPD was found in Offset1 as well as in Offset2 with 5.2 and 1 μg/kg, respectively. Thus, the addition of a spice mixture on the meat surface and, in this context, the addition of NaCl resulted in an increased content of 3- and 2-MCPD in the corresponding samples. Significantly higher levels of 3-MCPD have already been reported in seasoned or salted smoked fish samples compared to unsalted ones (Karl, Merkle, Kuhlmann, & Fritsche, 2016). Since the smoking experiments of Offset1 and Offset2 were performed simultaneously in the same smoking chamber and the positioning of seasoned and unseasoned meat pieces was homogeneous therein, the increased free 3-MCPD content in Offset1 cannot be attributed to differences in smoke density or smoking time. Instead, free 3-MCPD must be formed directly on the meat as a result of seasoning. A possible explanation for the significantly higher contents in the seasoned meat samples might be the reaction of a 3-MCPD precursor with chloride from the spice mixture on the slightly acidic meat surface. The chloride content in dry wood is low with a median of 40 mg/kg (Denner, 2018). Accordingly, the precursors resulting from the pyrolysis might not be quantitatively converted to 3-MCPD during smoke production and, therefore, could be transported to the meat by the smoke. The conversion to 3-MCPD takes place on the surface of the salted meat at temperatures of 120–140 °C. In another study (U.S. Department of Agriculture, 2005), fish samples were soaked in brine of different strengths and then simultaneously cold-smoked in the same oven. An increased formation of 3-MCPD in fish using higher concentrated brines was observed even at low temperatures of 30 °C in the smoking chamber, assuming that the reaction of the pyrolysis related precursor does not require higher temperatures. Furthermore, we determined the ratio of 3-MCPD to 2-MCPD in the smoked meat samples of Offset1 and Offset2 which differed significantly, with ratios of 15 and 23 respectively, according to the Wilcoxon test at $P < 0.05$. From other studies, lower 3- to 2-MCPD ratios are known which were attributed to other reaction mechanism under the influence of salt, fat, and increased temperature (Collier et al., 1991). Therefore, the lower 3- to 2-MCPD ratio in Offset1 might be the result of a combination of pyrolysis affected formation and heat induced formation in the presence of salt since during grilling experiments at comparable temperatures (mean values between 117 °C and 165 °C), a formation of free 3-MCPD was observed (Schallschmidt et al., 2012).

3.3. Penetration of OPAHs, PAHs, and 3-MCPD into home smoked meat samples

Since large pieces of meat with low surface to volume ratios are commonly used for home smoking, such samples are well suited for the investigation of possible penetration of the contaminants from the surface to the interior. Therefore, the samples from the setups Offset1 and Offset2 were divided into three layers (Section 2.2) and analyzed separately.

In the outer 5 mm-layer (I), 95% of the OPAHs and 79% of the PAHs were detected (Table 3). In the inner layer (III), only one OPAH (9FLO) but three PAHs (ANT, BaA, and FLU) were measured. Accordingly, the proportion of the lighter 3-ring systems was greater than the proportion of the heavier 4- and 5-ring systems in the middle (II) and inner layer. The effect that low molecular weight PAHs can migrate deeper into the product than high molecular weight ones has already been shown for uncommonly long (5–6 weeks) smoked meat (Chen et al., 2014). In addition, several studies demonstrated that most of the PAHs in smoked sausages are located in the casing and migrate only in small proportions to the interior (Gomes, Santos, Almeida, Elias, & Roseiro, 2013; Ledesma, Rendueles, & Diaz, 2014; Pöhlmann et al., 2013b). Based on the results shown here, this tendency is also observed for OPAHs. The observation that selected OPAHs are also detected in deeper layers of smoked meat (Chen et al., 2014) could not be confirmed in the present work applying practice-relevant conditions. Individual OPAHs were also detectable in layers II + III, but the proportion of PAHs was higher in these layers.

Table 3
Median proportions (in %) of OPAH, PAH, 3- and 2-MCPD in the three layers (I: outer layer; II: middle layer; III: inner layer) related to the complete samples of Offset1 and Offset2 (OPAH, PAH) or Offset1 (MCPD).

	n	Layer I	Layer II	Layer III
9FLO	8	94	4	2
ATQ	8	97	3	0
BbFLO	8	100	0	0
BZA	8	100	0	0
BaAQ	8	100	0	0
NAPHQ	8	100	0	0
BcdPO	8	100	0	0
OPAH4	8	100	0	0
OPAH8	8	95	3	2
FLU	8	74	14	13
ANT	8	80	10	9
BaA	8	86	8	7
CHR	8	100	0	0
BbF	8	100	0	0
BaP	8	100	0	0
PAH4	8	94	4	2
PAH6	8	79	11	10
3-MCPD	4	65	25	10
2-MCPD	3	61	27	12

Looking at the distribution of 3- MCPD in the different meat layers, 65% was found in the outer, 25% in the middle, and 10% in the inner layers. First indications of the good penetration of free 3-MCPD are described in the literature (Kuntzer & Weisshaar, 2006) and attributed to the small molecular size and good water solubility. In contrast to the mentioned study, a significant concentration gradient of 3-MCPD (Wilcoxon test, $P < 0.05$) was found between the layers in all Offset1 samples, with the content being highest in the outer layers. The same trend was observed with significance ($P < 0.05$) in Offset2 between outer and middle layers, but with no statistical significance between middle and inner layer samples. According to the 3- to 2-MCPD ratio, no different penetration behavior of 2-MCPD was observed as the ratio was the same in the different layers of meat. Consequently, MCPD penetrated noticeably into the interior of smoked meat in contrast to PAHs/OPAHs.

4. Conclusions

The choice of both smoking material and heating medium (wood logs or briquettes in conjunction with wood chips) significantly influenced the 3- and 2-MCPD contents and showed the highest impact on the OPAH and PAH contents of the meat samples. Logs were considered as more critical than charcoal briquettes since higher contents of contaminants were obtained. In order to mitigate the contents of parent and oxygenated PAHs and MCPD, further experiments should be carried out to cook the meat in the smoker without smoking as long as possible and to smoke it briefly towards the end of the cooking process by adding wood chips to a charcoal heating medium. The spatial separation of

smoke formation and food smoking, as demanded for industrial food production (Codex Alimentarius, 2009), did not influence the level of pyrolysis-related contaminants significantly as long as a barrier prevents fat or meat juices from dripping onto the heating medium. The addition of salt after smoking is recommended, since the salting of meat before smoking significantly increased the levels of 3- and 2-MCPD. The high correlation of PAH4 and OPAH4 seems to be a helpful rule of thumb to estimate the OPAH4 content in home smoking and should be evaluated for further applicability in industrial smoking. The observed low penetration of OPAH into the meat in home smoking conditions suggests that the selection of a peelable casing is not only a reasonable approach for reducing the PAH (Pöhlmann et al., 2013b) but also the OPAH contents.

CRediT authorship contribution statement

Lisa Zastrow: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Visualization. **Christopher Albert:** Conceptualization, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Karl Speer:** Supervision, Writing – review & editing. **Karl-Heinz Schwind:** Conceptualization, Methodology. **Wolfgang Jira:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing, Project administration, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

We would like to thank B. Breuning, S. Ganesha-Rajan and A. Igler for their excellent technical assistance and J. Haida for professionally performing the home smoking experiments.

References

Alimentarius, C. (2009). *Code of practice for the reduction of contamination of food with polycyclic aromatic hydrocarbons (PAH) from smoking and direct drying processes (CXC 68-2009*.

AOCS Official Method Cd 29b-13 (2017).

Bolton, J. L., Trush, M. A., Penning, T. M., Dryhurst, G., & Monks, T. J. (2000). Role of quinones in toxicology. *Chemical Research in Toxicology, 13*(3), 135–160. https://doi.org/10.1021/tx9902082

Chen, Y. C., Shen, G. F., Su, S., Shen, H. Z., Huang, Y., Li, T. C., & Tao, S. (2014). Contamination and distribution of parent, nitrated, and oxygenated polycyclic aromatic hydrocarbons in smoked meat. *Environmental Science and Pollution Research, 21*(19), 11521–11530. https://doi.org/10.1007/s11356-014-3129-8

Clergé, A., Le Goff, J., Lopez, C., Ledauphin, J., & Delépée, R. (2019). Oxy-PAHs: Occurrence in the environment and potential genotoxic/mutagenic risk assessment for human health. *Critical Reviews in Toxicology, 49*(4), 302–328. https://doi.org/10.1080/10408444.2019.1605333

Collier, P. D., Cromie, D. D. O., & Davies, A. P. (1991). Mechanism of formation of chloropropanols present in protein hydrolysates. *Journal of the American Oil Chemists Society, 68*(10), 785–790. https://doi.org/10.1007/bf02662173

Commission Regulation (EU). (2011). *No 835/2011 of 19 August 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for polycyclic aromatic hydrocarbons in foodstuffs*.

European Food Safety Authority. (2008). Polycyclic aromatic hydrocarbons in food - scientific opinion of the panel on contaminants in the food chain. *EFSA Journal, 724*, 1–114.

European Food Safety Authority. (2016). Risks for human health related to the presence of 3- and 2-monochloropropanediol (MCPD), and their fatty acid esters, and glycidyl fatty acid esters in food. *EFSA Journal, 14*(5), 4426.

European Food Safety Authority. (2018). Update of the risk assessment on 3-monochloropropane diol and its fatty acid esters. *EFSA Journal, 16*(1), 5083.

Gomes, A., Santos, C., Almeida, J., Elias, M., & Roseiro, L. C. (2013). Effect of fat content, casing type and smoking procedures on PAHs contents of Portuguese traditional dry fermented sausages. *Food and Chemical Toxicology, 58*, 369–374. https://doi.org/10.1016/j.fct.2013.05.015

IARC. (2012). 3-Monochloro-1,2-propanediol. *IARC Monographs, 101*, 349–374.

Jaffe, T. R., Wang, H. W., & Chambers, E. (2017). Determination of a lexicon for the sensory flavor attributes of smoked food products. *Journal of Sensory Studies, 32*(3). https://doi.org/10.1111/joss.12262

Kafouris, D., Koukkidou, A., Christou, E., Hadjigeorgiou, M., & Yiannopoulos, S. (2020). Determination of polycyclic aromatic hydrocarbons in traditionally smoked meat products and charcoal grilled meat in Cyprus. *Meat Science, 164*. https://doi.org/10.1016/j.meatsci.2020.108088

Karl, H., Merkle, S., Kuhlmann, J., & Fritsche, J. (2016). Development of analytical methods for the determination of free and ester bound 2-, 3-MCPD, and esterified glycidol in fishery products. *European Journal of Lipid Science and Technology, 118*(3), 406–417. https://doi.org/10.1002/ejlt.201400573

Kjallstrand, J., & Petersson, G. (2001). Phenolic antioxidants in wood smoke. *Science of the Total Environment, 277*(1–3), 69–75. https://doi.org/10.1016/s0048-9697(00)00863-9

Kuntzer, J., & Weisshaar, R. (2006). The smoking process - a potent source of 3-chloropropane-1,2-diol (3-MCPD) in meat products. *Deutsche Lebensmittel-Rundschau, 102*(9), 397–400.

Ledesma, E., Rendueles, M., & Diaz, M. (2014). Benzo(a)pyrene penetration on a smoked meat product during smoking time. *Food Additives & Contaminants Part A-Chemistry Analysis Control Exposure & Risk Assessment, 31*(10), 1688–1698. https://doi.org/10.1080/19440049.2014.949875

Lundstedt, S., White, P. A., Lemieux, C. L., Lynes, K. D., Lambert, L. B., Oberg, L., et al. (2007). Sources, fate, and toxic hazards of oxygenated polycyclic aromatic hydrocarbons (PAHs) at PAH-contaminated sites. *Ambio, 36*(6), 475–485. https://doi.org/10.1579/0044-7447(2007)36[475:Sfatho]2.0.Co;2

Mastanjevic, K. M., Kartalovic, B. D., Vranesevic, J. M., Novakov, N. J., & Habschied, K. J. (2020). Polycyclic aromatic hydrocarbons in traditionally smoked Slavonska kobasica. *Food Additives & Contaminants Part B-Surveillance, 13*(2), 82–87. https://doi.org/10.1080/19393210.2020.1712481

Ma, X., & Wu, S. M. (2022). Oxygenated polycyclic aromatic hydrocarbons in food: Toxicity, occurrence and potential sources. *Critical Reviews in Food Science and Nutrition*. https://doi.org/10.1080/10408398.2022.2146652

Ostermeyer, U., Merkle, S., Karl, H., & Fritsche, J. (2021). Free and bound MCPD and glycidyl esters in smoked and thermally treated fishery products of the German market. *European Food Research and Technology, 247*(7), 1757–1769. https://doi.org/10.1007/s00217-021-03746-6

Pöhlmann, M., Hitzel, A., Schwägele, F., Speer, K., & Jira, W. (2012). Contents of polycyclic aromatic hydrocarbons (PAH) and phenolic substances in Frankfurter-type sausages depending on smoking conditions using glow smoke. *Meat Science, 90*(1), 176–184. https://doi.org/10.1016/j.meatsci.2011.06.024

Pöhlmann, M., Hitzel, A., Schwägele, F., Speer, K., & Jira, W. (2013a). Influence of different smoke generation methods on the contents of polycyclic aromatic hydrocarbons (PAH) and phenolic substances in Frankfurter-type sausages. *Food Control, 34*(2), 347–355. https://doi.org/10.1016/j.foodcont.2013.05.005

Pöhlmann, M., Hitzel, A., Schwägele, F., Speer, K., & Jira, W. (2013b). Polycyclic aromatic hydrocarbons (PAH) and phenolic substances in smoked Frankfurter-type sausages depending on type of casing and fat content. *Food Control, 31*(1), 136–144. https://doi.org/10.1016/j.foodcont.2012.09.030

Schallschmidt, K., Hitzel, A., Pohlmann, M., Schwägele, F., Speer, K., & Jira, W. (2012). Determination of 3-MCPD in grilled meat using pressurized liquid extraction and gas chromatography-high resolution mass spectrometry. *Journal Fur Verbraucherschutz Und Lebensmittelsicherheit-Journal of Consumer Protection and Food Safety, 7*(3), 203–210. https://doi.org/10.1007/s00003-012-0775-y

Simon, R., de la Calle, B., Palme, S., Meier, D., & Anklam, E. (2005). Composition and analysis of liquid smoke flavouring primary products. *Journal of Separation Science, 28*(9–10), 871–882. https://doi.org/10.1002/jssc.200500009

Swaney-Stueve, M., Talavera, M., Jepsen, T., Severns, B., Wise, R., & Deubler, G. (2019). Sensory and consumer evaluation of smoked pulled pork prepared using different smokers and different types of wood. *Journal of Food Science, 84*(3), 640–649. https://doi.org/10.1111/1750-3841.14469

U.S. Department of Agriculture. (2005). *The origin and formation of 3-MCPD in foods and food ingredients*.

Velisek, J., Calta, P., Crews, C., Hasnip, S., & Dolezal, M. (2003). 3-Chloropropane-1,2-diol in models simulating processed foods: Precursors and agents causing its decomposition. [article]. *Czech Journal of Food Sciences, 21*(5), 153–161.

Yu, H. T. (2002). Environmental carcinogenic polycyclic aromatic hydrocarbons: Photochemistry and phototoxicity. *Journal of Environmental Science and Health - Part C: Environmental Carcinogenesis & Ecotoxicology Reviews, 20*(2), 149–183. https://doi.org/10.1081/gnc-120016203

Zastrow, L., Judas, M., Speer, K., Schwind, K. H., & Jira, W. (2022). Barbecue conditions affect contents of oxygenated and non-oxygenated polycyclic aromatic hydrocarbons in meat and non-meat patties. *Food Chemistry-X*, 14. https://doi.org/10.1016/j.fochx.2022.100351

Zastrow, L., Schwind, K. H., Schwägele, F., & Speer, K. (2019). Influence of smoking and barbecuing on the contents of anthraquinone (ATQ) and polycyclic aromatic hydrocarbons (PAHs) in frankfurter-type sausages. *Journal of Agricultural and Food Chemistry, 67*(50), 13998–14004. https://doi.org/10.1021/acs.jafc.9b03316

Zastrow, L., Speer, K., Schwind, K. H., & Jira, W. (2021). A sensitive GC-HRMS method for the simultaneous determination of parent and oxygenated polycyclic aromatic hydrocarbons in barbecued meat and meat substitutes. *Food Chemistry, 365*. https://doi.org/10.1016/j.foodchem.2021.130625

Zhang, Y. J., Chen, X. Q., & Zhang, Y. (2021). Analytical chemistry, formation, mitigation, and risk assessment of polycyclic aromatic hydrocarbons: From food

processing to in vivo metabolic transformation. *Comprehensive Reviews in Food Science and Food Safety, 20*(2), 1422–1456. https://doi.org/10.1111/1541-4337.12705