Polycyclic aromatic hydrocarbons (PAH) and phenolic substances in smoked Frankfurter-type sausages depending on type of casing and fat content

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Abstract

The contents of polycyclic aromatic hydrocarbons (15 + 1 EU priority PAH) and phenolic substances (guaiacol, 4-methylguaiacol, syringol, eugenol, and trans-isoeugenol) in smoked Frankfurter-type sausages were investigated depending on the type of casing and back fat content. Three types of casings (collagen casings, cellulose-peelable casings, and sheep casings) were tested in four smoking experiments. Furthermore, Frankfurter-type sausages with four different back fat contents (10%, 20%, 30%, and 39%) were produced and simultaneously smoked in 12 smoking experiments applying different smoking conditions (glow smoke). The type of casing and the back fat content of Frankfurter-type sausages had an influence on the PAH contents. The benzo[a]pyrene contents ranged between 0.08 µg/kg in peeled cellulose cased sausages and 0.81 µg/kg in sheep cased sausages and between 0.28 µg/kg (back fat content: 10%) and 1.37 µg/kg (back fat content: 39%). The sum contents of the five phenolic compounds depended on the type of casing and ranged between 38 mg/kg (collagen cased sausages) and 109 mg/kg (sheep cased sausages), but did not depend on the fat contents of the sausages.

Keywords: Polycyclic aromatic hydrocarbons, Phenolic substances, Frankfurter-type sausages, Type of casing, Fat content

1. Introduction

Polycyclic aromatic hydrocarbons (PAH) consist of two or more condensed aromatic carbon rings and are formed during the incomplete combustion of organic material (Smith, 1984). About 660 different compounds belong to the PAH group (Sander & Wise, 1997), some of them showing carcinogenic properties (IARC, 1987; IARC, 2010). Due to the carcinogenic properties, the Scientific Committee on Food (SCF) (2002) recommended that the PAH contents in food should be "as low as reasonably achievable" in adherence with the so-called ALARA-principle. Furthermore, the Codex Alimentarius Commission (2008) recommended the investigation and the identification of optimal smoking conditions for minimizing PAH contents.

The European Union suggested to analyse the contents of 15 + 1 PAH compounds, which are classified as priority in food (EC, 2005; JECFA, 2005). These 15 + 1 EU priority PAH are: benzo[c]fluorene (BcL), benzo[a]anthracene (BaA), cyclopenta[c,d]pyrene (CPP), chrysene (CHR), 5-methylchrysene (5 MC), benzo[b]fluoranthene (BbF), benzo[j]fluoranthene (BjF), benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), benzo[g,h,i]perylene (BgP), dibenzo[a,h]anthracene (DhA), indeno[1,2,3-cd]pyrene (IcP), dibenzo[a,e]pyrene (DeP), dibenzo[a,h]pyrene (DhP), dibenzo[a,i]pyrene (DiP), and dibenzo[a,l]pyrene (DlP). The EFSA (2008) concluded that BaP is not a suitable indicator for the occurrence of PAH in food and assessed that the sum content of the four PAH compounds BaP, CHR, BaA and BbF (PAH4) is the most suitable indicator of PAHs in food. Consequently, in addition to the still existing maximum level for BaP, a new maximum level for PAH4 in smoked meat products of 30 µg/kg (1/9/2012 to 31/08/2014) and, later, of 12 µg/kg was established in Commission Regulation (EC) No 1881/2006 amended by Commission Regulation (EU) No 835/2011.

In a previous study (Pöhlmann, Hitzel, Schwägele, Speer, & Jira, 2012) it was shown that a minimization of the PAH compounds in hot smoked sausages using glow smoke is possible. The most important parameter influencing the PAH contents was the smoke generation temperature, however, the ventilator velocity also had a noticeable influence on the PAH contents. Lowering the contents of the PAH compounds did not necessarily lead to a decrease in the amounts of phenolic substances which are of considerable importance for the organoleptic properties of smoked meat products (Bratzler, Spooner, Weatherspoon, & Maxey, 1969; Kjallstrand & Petersson, 2001) and show antimicrobial (Davidson & Branden, 1981) and antioxidative (Toth, 1982; Wittkowski, 1985) properties.

Besides the smoking conditions there is evidence that the surface of smoked meat products (fat content and type of casing)

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influences the PAH contents. The use of casings of different materials (e.g. cellulose or synthetic material) reduced the diffusion of BaP in the inner part of the meat product (Filipovic & Toth, 1971). Furthermore, a reduced penetration of PAHs in the inner part of smoked meat products was observed for synthetic casings compared to natural casings (Toth, 1973). In traditional Spanish smoked chorizo sausages the detected PAH contents in meat and in the collagen and tripe casings suggested that the collagen-based casings behaved as a better barrier to PAHs (Garcia-Falcon & Simal-Gandara, 2005). Preliminary tests on the influence of the type of casing on the PAH contents in Frankfurter-type sausages showed a better absorption of the PAHs by the sheep casings compared to cellulose-peelable casings (Ziegenhals, Müller, Jira, & Speer, 2008). Furthermore, different PAH adsorption capacities were observed for smoked beef and pork ham with similar surface/mass ratio (Djinovic, Popovic, & Jira, 2008) and for Portuguese traditional smoked meat products (Roseiro, Gomes, & Santos, 2011; Santos, Gomes, & Roseiro, 2011).

The main objective of this study was to analyse the contents of the 15+1 EU priority PAH and the phenolic substances guaiacol, 4-methylguaiacol, syringol, eugenol, and *trans*-isoeugenol in hot smoked Frankfurter-type sausages and to investigate possible correlations to the type of casings and the fat contents. For the experiments applying different types of casings Frankfurter-type sausages with collagen casings, cellulose-peelable casings, and sheep casings were produced. Four smoking experiments were performed for each type of casing. In all experiments, the same smoking time was applied. The reddening- and drying times were varied to obtain comparable smoking colours. Furthermore, Frankfurter-type sausages with four different fat contents (10%, 20%, 30%, and 39%) were produced and simultaneously smoked. For this purpose, 12 smoking experiments applying different smoking conditions were performed.

2. Materials and methods

2.1. Preparation of Frankfurter-type sausages

The basic formulations of the Frankfurter-type sausages and the casings for all experiments are shown in Table 1. For all the smoking experiments using different types of casings (A) the same formulation was used. For A1 cellulose casings (20-22 mm), for A2 sheep casings (18-20 mm), and for A3 collagen casings (20-22 mm) were used. The experiments (B) with different fat contents (10%, 20%, 30%, and 39%) were performed with Frankfurter-type sausages containing different portions of fresh pork, fresh beef, back fat, and crushed ice.

Table 1
Basic formulations and used casings of Frankfurter-type sausages for the different smoking experiments.

Experiment	Fresh pork (%)	Fresh beef (%)	Back fat (%)	Ice (%)	Casing
(A) Casing types					
A1 (4 experiments)	29.4	19.6	26.5	22.5	Cellulose
A2 (4 experiments)	29.4	19.6	26.5	22.5	Sheep
A3 (4 experiments)	29.4	19.6	26.5	22.5	Collagen
(B) Fat contents					
10% fat (12 experiments)	35.8	23.9	9.9	28.3	Sheep
20% fat (12 experiments)	32.0	21.3	19.6	25.0	Sheep
30% fat (12 experiments)	28.0	18.7	29.5	21.8	Sheep
39% fat (12 experiments)	24.1	16.1	39.1	18.7	Sheep
(C) Different positions in the smoking chamber					
Front (1 experiment)	29.4	19.6	26.5	22.5	Sheep
Centre e Front (1 experiment)	29.4	19.6	26.5	22.5	Sheep
Centre e Back (1 experiment)	29.4	19.6	26.5	22.5	Sheep
Back (1 experiment)	29.4	19.6	26.5	22.5	Sheep

Frankfurter-type sausages were smoked in four different positions in the smoking chamber (experiment C) with the same basic formulation as experiment A. For all experiments, the percentage contents of the other ingredients were in mean 1.38% salt (containing sodium nitrite ($NaNO_2$); 0.4%), 0.04% ascorbic acid, 0.17% dipotassium hydrogen phosphate (K_2HPO_4), and 0.43% spice mix "Goldwürstchen" from Raps (Kulmbach, Germany).

2.2. Smoking experiments

A T1900 smoking chamber obtained from Fessmann (Winnenden, Germany) was used for the smoking experiments. An explanation of the smoking conditions and details to the smoking chamber were previously published (Pöhlmann et al., 2012). The sausages were reddened for 10 min at 52 °C, afterwards dried for 12 min at 56 °C, and then smoked at 58 °C. For the experiments with different types of casings, the reddening- and drying times were adapted depending on the type of casing used.

The smoking time, the smoke density, and the ventilator velocity in the smoking chamber were varied as shown in Table 2. For the smoking experiment with Frankfurter-type sausages with different fat contents, the sausages with 10%, 20%, 30%, and 39% back fat were randomly positioned in the smoking chamber and smoked simultaneously. For the smoking experiments using cellulose casings and sheep casings, the Frankfurter-type sausages were also smoked in the same smoking procedure. The collagen cased Frankfurter-type sausages were smoked separately as longer reddening and drying times were required for these sausages to obtain comparable products. Overall, 21 smoking experiments were performed and 64 different sausages were analysed. For chemical analysis, about 1-2 kg of the smoked sausages were homogenized in a bowl chopper, placed in sterile side seal vacuum bags obtained from Gruber-Folien (Straubing, Germany), and stored in the dark at -18 °C. The sausages with cellulose casings were peeled and then homogenized. The cellulose casings were cut into small pieces (about 0.5 cm^2) and were used for analysis.

2.3. Measurement of the smoke generation temperature and the gases

The data acquisition of the gas detection in the smoking chamber and the temperature of wood combustion were performed with a 350-S flue gas analyser and a NiCrNi sensor from Testo (Lenzkirch, Germany). The concentrations of oxygen and CO_2 were measured in volume percent, and concentrations of CO were quantified in ppm. The gas concentrations were recorded during the entire smoking process, averaging one value every 5 s.

2.4. Measurement of the pH value and the colour

The pH-value of the smoked sausages was measured using a Portamess Type 911 pH meter from Knick (Berlin, Germany). A Minolta CR-400 colorimeter (Osaka, Japan) was used to determine the meat colour [L^* (lightness), a^* (redness) and b^* (yellowness)] of the produced sausages. In addition, pictures of the produced sausages were taken.

2.5. Reagents

The solvents n-hexane, iso-octane, and ethyl acetate were purchased from LGC Standards (Wesel, Germany) in Picograde® quality. The drying material used in the PLE cells poly(acrylic acid), partial sodium salt-graft-poly(ethylene oxide), cross-linked, 90-

Table 2
Different process parameters of the smoking experiments.

Experiment	Smoking time (min)	Smoke density	Ventilator velocity (rpm)	Extra information
(A) Casing types (12 samples, 8 smoking experiments)				
A1 a, b, c, d (4 experiments)	12	Intensive	3000	Sheep and cellulose casings
A2 a, b, c, d (4 experiments)				
A3 a, b, c, d (4 experiments)	12	Intensive	3000	Collagen casing
(B) Fat contents (48 samples, 12 smoking experiments)				
B1 a, b (2 experiments)	12	Intensive	3000	Fat contents (10e39%)
B2 a, b (2 experiments)	12	Medium	1500	Fat contents (10e39%)
B3 a, b (2 experiments)	15	Intensive	3000	Fat contents (10e39%)
B4 (1 experiment)	14	Intensive	3000	Fat contents (10e39%)
B5 (1 experiment)	13	Intensive	3000	Fat contents (10e39%)
B6 (1 experiment)	12	Intensive	750	Fat contents (10e39%)
B7 (1 experiment)	13	Intensive	750	Fat contents (10e39%)
B8 (1 experiment)	13	Intensive	1500	Fat contents (10e39%)
B9 (1 experiment)	14	Intensive	1500	Fat contents (10e39%)
(C) Different positions in the smoking chamber (4 samples, 1 smoking experiment)				
Front, CentreeFront, CentreeBack, Back	12	Intensive	3000	Different positions in smoking chamber

850 μm was obtained from Sigma-Aldrich (Steinheim, Germany), and the glass microfiber filters were purchased from Büchi (Flawil, Switzerland). Extracts were filtered through 1.0 μm PTFE syringe filters purchased from Alltech (Unterhaching, Germany) or through 0.45 μm PTFE OPTI-Flow syringe filters obtained from Wicom (Heppenheim, Germany). The GPC column was filled with Bio-Beads S-X3 (200e400 mesh) purchased from Bio-Rad Laboratories (Munich, Germany). The last clean up step was performed by means of Supelclean™ LC-Si SPE Tubes, 6 mL (1 g), obtained from Supelco (Bellefonte, USA). A standard mixture of the isotope labelled or fluorinated 15 + 1 EU priority PAHs i.e. benzo[a] anthracene-$^{13}C_6$, chrysene-$^{13}C_6$, 5-methylchrysene-d_3, benzo[b] fluoranthene-$^{13}C_6$, benzo[k]fluoranthene-$^{13}C_6$, benzo[a]pyr- ene-$^{13}C_4$, dibenzo[a,h]anthracene-d_{14}, indeno[1,2,3-cd]pyrene-d_{12}, benzo[g,h,i]perylene-$^{13}C_{12}$, dibenzo[a,e]pyrene-$^{13}C_6$, dibenzo[a,i] pyrene-$^{13}C_{12}$, 13-fluorodibenzo[a,l]pyrene, and 5-fluorobenzo[c] fluorene was prepared in isooctane mixing the solutions of the
single compounds purchased from LGC Standards [(^{13}C and ^{2}H labelled compounds) Wesel, Germany] and the Biochemical Institute for Environmental Carcinogens [(fluorinated compounds) Grosshansdorf, Germany], respectively. The PAH recovery standard mixture consisted of benzo[a]anthracene-d_{12}, benzo[a]pyrene-d_{12} and benzo[g,h,i]perylene-d_{12} (LGC Standards, Wesel, Germany) in isooctane. For the response factor calibration, the reference standard PAH-Mix 183, containing all 15 +1 EU priority PAHs obtained from Dr. Ehrenstorfer (Augsburg, Germany) was applied.

The native standards of guaiacol (2-methoxyphenol), 4-methylguaiacol (2-methoxy-4-methylphenol), syringol (2,6-dimethoxyphenol), eugenol (4-allyl-2-methoxyphenol), and iso-eugenol (cis/trans; 2-methoxy-4-(1-propenyl)phenol) were obtained exclusively from Alfa Aesar (Karlsruhe, Germany). Isotope labelled $^{13}C_6$-guaiacol was obtained from LGC Standards (Wesel, Germany), and guaiacol-d_4 was purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). All phenolic standards were dissolved in ethyl acetate. Lithium chloride was purchased from J.T. Baker (Deventer, Netherlands), and Na_2SO_4 and $NaHCO_3$ were both purchased from Merck (Darmstadt, Germany). Ethyl acetate was obtained from LGC Standards (Wesel, Germany), and diethyl ether was purchased from Acros organics (New Jersey, USA). BSTFA (N,O-bis(trimethylsilyl)trifluoroacetamide) was obtained from Supelco (Bellefonte, USA), and Hypersep Si SPE cartridges were purchased from ThermoFisher Scientific (Bellefonte, USA).

2.6. Analysis of the PAH contents

The PAHs were determined using a modified analytical method based on a previously published method (Pöhlmann et al., 2012).

2.6.1. Pressurized liquid extraction (PLE)

About 3 g of homogenized Frankfurter-type sausages or 3 g of cut cellulose casing were mixed with an equal amount of the drying material poly(acrylic acid), partial sodium salt-graft-poly(ethylene oxide). The resulting material was transferred into 40 mL cells, which were equipped with disposable glass-fibre filters and 3 g of drying material. Afterwards, 50 μL of a PAH standard mixture containing isotope labelled (^{13}C and ^{2}H) and fluorinated PAH compounds were added as internal standard. The PLE extraction was performed with a Speed Extractor E-916 obtained from Büchi (Flawil, Switzerland) and n-hexane as solvent. Two static cycles were accomplished (operating conditions: 70 °C, 70 bar, static time 10 min and purge time 120 s). The solvent of the extract was evaporated in a water bath (40 °C) using a nitrogen stream and the resulting fat was weighed.

2.6.2. Gel permeation chromatography (GPC)

The evaporated PLE extract was dissolved in 4.5 mL cyclohexane/ethyl acetate (1:1, v/v) and filtered through a polytetrafluoroethylene (PTFE) syringe filter of a pore size of 1 μm or 0.45 μm, if necessary. The GPC column (25 mm i.d.) was filled with 60 g Bio-Beads S-X3. The samples were eluted at a flow rate of 5 mL/min applying cyclohexane/ethyl acetate (1:1, v/v). The GPC solvent was removed by a rotary evaporator, and, finally, the eluate was dried in a nitrogen stream. Waste time was 0e36 min and collect time 36e65 min. The dried GPC eluate was dissolved in 1 mL cyclohexane.

2.6.3. Solid phase extraction (SPE)

The samples were transferred onto silica gel SPE cartridges, conditioned with 3 mL cyclohexane, and eluted with further 10 mL cyclohexane.

2.6.4. Preparation for GC/MS analysis

The dried eluate of SPE was dissolved in 1 mL isooctane and 50 μL of the PAH-recovery standard mixture and transferred to a 1 mL tapered vial. The remaining sample was carefully concentrated in a nitrogen stream to a volume of about 50 μL.

2.6.5. Fast-GC/HRMS analysis

Fast GC/HRMS (Ziegenhals et al., 2008) was performed using a Trace-GC chromatograph (ThermoFisher Scientific, Milan, Italy) equipped with a split/splitless injection port. A chromatographic separation of the 15 + 1 EU priority PAHs (with the exception of a separation of CHR and triphenylene (TP)) was performed on a TR-50MS column (10 m × 0.1 mm × 0.1 μm) (ThermoFisher Scientific, Bremen, Germany). The injection temperature was 260 °C and the

injection volume 1.5 µL (splitless). Helium with a constant flow of 0.6 mL/min was used as carrier gas. The following temperature program was applied: isothermal at 140 °C for 1 min, at 10 °C/min to 240 °C, at 5 °C/min to 270 °C, at 30 °C/min to 280 °C, at 4 °C/min to 290 °C, at 30 °C/min to 315 °C, and at 3 °C/min to 330 °C.

The identification of the PAHs by GC/HRMS was performed using a sector mass spectrometer DFS (ThermoFisher Scientific, Bremen, Germany) working in the electron impact (EI) positive ion mode, applying an electron energy of 45 eV. The temperatures of the source and the transfer line were heated up to 280 °C and 300 °C, respectively. The resolution of the MS was tuned to 8000 (10% valley definition).

2.7. Analysis of phenolic compounds

The five phenolic compounds were determined using a method based on a previously published method (Pöhlmann et al., 2012). 3 g of homogenized sausage or 3 g of cellulose casing were used for analysis. The phenolic compounds were distilled with an aqueous LiCl solution (30%) in an Antonacopoulos apparatus (Antonacopoulos, 1960; Toth, 1982). After adjusting to pH 5, the distillate was extracted three times with diethyl ether in a separatory funnel. The solvent was removed with a rotary evaporator, and the phenolic compounds were dissolved in ethyl acetate. For cleaning up, the extract was applied to a silica cartridge and eluted with ethyl acetate. The eluate was derivatised with BSTFA. The trimethylsilylated phenolic extract was analysed by GC/MS using an Agilent 7890A GC coupled with an Agilent 5975C inert mass spectrometric detector. The GC was equipped with a DB-5MS capillary column (30 m × 0.25 mm i.d. × 0.25 µm) obtained from Agilent (Waldbronn, Germany). The GC/MS conditions are described elsewhere (Pöhlmann et al., 2012).

2.8. Statistical analysis

The statistical analysis was performed using the Statistica 7.1 software (StatSoft Inc., 2005).

3. Results and discussion

3.1. Results of pH, weight loss, colour analysis, and fat determination

The mean pH-value of the Frankfurter-type sausages of all the experiments (A, B, and C) was 6.08 ± 0.06, and the mean weight loss was 6.6% for A1 (cellulose), 7.8% for A2 (sheep), and 6.0% for A3 (collagen). The higher the fat content was in the Frankfurter-type sausages, the lower was the weight loss. The lightness (L^*-value), redness (a^*-value) and the yellowness (b^*-value) of the Frankfurter-type sausages are shown in Table 3. Although the sausages with different fat contents were smoked simultaneously under the same smoking conditions, the colours of the sausages were different as the different formulations of the sausages had a significant influence on the colour. The mean L^*-value for all smoked sausages with different casings (A1-3) was 55.5, the a^*-value 19.2, and the b^*-value 31.5. The mean fat contents of the smoked sausages with different back fat content are shown in Table 3.

3.2. Smoke generation temperatures and gas concentrations of the different smoking experiments

The maximum of smoke generation temperature for the smoking experiments with different casings (A) ranged from 589 °C to 775 °C. The highest maxima of the smoke generation temperatures for the experiments with different fat contents were 848 °C and 816 °C for smoking experiments B3a and B3b (intensive smoke, 3000 rpm, 15 min smoking time), and the lowest maxima of the smoke generation temperatures were 550 °C and 551 °C for smoking experiments B2a and B2b (medium smoke, 1500 rpm, 21 min smoking time). The smoke generation temperature increased with longer smoking times, lower ventilator velocities, and higher smoke density.

The concentrations of CO and CO_2 in the smoking chamber were higher for intensively smoked sausages than for medium smoked sausages. Longer smoking times and lower ventilator velocities also increased the concentration of CO and CO_2.

3.3. Effect of the casings

3.3.1. Correlation between the PAH contents and the type of casing

The contents of the analysed PAH compounds in Frankfurter-type sausages with different casings are shown as mean, min and max values in Table 4. In the experiments A1a-A3d, three types of casings (peelable cellulose, sheep, and collagen) were used. The mean contents of experiments A1a-A3d in the edible part of the smoked Frankfurter-type sausages were 6.45 ± 3.74 µg/kg (15 + 1 EU priority PAHs), 2.44 ± 1.44 µg/kg (PAH4), and 0.35 ± 0.24 µg/kg (BaP). Frankfurter-type sausages with peelable cellulose casings (A1a-d) showed PAH contents of 1.98 ± 0.41 µg/kg (15 + 1 EU priority PAH), 0.75 ± 0.19 µg/kg (PAH4), and 0.09 ± 0.03 µg/kg (BaP). The sausages with the sheep casings had higher PAH contents than the peeled cellulose sausages and contained 8.80 ± 2.75 µg/kg (15 + 1 EU priority PAHs), 3.59 ± 1.09 µg/kg (PAH4), and 0.57 ± 0.21 µg/kg (BaP). The collagen casings showed a similar tendency (15 + 1 EU priority PAHs: 8.80 ± 1.85 µg/kg, PAH4: 2.98 ± 0.63 µg/kg and BaP: 0.40 ± 0.12 µg/kg) (Fig. 1).

The cellulose casings of the experiments A1a-d were also analysed and contained high PAH contents compared to the PAH contents in the sausages (15 + 1 EU priority PAHs: 179 ± 66 µg/kg, PAH4: 81 ± 31 µg/kg and BaP: 23 ± 11 µg/kg). A cellulose casing accounted for about 1.5% of the weight of a total Frankfurter-type sausage before peeling. Considering the different weight proportions of the cellulose casing and the edible part of the sausage in relation to the PAH content, the complete Frankfurter-type

Table 3
Results of pH, weight loss, colour analysis, and fat determination for the different smoking experiments.

	A Casing types			B Fat contents				C Different positions
	Cellulose	Sheep	Collagen	9.9%	19.6%	29.5%	39.1%	
Mean fat content of smoked sausages (%)				14.6	22.0	31.0	38.2	
pH-value	6.05	6.12	6.04	6.06	6.07	6.06	6.07	6.2
Weight loss (%)	6.6	7.8	6.0	9.3	8.7	7.2	6.5	7.8
Colour								
L^*-value	56.6	57.0	54.3	49.0	52.3	56.3	57.8	57.3
a^*-value	18.7	18.2	20.1	22.1	21.0	19.1	18.0	18.2
b^*-value	29.2	31.5	33.9	30.9	32.4	33.5	33.2	31.8

Table 4
PAH contents [µg/kg] in smoked sausages with different casings (A).

Experiment	Casing	BaP [µg/kg]	PAH4 [µg/kg]	15 + 1 EU-PAH [µg/kg]
A1 mean	Cellulose	0.09	0.75	1.98
A1 min	Cellulose	0.08	0.58	1.63
A1 max	Cellulose	0.14	1.03	2.56
A2 mean	Sheep	0.57	3.59	8.80
A2 min	Sheep	0.37	2.60	6.21
A2 max	Sheep	0.81	4.77	11.74
A3 mean	Collagen	0.40	2.98	8.59
A3 min	Collagen	0.29	2.43	6.86
A3 max	Collagen	0.54	3.87	11.17

sausages (with cellulose casings) would have contained 1.95 ± 0.58 µg/kg (PAH4), 0.62 ± 0.16 µg/kg (BaA), 0.58 ± 0.14 µg/kg (CHR), 0.32 ± 0.12 µg/kg (BbF), and 0.43 ± 0.19 µg/kg (BaP). Consequently, the cellulose casings contained 61 ± 11% of the total PAH4 content, 55 ± 12% of the total BaA content, 49 ± 14% of the total CHR content, 71 ± 10% of the total BbF content, and 77 ± 7% of the total BaP content of an unpeeled Frankfurter-type sausage. The tendency of being accumulated in the cellulose casings was stronger for the five-ring molecules BbF and BaP than for the four-ring molecules BaA and CHR.

The edible part of the Frankfurter-type sausages of experiments A1a-d, the sausages filled in collagen casings (A2a-d), and the sausages filled into sheep casings (A3a-d) showed similar contributions of the single compounds to the PAH4 contents (A1a-d: 36% BaA, 40% CHR, 12% BbF, and 13% BaP; A2a-d: 40% BaA, 32% CHR, 12% BbF and 16% BaP; A3a-d: 41% BaA, 34% CHR, 12% BbF, and 13% BaP). However, the cellulose casings of experiments A1a-d had another composition of the PAH4: 29% BaA, 24% CHR, 19% BbF, and 28% BaP. Compared to the peeled sausages, this corresponded to the results of the favoured accumulation of the five-ring molecules BbF and BaP in the cellulose casing.

3.3.2. Correlation between the contents of phenolic substances and the type of casing

The contents of the analysed phenolic compounds in Frankfurter-type sausages with different casings are shown as mean, min and max values in Table 5. The quantification was performed by a response factor calibration. All compounds were quantified using $^{13}C_6$-guaiacol as internal standard and guaiacol-d_4 as recovery standard.

The cellulose-cased sausages were peeled after the smoking procedure, and the peeled sausage as well as the casing were analysed separately. The contents of the phenolic compounds depended on the type of casing. In sausages with sheep casings, the highest sum contents of the five phenolic compounds (mean 93.6 mg/kg) were analysed (Fig. 1). The corresponding contents of the sausages with collagen and cellulose casings were considerably lower. In sausages with cellulose casings, the sum content of the five phenolic compounds was in mean 48.8 mg/kg and for collagen casings 46.6 mg/kg. The mean percentage contributions of the single phenolic compounds to the sum content of the five phenolic substances for all casing types were as follows: *trans*-isoeugenol (47 ± 6%), syringol (32 ± 7%), 4-methylguaiacol (9 ± 2%), guaiacol (6 ± 1%), and eugenol (5 ± 1%). The percentage contributions of guaiacol, 4-methylguaiacol, and eugenol were similar for all types of casings. In cellulose-cased sausages, the percentage contribution of *trans*-isoeugenol (37%) was a little lower and the percentage content of syringol (42%) was a little higher than for the other casings (in mean for collagen and sheep casings: syringol (30%) and *trans*-isoeugenol (50%)).

The weight proportion of the casing was about 1.5% of the total sausage. The mean sum content of the five phenolic compounds in the peeled cellulose casing was 30.3 mg/kg and, consequently, in consideration of the weight proportion only 1.0% of the sum content of the complete sausage (48.8 mg/kg). The different distributions of the single phenolic compounds between the casing and the peeled sausages were also calculated. Guaiacol and eugenol had a percentage content of 0.7% in the cellulose casing and 99.3% in the peeled sausage, and 4-methylguaiacol and *trans*-isoeugenol had a percentage content of 0.5% in the cellulose casing and 99.5% in the peeled sausage. The percentage content of syringol was 1.7% in the casing and 98.3% in the peeled sausage. Furthermore, the percentage contributions of syringol, *trans*-isoeugenol, and 4-methylguaiacol to the sum content of the five phenolic

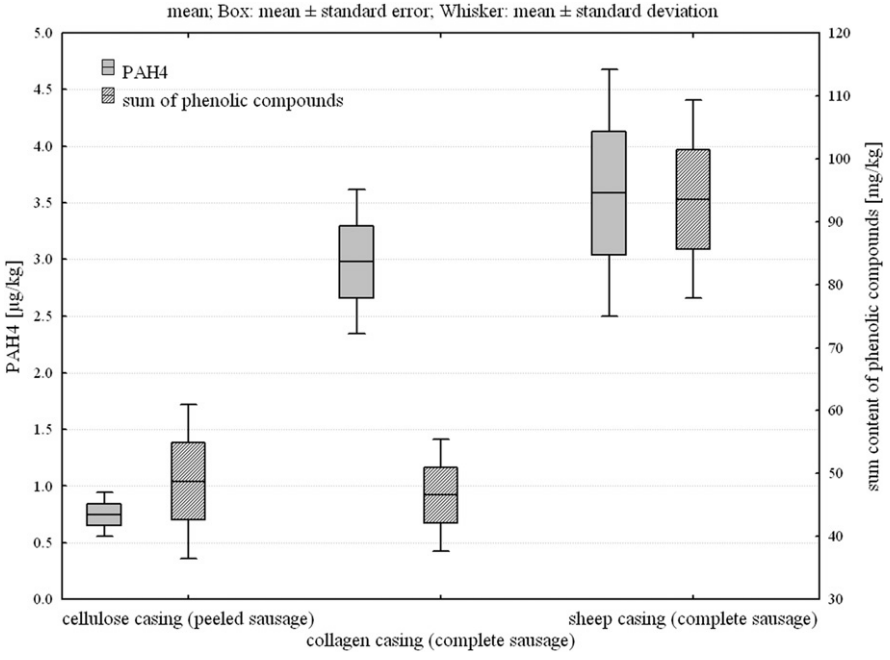

Fig. 1. PAH4 contents [µg/kg] and sum contents of the five phenolic compounds [mg/kg] in Frankfurter-type sausages in cellulose casing (peeled sausage), collagen casing (complete sausage), and sheep casing (complete sausage) (N ¼ 4).

Table 5
Contents of phenolic substances [mg/kg] in smoked sausages with different casings (A).

Experiment	Casing	Guaiacol [mg/kg]	4-Methylguaiacol [mg/kg]	Syringol [mg/kg]	Eugenol [mg/kg]	*Trans*-isoeugenol [mg/kg]	Sum of 5 phenols [mg/kg]
A1 mean	Cellulose	2.9	4.9	17.3	2.6	21.0	48.8
A1 min	Cellulose	2.4	3.5	16.5	1.9	15.0	40.2
A1 max	Cellulose	3.7	7.9	18.1	4.2	34.4	66.8
A2 mean	Sheep	6.0	10.1	25.9	5.0	46.6	93.6
A2 min	Sheep	5.0	7.9	20.1	3.7	34.2	77.9
A2 max	Sheep	7.7	12.4	31.3	6.7	60.3	109.2
A3 mean	Collagen	2.0	3.6	15.0	2.6	23.3	46.6
A3 min	Collagen	1.2	2.5	11.6	2.5	19.8	37.6
A3 max	Collagen	2.7	4.5	18.8	2.7	26.6	55.5

substances in the sausage and the casing were different: In the casing, the percentage contribution of syringol was 65% (peeled sausage: 42%), of *trans*-isoeugenol 21% (peeled sausage: 37%), and of 4-methylguaiacol 6% (peeled sausage: 10%).

3.4. Effect of the fat contents

3.4.1. Correlation between the PAH contents and the fat contents in Frankfurter-type sausages

The contents of the analysed PAH compounds in Frankfurter-type sausages with different back fat contents are shown in Table 6. For the experiments B1a,b e B9, the mean content of the 15 + 1 EU priority PAH was 8.3 ± 3.0 µg/kg and of PAH4 3.5 ± 1.1 µg/kg. The mean contents of the single compounds of PAH4 were as follows: BaA 1.1 ± 0.4 µg/kg, CHR 1.0 ± 0.4 µg/kg, BaP 0.8 ± 0.4 µg/kg, and BbF 0.5 ± 0.2 µg/kg. The highest PAH4 and CHR contents were analysed in the samples of experiment B3a (intensive smoke, ventilator velocity 3000 rpm, smoking time: 15 min), and 19.6% back fat (6.2 µg/kg (PAH4); 2.0 µg/kg (CHR)). The Frankfurter-type sausages in experiment B3a with 39.1% back fat showed the highest 15 + 1 EU priority PAH contents (16.0 µg/kg) and BaP contents (1.4 µg/kg). Also for BbF, the highest contents were analysed in B3a, but for 29.5% back fat (0.9 µg/kg). For BaA, the maximum content of 2.0 µg/kg resulted for experiment B1b (intensive smoke, ventilator velocity 3000 rpm, smoking time: 12 min), and 39.1% back fat.

Table 6
Contents of PAH [µg/kg] in smoked sausages with different fat contents applying different smoking conditions (B) and different positions in the smoking chamber (C).

Experiment	BaP [µg/kg]	PAH4 [µg/kg]	15 + 1 EU-PAH [µg/kg]
B1-10%	0.41	2.14	5.14
B1-20%	0.55	2.99	7.25
B1-30%	0.67	4.28	10.56
B1-39%	0.76	4.78	11.59
B2-10%	0.29	1.71	4.11
B2-20%	0.39	2.58	6.35
B2-30%	0.43	2.97	7.44
B2-39%	0.43	3.17	7.88
B3-10%	0.76	3.47	8.58
B3-20%	1.06	5.36	13.95
B3-30%	1.13	5.05	15.57
B3-39%	1.05	4.77	13.17
B4-10%	0.51	2.81	6.68
B4-20%	0.67	3.75	9.36
B4-30%	0.73	3.41	9.16
B4-39%	0.76	4.15	9.80
B5-10%	0.50	2.74	6.28
B5-20%	0.56	3.04	7.30
B5-30%	0.82	3.92	11.35
B5-39%	0.75	3.44	9.20
B6-10%	0.45	2.28	5.52
B6-20%	0.85	3.31	8.79
B6-30%	0.70	3.52	9.40
B6-39%	0.80	3.52	8.88
B7-10%	0.31	1.75	3.91
B7-20%	0.32	2.12	5.38
B7-30%	0.52	2.84	7.12
B7-39%	0.66	3.50	9.12
B8-10%	0.49	2.19	5.80
B8-20%	0.42	2.38	5.81
B8-30%	0.59	3.03	6.63
B8-39%	0.72	3.49	8.59
B9-10%	0.40	1.87	4.49
B9-20%	0.43	2.05	5.05
B9-30%	0.56	2.86	6.92
B9-39%	0.62	3.02	7.23
C-front	1.05	4.22	10.16
C-centreefront	1.01	3.90	9.35
C-centreeback	1.10	4.57	10.73
C-back	0.88	3.62	8.36
C mean ± stdev.	1.01 ± 0.09	4.08 ± 0.41	9.65 ± 1.03

The lowest contents of 15 + 1 EU priority PAHs (3.7 µg/kg), PAH4 (1.5 µg/kg), BaA (0.5 µg/kg), CHR (0.5 µg/kg), BbF (0.2 µg/kg), and BaP (0.3 µg/kg) were all detected in the samples of experiment B2b (medium smoke, ventilator velocity 1500 rpm, smoking time: 21 min) with a back fat content of 9.9%. The different contents of back fat in the Frankfurter-type sausages resulted in different PAH contents. Increasing the back fat content from 9.9% to 19.6%, to 29.5%, and up to 39.1% the mean PAH4 content also increased from 2.4 ± 0.7 µg/kg to 3.2 ± 1.2 µg/kg, to 3.7 ± 0.9 µg/kg, and up to 3.9 ± 0.9 µg/kg. The same tendencies were observed for BaA, CHR, BbF, and BaP.

The percentage contributions of BaA, CHR, BbF, and BaP to PAH4 in Frankfurter-type sausages with 9.9%, 19.6%, 29.5%, and 39.1% back fat were in mean 35% BaA, 32% CHR, 13% BbF, and 19% BaP. The sausages with the lowest back fat content showed slightly higher percentage contributions of the heavier five ring molecules BbF and BaP to PAH4. On the other hand, sausages with higher back fat contents (19.6%, 29.5%, and 39.1%) showed slightly higher percentage contributions of the lighter four ring molecules BaA and CHR.

For a better comparability of different smoking conditions, the PAH4 content in Frankfurter-type sausages with a back fat content of 39.1% was set to 100%, and the other three remaining PAH4 contents of the same smoking experiment were compared to this value in percent.

To check the influence of the back fat contents of the sausages on the PAH contents in dependence on the absolute PAH4 content, three groups were formed: the first group consisted of the four lowest PAH4 contents for sausages with 9.9% back fat (B2a,b; B7; B9; PAH4 <2 µg/kg), the second group of the four medium PAH4 contents for sausages with 9.9% back fat (B1a,b; B6; B8; 2 µg/kg < PAH4 < 2.5 µg/kg), and the third group of the four highest PAH4 contents for sausages with 9.9% back fat (B3a,b; B4; B5; PAH4 >2.5 µg/kg). The relative PAH4 contents of the first group with low PAH4 contents and the second group with medium PAH4 contents increased with increasing back fat contents, showing a similar behaviour: The PAH4 contents increased from 55% (back fat content: 9.9%) to 72% (back fat content: 19.6%) and up to 91% (low) and 93% (medium), respectively (back fat content: 29.5%) (Fig. 2). In

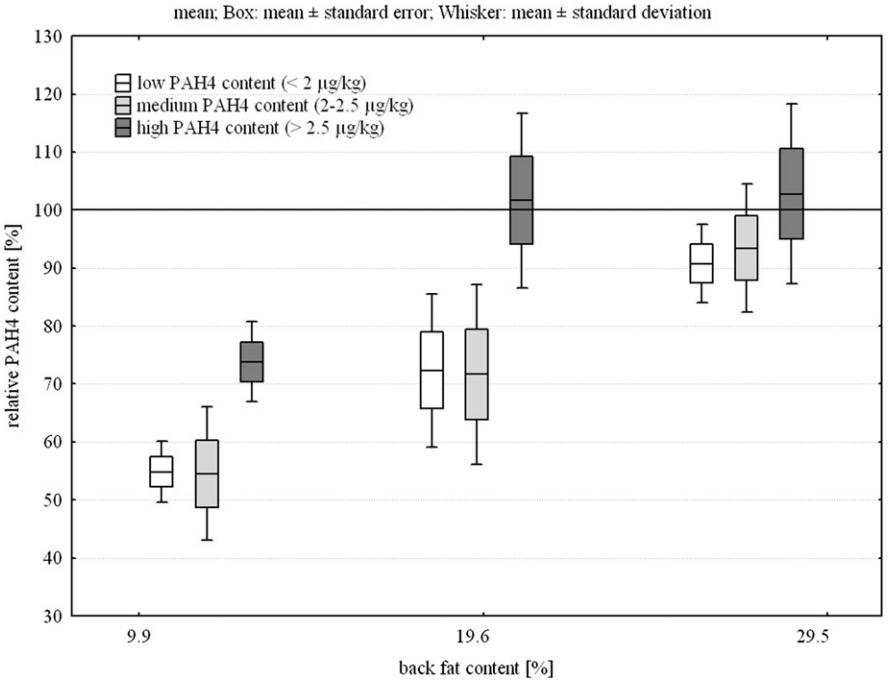

Fig. 2. Correlation between normalised PAH4 contents (39.1% fat ¼ 100%) and fat content in Frankfurter-type sausages with low (<2 µg/kg), medium (2e2.5 µg/kg), and high (>2.5 µg/kg) PAH4 contents (*N* ¼ 4, each).

contrast, sausages of the third group with high absolute PAH4 contents showed similar relative PAH4 contents for back fat contents of 19.6% and 29.5% (102% and 103%, respectively); as for back fat contents of 39.1%, only sausages with 9.9% back fat had a lower relative PAH4 content (74%).

The weight loss of sausages with lower back fat content was higher than in sausages with high back fat content. However the PAH contents were lower in sausages with higher weight loss than in sausages with lower weight loss. This effect is most likely due to the decreasing lipophilic character of the surface of the sausage from lowering the back fat contents in the formulation. However, this effect is less pronounced for intensively smoked sausages with higher PAH contents (PAH4 >2.5 µg/kg). For these sausages, a noticeable reduction of the PAH contents was observed by lowering the back fat content from 20% to 10%. For sausages with back fat contents of 20%, 30%, and 39%, the PAH contents were nearly at the same level. For Frankfurter-type sausages with low and medium PAH contents, a noticeable reduction of the PAH contents was observed by lowering the back fat contents from 30% to 20%.

3.4.2. Correlation between the contents of phenolic substances and the fat contents in Frankfurter-type sausages

The contents of the phenolic compounds in the smoked Frankfurter-type sausages with different back fat contents are shown in Table 7. The highest sum content of the five phenolic compounds was analysed in the intensively smoked sausages applying a smoking time of 15 min (B3a and b) (107 mg/kg), and the lowest amounts were detected in intensively smoked sausages applying a ventilator velocity of 750 rpm and a smoking time of 12 min (41 mg/kg). The mean percentage contribution of the single phenolic compounds to the sum content of the five phenolic substances was as follows: eugenol (5%), guaiacol (8%), 4-methyl-guaiacol (11%), *trans*-isoeugenol (36%), and syringol (39%). Between sausages with different fat contents, no differences in the sum contents of the five phenolic compounds were observed. The mean sum contents were as follows: 62.2 mg/kg (fat content: 9.9%), 62.0 mg/kg (fat content: 19.6%), 63.0 mg/kg (fat content: 29.5%), and 64.6 mg/kg (fat content: 39.1%). The percentage contributions of the single phenolic compounds to the sum content of the five phenolic compounds showed a dependency on the fat content. The percentage contribution of syringol decreased with higher fat content from 44% (fat content: 9.9%) to 34% (fat content: 39.1%) and, in contrast, the percentage of *trans*-isoeugenol increased from 30% to 40%.

In order to check the correlation between the contents of phenolic substances and the fat contents in Frankfurter-type sausages applying different smoking conditions, the contents of the phenolic compounds of the sausages with a fat content of 39.1% were normalised to 100%. Consequently, it was possible to check the influence of the back fat contents on the contents of phenolic compounds independent of the applied smoking conditions. The normalised content of syringol decreased with higher fat contents from 130% (10% fat content), to 123% (19.6% fat content), to 108% (29.5% fat content), and finally to 100% (39.1% fat content). In contrast, the normalised *trans*-isoeugenol content increased from 79% (9.9% fat content) to 94% (19.6% fat content), 100% (29.5% fat content), and at last 100% (39.1% fat content). However, the normalised sum content of the five phenolic compounds was constant from 9.9% fat content (101%) to 19.6% fat content (102%), to 29.5% fat content (100%), and up to 39.1% fat content (100%).

3.5. Effect of the position in the smoking chamber

The PAH4 content for the different positions in the smoking chamber was in mean 4.1 ± 0.4 µg/kg. The percentage standard deviations for BaA, CHR, BbF and BaP were below 12%.

The mean sum content of the phenolic compounds in Frankfurter-type sausages smoked in different positions in the smoking chamber was 69.7 ± 4.3 mg/kg. The percentage standard deviation for all phenolic compounds was below 10%. The four different positions showed no effect on the phenolic contents and the pattern of phenolic compounds.

Table 7
Contents of phenolic substances [mg/kg] in smoked sausages with different fat contents applying different smoking conditions (B) and different positions in the smoking chamber (C).

Experiment	Guaiacol [mg/kg]	4-Methylguaiacol [mg/kg]	Syringol [mg/kg]	Eugenol [mg/kg]	*Trans*-isoeugenol [mg/kg]	Sum of 5 phenols [mg/kg]
B1-10%	2.7	3.9	18.2	2.3	15.4	42.5
B1-20%	2.3	3.9	16.2	2.5	21.2	46.2
B1-30%	2.8	4.9	15.2	3.3	25.4	51.6
B1-39%	3.0	5.2	13.9	3.3	24.7	50.1
B2-10%	3.6	4.2	20.2	1.8	13.2	43.0
B2-20%	3.3	4.3	18.5	2.2	16.5	44.8
B2-30%	2.3	4.3	15.9	2.2	18.4	43.0
B2-39%	2.4	4.2	16.1	2.1	22.4	47.1
B3-10%	8.4	10.5	39.7	3.9	33.4	95.9
B3-20%	7.5	11.0	35.5	4.5	38.7	97.2
B3-30%	7.7	11.2	34.7	4.7	38.7	97.0
B3-39%	8.6	13.3	35.0	5.7	46.5	109.1
B4-10%	7.5	9.9	39.6	3.4	29.1	89.4
B4-20%	8.0	11.4	37.0	4.2	28.3	88.9
B4-30%	8.0	11.6	33.9	4.4	40.4	98.3
B4-39%	9.2	14.2	35.9	5.3	29.1	93.7
B5-10%	7.2	9.3	37.0	3.7	31.9	89.1
B5-20%	7.6	10.4	35.6	3.9	9.5	67.0
B5-30%	7.6	11.4	35.8	4.4	36.0	95.2
B5-39%	7.7	11.6	32.6	4.9	41.3	98.1
B6-10%	6.8	5.8	20.0	2.5	13.9	49.0
B6-20%	4.3	5.0	21.2	2.5	19.8	52.9
B6-30%	3.2	4.4	14.7	2.6	3.7	28.6
B6-39%	3.3	4.3	12.8	2.6	11.1	34.0
B7-10%	9.1	7.9	25.3	4.1	15.7	62.2
B7-20%	5.8	6.0	24.4	2.5	21.7	60.5
B7-30%	3.5	4.5	17.0	2.2	18.4	45.7
B7-39%	3.7	5.1	20.9	2.9	22.0	54.6
B8-10%	5.5	6.2	24.0	2.8	3.2	41.7
B8-20%	4.4	5.5	18.4	2.7	13.0	43.9
B8-30%	4.5	6.0	16.8	3.1	20.2	50.5
B8-39%	4.3	5.8	17.7	3.4	14.4	45.6
B9-10%	4.8	5.4	23.3	3.1	15.8	52.4
B9-20%	4.8	6.6	25.9	2.8	15.0	55.0
B9-30%	5.0	7.5	23.2	3.3	16.6	55.6
B9-39%	5.0	6.5	12.6	3.2	9.4	36.7
C-front	4.4	6.6	24.5	3.2	25.3	63.9
C-centreefront	4.5	7.1	27.7	3.9	30.5	73.8
C-centreeback	4.4	6.8	27.8	3.8	28.9	71.7
C-back	4.6	6.8	25.8	3.5	28.7	69.4
C mean ± stdev.	4.5 ± 0.1	6.8 ± 0.2	26.4 ± 1.6	3.6 ± 0.3	28.4 ± 2.2	69.7 ± 4.3

4. Conclusions

The selection of a cellulose-peelable casing is a reasonable approach for reducing the PAH contents in hot smoked sausages as a high percentage of the PAHs (BaP: 77%; PAH4: 61%) remains in the peelable casing and does not penetrate the inside of the meat product. In contrast, the major part of the phenolic compounds (about 99%) penetrates the inside of the sausage. A comparison of the sausages in the sheep casing and the collagen casing showed that the sum content of phenolic compounds was twice as high for sheep casings and the PAH contents were nearly at the same level.

A reduction of the PAH compounds in hot smoked Frankfurter-type sausages by lowering the back fat contents in the formulation of the sausages is also possible. In spite of the higher weight losses of sausages with lower fat contents, the PAH contents in these sausages were lower. A decrease in the amounts of phenolic substances was not observed by lowering the back fat contents in Frankfurter-type sausages as very similar contents of phenolic compounds were detected in sausages of all fat contents.

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