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Occurrence and exposure to polycyclic aromatic hydrocarbons (PAHs) in traditional dry-cured or smoked meat products from Brazil

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) are classified as environmental and food contaminants, with several adverse health effects, especially genotoxic and carcinogenic actions. In processed meats, they are the major contaminants, especially those subjected to smoking processes. Considering that the population is exposed to PAHs through several routes, and that the ingestion of contaminated food is considered the main one, evaluating the concentrations of these contaminants in food becomes essential, as well as the exposure and risk to the population at different ages through ingestion of the evaluated products. In the current study, the levels of nine PAHs (PAH9) were measured in 205 processed meat products commercially available and with high consumption in Brazil. The methodology involved saponification, extraction with n-hexane, purification with solid-phase extraction (SPE) silica cartridges, and quantification by liquid chromatography with fluorescence detection. In general, 83% of all samples were contaminated with at least one of the studied PAHs, and the measured PAH9 levels ranged between < LOQ-108.24 µg/kg. The highest mean of total PAHs was found in smoked sausage (108.24 µg/kg), while the lowest content was found in ham (1.83 µg/kg). Benzo[a]pyrene (BaP) and PAH4 (benz[a]anthracene, chrysene, benzo[b]fluoranthene, and BaP) exceeded the maximum permissible limits of the European Union (EU) in three (1.5%) and 18 samples (8.7%), respectively. The results of margin of exposure results (MOE ≥ 10,000) and incremental lifetime cancer risk (ILCR) values ($10-6 < ILCR < 10-4$) in all ten types of meat indicated there were low significant potential health problems related to meat products consumption for the Brazilian population.

Keywords Meat, Benzo[a]pyrene, Health risk assessment, Food safety, Chrysene

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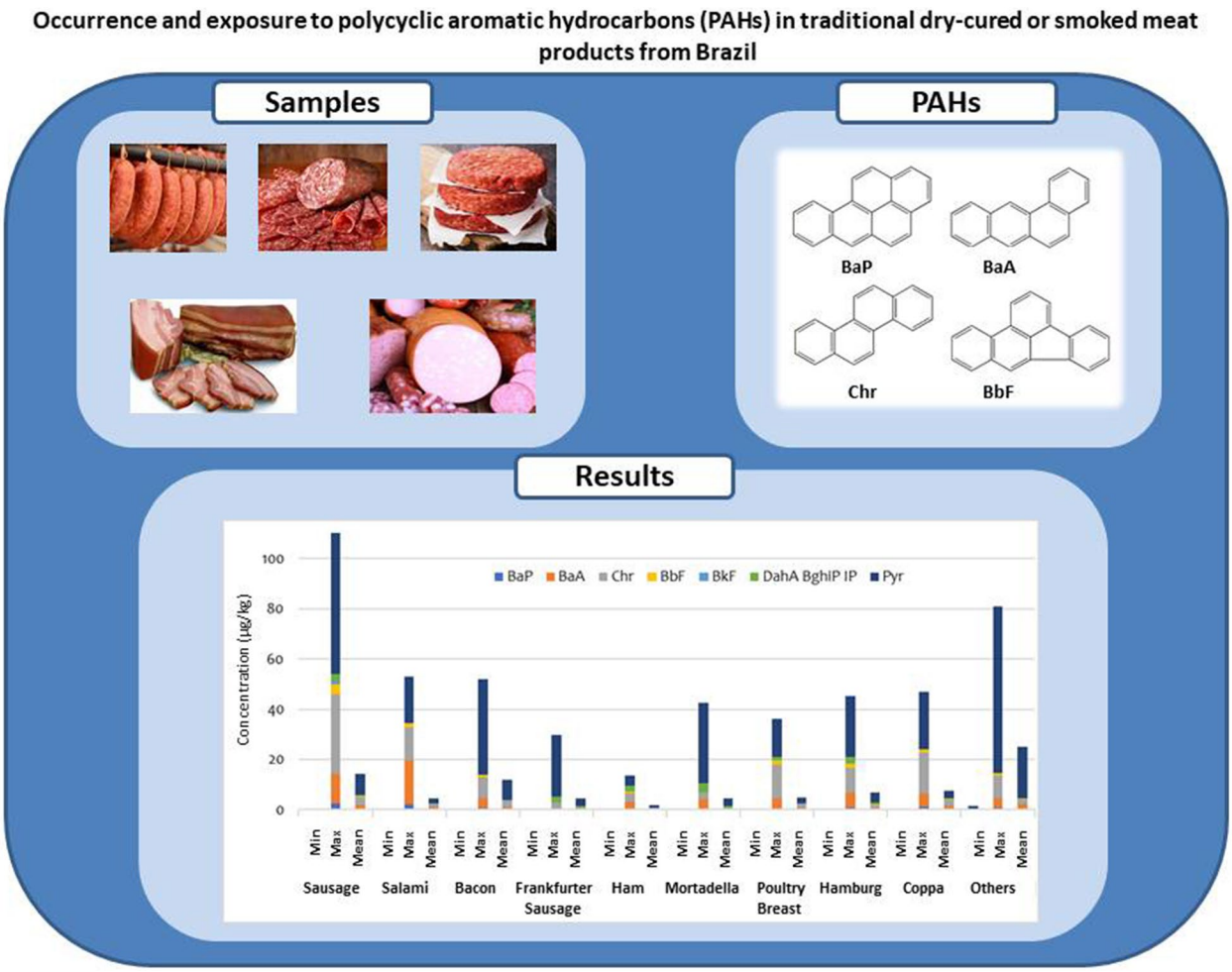
Graphical Abstract



Introduction

Meat is an important source of protein in the human diet, providing essential amino acids along with vitamins, minerals, and fatty acids (Dutta et al. 2022; Whitton et al. 2021). Processed meats, including sausage and others, are often associated with industrially produced products that undergo curing and/or smoking processes, potentially contributing to the formation of carcinogenic compounds such as polycyclic aromatic hydrocarbons (PAHs) that can lead to adverse health effects (Geiker et al. 2021).

Polycyclic aromatic hydrocarbons (PAHs) are contaminants composed of fused carbon and hydrogen aromatic rings, formed through the incomplete combustion of organic materials, and are classified as priority contaminants. In the environment, PAHs originate from both natural sources, such as forest fires and volcanic emissions, and predominantly from anthropogenic sources, including coal combustion, vehicle emissions, lubricating oils used in engines, and cigarette smoke. Humans are exposed to PAHs through inhalation, skin contact, and diet; however, dietary intake significantly impacts non-occupational exposure, with food consumption accounting for over 70% of exposure in non-smokers (Mallah et al. 2022; Sampaio et al. 2021; Sun; Wu; Gong, 2019).

The effects of PAHs on human health depend on various factors, including the duration and route of exposure, concentration, and toxicity (IARC 2010, 2012; Sampaio et al. 2021). Most studies associate PAHs effects with carcinogenicity, and recent data suggest that frequent exposure may increase the risk of

oxidative stress, thrombosis, hypertension, myocardial infarction, and cardiovascular disease (Mallah et al. 2022; Mirzababaei et al. 2022; Sampaio et al. 2021; Zhang et al. 2020).

The International Agency for Research on Cancer (IARC) classified carcinogenic substances into four categories: Group 1 (carcinogenic to humans), Group 2A (probably carcinogenic to humans), Group 2B (possibly carcinogenic to humans), and Group 3 (not classifiable as to its carcinogenicity to humans). Benzo[a]pyrene (BaP) belongs to Group 1 and has been investigated for several decades due to its toxic effects, particularly with regard to genotoxicity and carcinogenicity. Other compounds such as benz[a]anthracene (BaA), chrysene (Chr), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), and indeno[1,2,3-c,d]pyrene (IcdP) belong to Group 2B, and dibenz[a,h]anthracene (DahA) belongs to Group 2A (IARC 2010, 2012; Duedahl-Olesen et al. 2015; Mallah et al. 2022).

Regarding the occurrence of PAHs in food, various categories have been investigated, with meat products being the most commonly studied. In these, PAHs can be deposited during processing, such as smoking or drying. Smoking can be carried out using direct or indirect methods. In the direct techniques, also known as traditional techniques, the meat is smoked in the same chamber where combustion occurs, and PAHs can be formed due to incomplete combustion of the fuel used. Consequently, these compounds may be deposited on the food surface (Codex 2009; Ledesma et al. 2016). The indirect method involves using smoke generated by a friction generator or liquid smoke. Additionally, meat subjected to a conventional drying process, using sunlight, wind or hot air, goes through a slow procedure that may result in consequences stemming from prior environmental contamination or the combustion of combustible material to heat the air (Codex 2009; Ledesma et al. 2016; Aykın-Dinçer 2021).

In 2008, the European Food Safety Authority (EFSA) CONTAM Panel recommended that the analysis of BaP alone was not a sufficient indicator for all the genotoxic and carcinogenic PAHs present in food. Instead, they advised evaluating four PAHs (PAH4), namely BaA, Chr, BbF and BaP. Additionally, they noted that there were no significant differences in the concentrations of PAH4 and eight PAHs (PAH8) (PAH4, BkF, IcdP, DahA, and benzo[g,h,i]perylene (BghiP) (EFSA 2008). The European Commission considers PAH4 as indicative of the occurrence of these compounds in foods, and a separate maximum level for BaP is also established for future data comparison (EFSA 2008). According to Regulation No. 835/2011, the maximum levels of BaP and PAH4 in smoked meat were set at 2 and 12 μg/kg, respectively (EC 2011a).

International research on PAHs has been extensive, indicating that meat products are among the food items with the highest concentrations of PAHs (Chiang et al. 2021; Martorell et al. 2010). However, to our knowledge, national studies pertaining to these specific foods are still limited (Camargo; Toledo 2001; Schwert et al. 2020; Merlo et al. 2021; Silva et al. 2023), and none of them have focused on the diversity of categories presented in our study. Given the potential association between PAHs consumption and cancer development as a public health concern, it is crucial to assess PAHs levels and associated risks of chronic exposure through dietary intake.

The aim of this study was to investigate the concentrations of 9 PAHs in processed meat products available in Brazil. Additionally, health risk assessments were conducted for various age groups, estimating dietary daily intake PAHs exposure, incremental lifetime cancer risk (ILCR), and margin of exposure (MOE).

Materials and methods

Samples

From 2019 to 2021, a total of 205 samples of meat products were obtained from supermarkets and local markets in Brazil. The collected samples were classified into the following ten categories: fresh and smoked sausages ($n=55$), salami ($n=30$), smoked bacon ($n=23$), frankfurter sausage ($n=18$), ham ($n=16$), mortadella ($n=16$), cured poultry breast including turkey, chester and chicken ($n=15$), hamburgers ($n=15$), coppa ($n=10$), and other products [including shoulder clod ($n=2$), morcilla ($n=2$), pastrami ($n=1$), salmon ($n=1$), black butifarra ($n=1$)]. The samples were triturated, homogenized, and stored in labeled packages protected from light at -18 °C. Prior to analysis, samples were thawed at room temperature after being removed from the freezer.

Chemical and materials

A certified reference standard mixture of the PAH16 (2000 μg/mL in methylene chloride: benzene) was purchased from Supelco (St. Louis, MO, USA) and used for analysis of nine PAHs (PAH9), including Pyr, BaA, Chr, BbF, BkF, BaP, IcdP, DahA, and BghiP. Individual solutions of PAHs were used for PAH4 identification and quantification as BaA, Chr, BbF, and BaP (Supelco, St. Louis, MO, USA). Working standard mixtures were prepared by diluting in acetonitrile to a concentration of 200 ng/mL. Ultrapure water was obtained using a Millipore Milli-Q Ultra-pure water system (Millipore, Bedford, MA, USA). Acetonitrile (MeCN), ethanol, methanol (MeOH) and n-hexane were high-performance liquid chromatography (HPLC)-grade from

Loba Chemie (Mumbai, IND) and Merck (Darmstadt, Germany), and all other chemicals used were of analytical grade. Solid-phase extraction (SPE) cartridges with silica sorbent (Discovery DSC-Si 500 mg/6 mL from Supelco) were employed along with an automated solid-phase extraction system (GX-274 ASPEC system, Gilson Inc., Middleton, WI, USA). The solution was filtered through polytetrafluoroethylene (PTFE) syringe filters (15 mm, 0.2 µm).

PAHs analysis

PAHs were extracted according to the method described by Silva et al. (2023). Approximately 2.0 g of sample were weighed and placed into a glass tube. Subsequently, 5 mL of 2 M ethanolic KOH solution was added, and the mixture was hydrolyzed in a water bath for 3 h at 40 °C. After cooling, 5 mL of water were added, and then the PAHs were extracted three times with 10 mL of n-hexane. The combined extracts were transferred to a 125 mL separating funnel and mixed successively with 30 mL MeOH:H_2O (4:1, v/v), 30 mL MeOH:H_2O (1:1, v/v), and 30 mL of water. The mixture was then filtered over Na_2SO_4. The organic phase was evaporated to dryness using a stream of nitrogen gas, and the residue was suspended in a solution composed of the concentrate plus 3.0 mL of MeCN. The SPE cartridge was conditioned with 3 mL of MeCN, and 3 mL of diluted residue was loaded onto the cartridge (maximum flow rate: 2.0 mL/min). The cartridge was washed with 3 mL of MeCN, and eluate was evaporated to dryness at 30 °C using an Eppendorf 5301 vacuum concentrator (Eppendorf, Hamburg, Germany). The residue was reconstituted with 1.0 mL of MeCN, filtered through a PTFE syringe filter, and stored in a vial for chromatographic analysis.

A system of ultra-high-performance liquid chromatographic (UHPLC) Shimadzu Nexera® (Shimadzu, Kyoto, Japan) was used, consisting of a LC-30AD pump, SIL-30AC automatic injector, DGU-20A degasser, CTO-20A column oven, and RF-20A fluorescence detector. The data elaboration was performed by the LabSolution® software (Shimadzu). Chromatographic separation was carried out on a Zorbax Eclipse PAH column (100×2.1 mm, 1.8 µm, Agilent, Palo Alto, CA, USA), and Eclipse Plus guard column (5×2.1 mm, 1.8 µm, Agilent) at 30 °C using MeCN and water as the mobile phase for gradient elution: 0–0.9 min, 55% MeCN; 0.9–7.0 min, 75% MeCN; 7.0–10.0 min, 75%; 10.0–18.0 min, 100% MeCN; 18.0–23.0 min, 100% MeCN; 23.0–27.0 min, 55% MeCN; and 27.0–34.0 min, 55% MeCN. The mobile phase flow rate was 0.4 mL/min. The injection volume was 2 µL. The detection was performed using fluorescence detection and a program to excitation and emission wavelength was applied: 270/390 nm (for Pyr, BaA, and Chr), and 290/430 nm (for BbF, BkF, BaP, IcdP, DahA, and BghiP).

Quality assurance and quality control

The parameters evaluated included linearity, accuracy, precision (repeatability), limits of detection (LOD), and quantification (LOQ) (EC 2011b; INMETRO 2020). The matrix effect was investigated by comparing the slopes of curves in a fortified matrix and curves in solvent (MeCN). Standard curves were prepared using five different concentrations of PAHs (ranging from 0.50 to 20.0 ng/mL) in MeCN, in triplicate, for the standard curves, and linearity was assessed using peak areas measurements and concentrations determined by the external calibration curve method for the nine PAHs (PAH9). Recoveries were determined using the standard addition technique at three concentrations of spiked black matrix at low, medium, and high levels (three independent replicates), and were used to evaluate the accuracy (%) and precision by relative standard deviation (RSD, %). The LOD was calculated as three times the standard deviation (SD) of six replicate measurements of lowest concentrations of fortified sample (ranging from 0.25 to 1.0 µg/kg), and the LOQ was defined as ten times the SD, representing the lowest point of the calibration curve.

Exposure assessment and risk characterization

The risk indicators used in this study were: Dietary Daily Intake (ID), Incremental Lifetime Cancer Risk (ILCR), and Margin of Exposure (MOE). In this study, MOE was calculated for BaP, PAH4 and PAH8, while ILCR was determined for PAH4. If the concentrations were lower than the limit of quantification (LOQ), results such as half of the LOQ were used (Arisseto el al. 2017).

The population was divided into three age groups according to Instituto Brasileiro de Geografia e Estatística (IBGE) classifications: adolescents (10–19 years old), adults (20–59 years old), and seniors (≥60 years old),with anthropometric and food consumption data also incorporated (IBGE 2010, 2020a, 2020b).

The ID (ng/day) was estimated based on the concentrations of PAHs and the intake rate using the equation:

$$ID = Ci \times IR$$

where: Ci = individual PAHs concentration (ng/g); IR = meat product intake rate, for ham: 0.4 g/day, mortadella: 0.6 g/day, sausage (fresh and smoked): 3.9 g/day, frankfurter sausage: 1.1 g/day, hamburger (as beef-based

preparation): 7.5 g/day, and other products (as other cold cuts and sausages): 0.3 g/day (IBGE 2020a).

The MOE was determined using the following equation:

$$MOE = \frac{BMDL10 \times BW}{ID}$$

where: BMDL10 = lower bound of a 95% confidence interval of the benchmark dose of PAHs indicators, that caused a 10% tumor incidence in animal tests (0.07, 0.34 and 0.49 mg kg^{-1} bw day^{-1} for BaP, PAH4, and PAH8 respectively) (EFSA 2008); BW = average body weight (for adolescents: BW = 50.5 kg; for adults: BW = 67.5 kg; for seniors: BW = 64.9 kg) (IBGE 2010); ID = Dietary Daily Intake (ng/day).

The ILCR of Brazilian population was calculated using the equation:

$$ILCR = \frac{TEQ \times IR \times EF \times ED \times SF \times CF}{BW \times AT}$$

where: TEQ = toxic equivalency quotients (μg/kg); IR = ingestion of meat product (g/day) IBGE (2020a); EF = exposure frequency = 365 days/year; ED = exposure duration (for adolescents: ED = 10 years; for adults: ED = 40; for seniors: ED = 17) (IBGE 2010, 2020a, 2020b); SF = carcinogenic slope factor for ingestion of BaP (7.3 mg/kg. day) (Wang et al. 2021); CF = unit transformation factor (10^{-6} mg/ng); BW = average body weight (for adolescents: BW = 50.5 kg; for adults: BW = 67.5 kg; for seniors: BW = 64.9 kg) (IBGE 2010); AT = average life expectancy (76.6 years in Brazil) (IBGE 2020b).

The TEQ (ng/kg) was estimated using the equation:

$$TEQ = \Sigma[Ci \times TEFi]$$

where: Ci = individual PAHs concentration (ng/g); TEF = 0.1 (BaA and BbF), 0.01 (Chr) and 1 (BaP).

Statistical analysis

Data of commercial samples were analyzed in duplicate experiments. All results were presented as mean. The statistical evaluation was performed using Action 2.5 Software, Microsoft Office Excel, and Statistica 13.4 Software (TIBCO).

Results and discussion

Quality assurance and quality control

The method validation parameters are presented in Table 1 and were evaluated following the guidelines provided by the European Commission (EC 2011b) and INMETRO (2020). Figure 1 illustrates the chromatograms of PAH9 standards and a blank spiked sample.

No matrix effect was observed for all PAH9, and solvent-based calibration curves were used for the linearity study based on least-squares methods. The square correlation coefficients (r^2) ranged from 0.9964 to 0.9991, indicating good linear regressions. Accuracy rates and precision were evaluated by adding three concentrations (2.5, 5.0 and 10.0 μg/kg) of PAH9. The average recoveries ranged from 84.60–101.30%, with relative standard deviations (RSD) less than 10%, considered adequate, within performance criteria (EC 2011b). The LOD ranged from 0.15 to 0.30 μg/kg, and the LOQ ranged from 0.50 to 1.00 μg/kg. All criteria indicated a method with good precision, accuracy, and sensibility for the determination of PAH9 in different meat products.

PAHs in processed meat samples

The occurrence of PAH9 was investigated in 205 marketed meat products, and 83% (170/205) of the samples analyzed were contaminated with at least one PAH. The overall frequency (% of quantitative samples / Total tested samples × 100), detected range, mean, and median values are presented in Table 2. PAHs were quantified especially in the categories of hamburger (15/15) and

Table 1 Parameters for analysis of PAH9 in meat products

Analyte	Calibration curve		LOD (μg/kg)	LOQ (μg/kg)	Recovery (%)	Precision RSD (%)
	Linear range (ng/mL)	r^2				
Pyr	1.00—20.00	0.9989	0.30	1.00	101.30	8.27
BaA	2.00—20.00	0.9964	0.30	1.00	94.18	0.63
Chr	1.00—15.00	0.9989	0.15	0.50	92.71	3.70
BbF	1.00—15.00	0.9990	0.15	0.50	84.60	2.68
BkF	1.00—20.00	0.9991	0.30	1.00	97.28	9.02
BaP	1.00—15.00	0.9981	0.15	0.50	90.43	1.80
IcdP + DahA + BghiP	1.00—20.00	0.9990	0.30	1.00	97.68	1.80

Pyr pyrene, *BaA* benz[a]anthracene, *Chr* chrysene, *BbF* benzo[b]fluoranthene, *BkF* benzo[k]fluoranthene, *BaP* benzo[a]pyrene, *IcdP* indeno[1,2,3-c,d]pyrene, *DahA* dibenz[a,h]anthracene, *BghiP* benzo[g,h,i]perylene, r^2 coefficient of determination

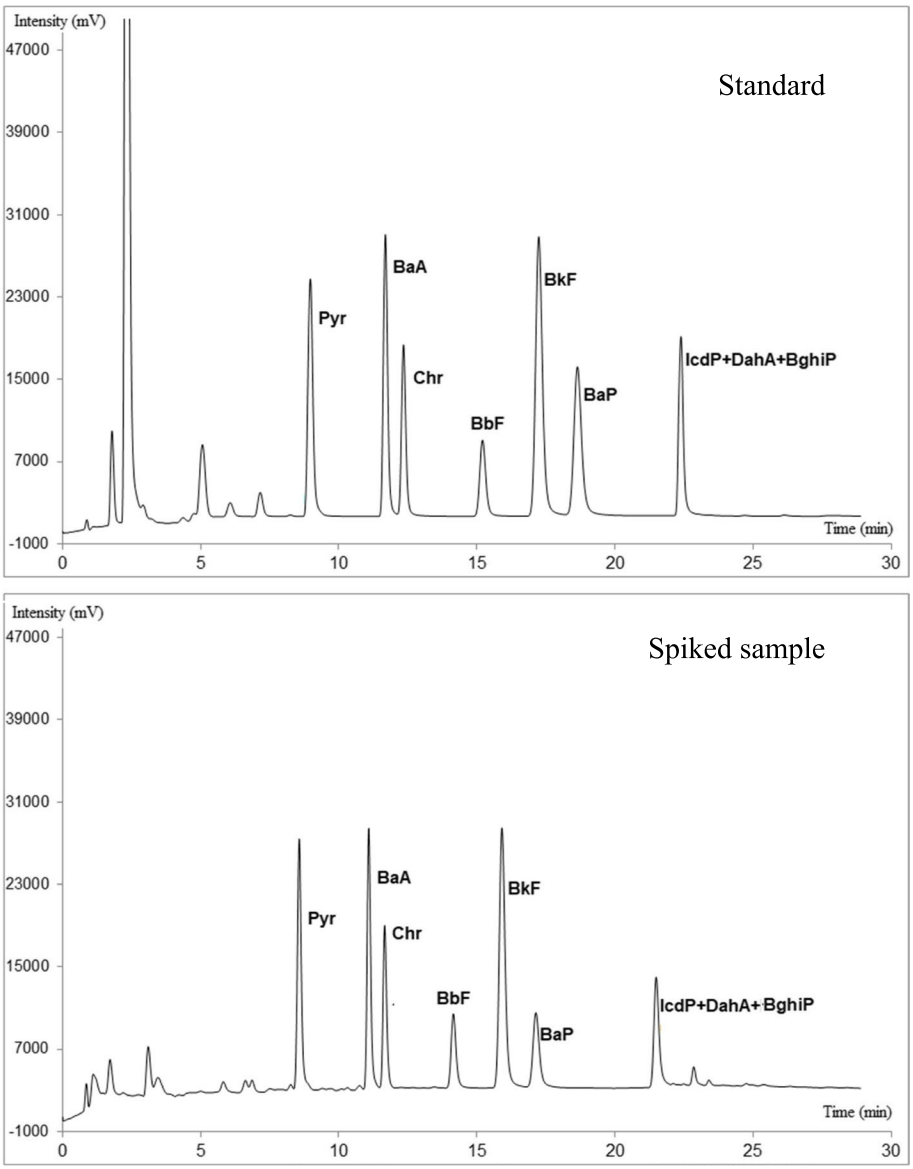


Fig. 1 Chromatograms of PAH9 standards (5.0 ng/mL) and a sample of ham spiked with 5.0 ng/g. *Pyr* pyrene, *BaA* benz[a]anthracene, *Chr* chrysene, *BbF* benzo[b]fluoranthene, *BkF* benzo[k]fluoranthene, *BaP* benzo[a]pyrene, *IcdP* indeno[1,2,3-c,d]pyrene, *DahA* dibenz[a,h]anthracene, *BghiP* benzo[g,h,i]perylene

bacon (23/23), but were also found in frankfurter sausages (17/18), coppa (8/10), cured poultry breast (12/15), fresh and smoked sausage (52/55), ham (12/16), mortadella (11/16), and salami (16/30), with individual levels ranging from < LOQ to 76.76 µg/kg.

The highest level of PAH9 was detected in smoked sausage (108.24 µg/kg), followed by morcilla (70.56 µg/kg), and bacon (51.24 µg/kg). In contrast, the category of ham showed the lowest concentration of PAH9 (with a mean of 1.83 µg/kg, and a median of 1.48 µg/kg).

The mass quantification rates of individual PAHs in the total samples are shown in Fig. 2. It was found that ten types of meat had a similar distribution of PAHs, with Pyr and Chr being the most prevalent. In all categories, the three quantified PAHs at higher concentrations were Pyr (< LOQ-76.76 µg/kg), Chr (< LOQ-31.66 µg/kg), and BaA (< LOQ-17.42 µg/kg). BkF presented the lowest concentration (< LOQ-1.63 µg/kg).

BaP and PAH4 concentrations were < LOQ-2.44 µg/kg and < LOQ-48.98 µg/kg, respectively. In the evaluation of samples according to Regulation No. 835/2011 of the

Table 2 Summary of PAHs concentration in different types of meat products sold in Brazil

Meat product		Pyr	BaA	Chr	BbF	BkF	BaP	IcdP + DahA + BghiP	PAH4	PAH8	PAH9
Sausage (*n* = 55)	*> LOQ (%)*	80	9	16	0	0	0	13	5	5	13
	Min–Max	< LOQ-76.76	< LOQ-11.77	< LOQ-31.66	< LOQ-3.93	< LOQ-1.63	< LOQ-2.44	< LOQ-2.63	< LOQ-48.98	< LOQ-50.61	< LOQ-108.24
	Mean	8.39	1.61	3.28	< LOQ	< LOQ	< LOQ	< LOQ	5.40	5.96	14.36
	Median	1.89	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Salami (*n* = 30)	*> LOQ (%)*	47	20	33	3	0	7	0	17	13	30
	Min–Max	< LOQ-18.45	< LOQ-17.42	< LOQ-13.37	< LOQ-1.59	< LOQ	< LOQ-2.12	< LOQ	< LOQ-29.07	< LOQ-29.07	< LOQ-30.71
	Mean	1.97	< LOQ	1.57	< LOQ	< LOQ	< LOQ	< LOQ	2.62	2.62	4.58
	Median	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	1.09
Smoked bacon (*n* = 23)	*> LOQ (%)*	87	48	87	22	0	22	0	52	26	52
	Min–Max	< LOQ-37.89	< LOQ-3.59	< LOQ-8.58	< LOQ-0.87	< LOQ	< LOQ-0.89	< LOQ	< LOQ-13.38	< LOQ-13.38	< LOQ
	Mean	7.92	1.05	2.47	< LOQ	< LOQ	< LOQ	< LOQ	3.83	3.83	11.75
	Median	4.35	< LOQ	1.55	< LOQ	< LOQ	< LOQ	< LOQ	3.03	3.03	6.93
Frankfurter sausage (*n* = 18)	*> LOQ (%)*	89	0	56	0	0	0	28	11	0	11
	Min–Max	< LOQ-24.73	< LOQ	< LOQ-2.93	< LOQ	< LOQ	< LOQ	< LOQ-2.17	< LOQ-2.93	< LOQ-2.93	< LOQ-25.32
	Mean	3.26	< LOQ	0.87	< LOQ	< LOQ	< LOQ	< LOQ	0.87	1.31	4.57
	Median	1.97	< LOQ	0.64	< LOQ	< LOQ	< LOQ	< LOQ	0.64	1.36	3.70
Ham (*n* = 16)	*> LOQ (%)*	56	13	31	6	0	6	6	19	0	6
	Min–Max	< LOQ-3.99	< LOQ-2.58	< LOQ-3.44	< LOQ-0.64	< LOQ	< LOQ-0.55	< LOQ-2.40	< LOQ-3.44	< LOQ	< LOQ
	Mean	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	0.75	0.90	1.83
	Median	1.08	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	1.48
Mortadella (*n* = 16)	*> LOQ (%)*	63	6	31	0	0	0	25	13	6	25
	Min–Max	< LOQ-32.07	< LOQ-4.11	< LOQ-2.60	< LOQ	< LOQ	< LOQ	< LOQ-3.73	< LOQ-6.58	< LOQ-6.58	< LOQ-38.66
	Mean	3.19	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	0.75	1.42	4.61
	Median	1.23	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	0.32	2.09
Cured poultry breast (*n* = 15)	*> LOQ (%)*	67	20	40	7	0	0	7	33	13	13
	Min–Max	< LOQ-15.45	< LOQ-4.60	< LOQ-13.41	< LOQ-1.52	< LOQ	< LOQ	< LOQ-1.34	< LOQ-18.01	< LOQ-18.01	< LOQ-19.27
	Mean	2.13	< LOQ	1.68	< LOQ	< LOQ	< LOQ	< LOQ	2.45	2.54	4.67
	Median	1.44	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	1.34	3.43
Hamburger (*n* = 15)	*> LOQ (%)*	93	27	40	13	0	7	33	20	13	27
	Min–Max	< LOQ-24.29	< LOQ-6.20	< LOQ-9.64	< LOQ-1.86	< LOQ	< LOQ-0.67	< LOQ-2.63	< LOQ-17.70	< LOQ-18.77	< LOQ-29.65
	Mean	3.97	< LOQ	1.00	< LOQ	< LOQ	< LOQ	< LOQ	2.10	2.71	6.69
	Median	2.19	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	1.33	3.72

Table 2 (continued)

Meat product		Pyr	BaA	Chr	BbF	BkF	BaP	IcdP + DahA + BghiP	PAH4	PAH8	PAH9
***Coppa* (*n* = 10)**	*> LOQ (%)*	80	30	40	10	0	10	0	40	20	30
	Min–Max	< LOQ-22.79	< LOQ-5.61	< LOQ-16.19	< LOQ-1.31	< LOQ	< LOQ-0.97	< LOQ	< LOQ-20.58	< LOQ-20.58	< LOQ-22.79
	Mean	3.18	1.31	2.83	< LOQ	< LOQ	< LOQ	< LOQ	4.36	4.36	7.54
	Median	1.19	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	0.70	0.70	3.42
Other Products (*n* = 7)	*> LOQ (%)*	100	57	86	14	0	14	0	57	29	71
	Min–Max	1.31–66.35	< LOQ-3.67	< LOQ-9.08	< LOQ-0.88	< LOQ	< LOQ-0.81	< LOQ	< LOQ-14.44	< LOQ-14.44	2.0370.56
	Mean	20.70	1.98	2.14	< LOQ	< LOQ	< LOQ	< LOQ	4.36	4.36	25.06
	Median	3.90	3.34	1.16	< LOQ	< LOQ	< LOQ	< LOQ	3.89	3.89	15.83
Total (*n* = 205)	*> LOQ (%)*	74	26	53	9	2	8	15	30	16	32
	Min–Max	< LOQ-76.76	< LOQ-17.42	< LOQ-31.66	< LOQ-3.93	< LOQ-1.63	< LOQ-2.44	< LOQ-3.73	< LOQ-48.98	< LOQ-50.61	< LOQ-108.24
	Mean	5.54	< LOQ	1.94	0.15	< LOQ	< LOQ	< LOQ	3.15	3.45	8.99
	Median	1.57	< LOQ	0.57	< LOQ	< LOQ	< LOQ	< LOQ	0.78	1.34	3.43

BaA benz[a]anthracene, *Chr* chrysene, *BbF* benzo[b]fluoranthene, *BkF* benzo[k]fluoranthene, *BaP* benzo[a]pyrene, *IcdP* indeno[1,2,3-c,d]pyrene, *DahA* dibenz[a,h]anthracene, *BghiP* benzo[g,h,i]perylene, *PAH4*BaA + Chr + BbF + BaP, *PAH8* PAH4 + BkF + DahA + BghiP + IcdP, *PAH9* PAH8 + Pyr

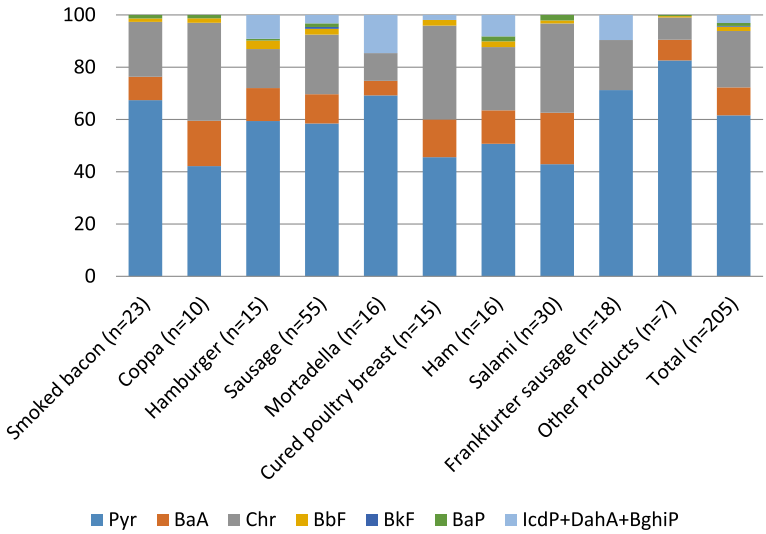


Fig. 2 Mass quantification rates of PAHs in the meat products. *Pyr* pyrene, *BaA* benz[a]anthracene, *Chr* chrysene, *BbF* benzo[b]fluoranthene, *BkF* benzo[k]fluoranthene, *BaP* benzo[a]pyrene, *IcdP* indeno[1,2,3-c,d]pyrene, *DahA* dibenz[a,h]anthracene, *BghiP* benzo[g,h,i]perylene

European Commission (EC 2011a), three samples (1.5%) showed unsatisfactory levels for BaP (> 2.0 µg/kg), two smoked sausages, and one salami. Regarding PAH4, 18 samples (8.7%) had levels above 12.0 µg/kg, the majority of which consisted of smoked sausage (6 samples), followed by salami (3 samples), fresh sausage (2 samples), smoked bacon (2 samples), coppa (2 samples), hamburger (1 sample), breast turkey cured (1 sample), and smoked salmon (1 sample) (Table 2). For PAH4, the most unsatisfactory samples were affected by the highest amounts of Chr, followed by BaA.

The samples of frankfurter sausages, ham, mortadella, breast cured (chester and chicken) and other smoked products (morcilla, shoulder clod, pastrami, and black butifarra) showed satisfactory results, within the safe limits (EC 2011a). In general, the highest percentage of unsatisfactory results was detected in sausages, especially smoked sausages, with PAH4 concentrations ranging from < LOQ to 48.98 µg/kg, four times higher than the level by the European Commission (EC 2011a).

The results for BaP in this study showed lower values than those reported in other published studies. Kafouris et al. (2020) evaluated the presence of PAHs in meat smoked using the traditional charcoal method,with a mean BaP concentration of 0.77 µg/kg. In Latvia, BaP levels in smoked pork meats ranged between LOQ (0.05 µg/kg) and 6.03 µg/kg (Rozentāle et al. 2015). Zhang et al. (2022) found concentrations in the range of 0.51 to 4.57 µg/kg. In 2015, the proportion of unsatisfactory samples according to Regulation No. 835/2011, for BaP (> 2 µg/kg) in 128 smoked meat samples from Latvian was almost 14% (Rozentāle et al. 2015), while our result was 1.5%, indicating lower contamination and better control of the smoking and drying process.

For PAH4, the results were similar to those reported by Zhang et al. (2022), who found a range between 2.40 and 53.56 µg/kg. This range was lower than that reported by Kafouris et al. (2020) (mean value 9.40 µg/kg), and higher than that reported by Lu et al. (2017) (mean value 1.75 µg/kg).

Higher concentrations of BaA and Chr were observed in the PAH4. Similar behavior was reported by Rozentāle et al. (2015), where the median values of BaA and Chr were 0.76 and 0.82 µg/kg, respectively. The most commonly observed compound in smoked meat products studied by Onopiuk et al. (2022) was benz[a] anthracene.

For the determination of 16 PAHs in *Slavonska slanina* (traditionally smoked bacon), produced under industrial conditions, the concentrations of Pyr, BaA, BbF, BkF, and BaP were below the LOQ (Kartalović et al. 2022), which were lower than the concentrations found in this study.

Considering our data for PAH4 and PAH8, the maximum, mean, and median values were similar. These results are consistent with those presented by EFSA (2008), where the CONTAM Panel evaluated that when PAH4 are detected, PAH8 are almost always present. Therefore, PAH4 is a good indicator of the presence of carcinogenic PAHs.

The satisfactory results for ham and smoked and fresh sausages differ from those of some authors, which may be justified due to the method of smoking used. Zachara, Galkowska and Juszczak (2017), when analyzing

smoked fish and meat products available in the Polish market, found that sausages smoked in the traditional way, directly over a wood fire, were characterized by higher PAH4 values (35.90 ± 7.18 µg/kg), compared to samples from industrial smoking (16.83 ± 3.37 µg/kg). The same ratio was found in the ham samples (traditional smoking: 15.47 µg/kg and industrial smoking: 4.77 µg/kg).

Kartalović et al. (2022) found that in *Slavonska slanina* smoked under industrial conditions, the results for BaP and PAH4 were below the LOQ. However, in samples smoked using an open fire (the traditional method), the PAH4 were as follows: 14.84 µg/kg in the middle, 10.60 µg/kg on the surface and 17.37 µg/kg in the skin.

Thus, it can be observed that the smoking method is an important factor in the formation of PAHs in meat products. Both direct and indirect smoking methods can be utilized, and in the direct method, contamination may be greater due to the deposition of PAHs produced by the incomplete combustion of materials used in food (Onopiuk et al. 2021).

Food composition is a factor that influences the formation of PAHs when meat products undergo smoking processes, as foods with high-fat content are more susceptible to contamination due to their affinity for lipophilic PAHs. As suggested by Lu et al. (2017), reducing the fat content from 30 to 20% in smoked pork sausages resulted in a decrease in PAHs levels in the samples.

During the smoking of meat products, several factors influence PAHs contamination, including the process and characteristics of the meat such as its type, lipid content, moisture levels, fuel type, smoking time, temperature, airflow, presence of food additives, distance and position of the product relative to the heat source, cleaning and maintenance of the equipment, design of the smoking chamber, the type of smoke generator, and equipment used to mix smoke and air (Ledesma et al. 2016; Onopiuk et al. 2021).

In a direct way, Racovita et al. (2020) observed that concentrations generally increased when the temperature and smoking time were higher in smoked pork sausages, demonstrating the importance of controlling conditions during the process. Regarding the type of wood used, the *Betulaceae* species (alder and birch) showed higher PAHs levels than *Fagaceae* species (beech and oak).

With modern technology in the industry, smoking is commonly controlled in various processes. Adaptations have been made to the process, such as using smoke generated in external chambers, shortening the smoking time, or using smoke flavorings. These are considered good manufacturing practice strategies that can significantly reduce PAHs content in foods while maintaining flavor (Kafouris et al. 2020; Ledesma et al. 2016; Onopiuk et al. 2021; Rozentāle et al. 2015). The CAC/RCP 68/2009 of *Codex Alimentarius* is the principal code for national authorities and manufacturers. It defines important points and provides guidance for recommendations to prevent and reduce contamination of food with PAHs from smoking and direct drying processes (Codex 2009).

Regarding flavorings, liquid smoke can be produced by condensing gases generated through oxygen-controlled pyrolysis of hardwood chips. Compared to traditional smoking techniques, it can reduce the PAH content in food. Additionally, it offers other industrial advantages such as rapid addition, consistent solution, and reproducibility of the final product (Shao et al. 2023; Zhu et al. 2022).

The type of casing used for smoked meat products can also influence the level of PAHs. In a study by Henríquez-Hernández et al. (2016), sausages wrapped in natural casings showed higher BaP content compared to those wrapped in cellulose casings. This difference is attributed to the lipophilic nature of natural casings, which, like PAHs, allows these compounds to permeate into the food. The use of nanoparticles in casings could be a strategy to further reduce the contamination of smoked products. Farhadi et al. (2022) demonstrated that sugarcane bagasse casings containing nanoclay adsorbed more PAH4 compared to a control film (fibrous casings without adsorbent).

Exposure assessment and risk characterization

The ILCR method is commonly used in PAHs risk assessment. The MOE method is suggested by the EFSA CONTAM Panel because the Scientific Committee on Food (SCF) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) concluded that the toxic equivalency factor (TEF) had limitations in the available data, and different PAHs have various mechanisms of action (EFSA 2008). Some authors include both methods in their articles for comparison purposes (Wang et al. 2021; Yan et al. 2022).

The main human exposure to PAHs occurs through the consumption of contaminated food. In all environmental compartments, food, air, water, soil, and cigarettes, consumption of PAHs-contaminated foods represents 96.2% of the exposure route in non-smokers, while it varies between 27–37% in smokers (Menzie and Potocki 1992; Paris et al. 2018).

Table 3 presents the ID, MOE, and ILCR results. The products that exhibited the greatest significance in daily intake were sausages (smoked and fresh), followed by hamburgers. For sausages, the mean ID values of BaP, PAH4, and PAH8 were 1.65, 24.35, and 29.95 ng/day, respectively. For hamburgers, the mean ID values of BaP, PAH4, and PAH8 were 2.08, 23.03, and 34.36 ng/day,

Table 3 Mean values for dietary daily intake (ID), margin of exposure (MOE), and Incremental lifetime cancer risk (ILCR) in different meat products

Products	ID (ng/day)			$MOE \times 10^{+6}$			$ILCR \times 10^{-9}$ for PAH4		
	BaP	PAH4	PAH8	BaP	PAH4	PAH8	Adolescents	Adults	Seniors
Smoked and fresh sausage (*n* = 55)	1.65	24.35	29.95	2.59	0.85	1.00	4.10	9.22	5.43
Salami (*n* = 30)	0.10	1.10	1.40	43.14	18.89	21.35	5.30	11.91	7.01
Smoked bacon (*n* = 23)	0.11	1.41	1.72	40.40	14.76	17.39	7.79	17.50	10.31
Frankfurter sausage (*n* = 18)	0.28	2.19	3.69	15.52	9.49	8.10	3.82	8.59	5.06
Ham (*n* = 16)	0.11	0.75	1.21	39.67	27.65	24.65	2.53	5.68	3.34
Mortadella (*n* = 16)	0.15	1.47	2.41	28.46	14.11	12.38	2.79	6.27	3.69
Cured poultry breast (*n* = 15)	0.08	1.05	1.37	56.91	19.82	21.78	3.13	7.04	4.14
Hamburger (*n* = 15)	2.08	23.03	34.36	2.05	0.90	0.87	4.17	9.37	5.52
***Coppa* (*n* = 10)**	0.10	1.59	1.91	44.24	13.01	15.65	3.09	6.94	4.08
Other products (*n* = 7)	0.98	1.51	1.81	43.08	13.71	16.49	3.42	7.69	4.53

BaP benzo[a]pyrene, PAH4 BaA, Chr, BbF, and BaP, PAH8 PAH4, *BkF* benzo[k]fluoranthene, *IcdP* indeno[1,2,3-c,d]pyrene, *DahA* dibenz[a,h]anthracene, *BghiP* benzo[g,h,i] perylene

respectively. The daily intakes of PAH4 and PAH8 were approximately 7 to 20 times higher than that of BaP.

The ILCR health risk assessment indicated that these products do not have a considerable health risk to adolescents, adults and seniors (Table 3). According to the U.S.EPA standard, ILCR values below 10^{-6} are considered inconsequential risk (Wang et al. 2021). The MOE values (Table 3) of the evaluated meat products were higher than 10,000, indicating minimal adverse health effects and a low risk to public health (EFSA 2008).

Wang et al. (2021) also identified low health risks associated with the consumption of fried and grilled fish from Shandong, China, among children, adolescents, and seniors, based on MOE and ILCR values. Similarly, smoked fish from Romania showed low significant cancer risk, with MOE values ranging from $0.59–30.21 \times 10^{+6}$ and ILCR values ranging from $0.17–16.60 \times 10^{-6}$ (Racovita et al. 2021). Additionally, Rozentāle et al. (2015) demonstrated that the intake of smoked meat from Latvian did not raise any significant toxicological concerns based on MOE evaluations.

Bian et al. (2023) evaluated the occurrence and health risks posed by heavy metals in crayfish (*Procambarus clarkii*) from Jiangsu, China. The study underscores the significance of monitoring contaminants and regulating metal levels in food sources. Additionally, estimated daily intake values of these metals were found to be below the provisional tolerable daily intakes established by regulatory authorities, indicating relatively low health risks associated with crayfish consumption. These results are similar to those found in our work, suggesting a low potential risk to consumer health.

Conclusion

In this study, a method utilizing SPE cleanup and UHPLC fluorescence quantification was successfully employed to determine nine PAHs across ten categories in 205 commercial meat products distributed in Brazil. Sausages exhibited the highest concentrations of PAHs, followed by hamburgers. Chrysene was the most frequently detected contaminant, with Pyr (< LOQ-76.76 µg/kg) and Chr (< LOQ-31.66 µg/kg) being the PAHs found in the highest concentrations. BkF exhibited the lowest levels (< LOQ-1.63 µg/kg). A small percentage of meat products (1% of the sample for BaP and 9% for PAH4) exceeded permitted levels in the European Union. However, considering the margin of exposures (MOE) values (upper to 10^{+4}) and the incremental life cancer risk (ILCR) values (between 10^{-6} and 10^{-4}), the risk assessment suggests a low potential health risk to consumers of meat products. While many PAHs are recognized as carcinogens, mutagens, and teratogens, our findings emphasize the importance of continuous monitoring of PAHs. Data collected from commercial samples can inform regulatory agencies in establishing and revising safe limits and managing risks to the population.

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Authors' contributions

Conceptualization, SAS, APA, EAFST and GRS; methodology, SAS, GZD, APA and GRS; validation, SAS, GZD, APA; Data curation, SAS, GZD, APA and GMG; Writing—original draft preparation, SAS, GZD, APA, GMZ, MMR, EAFST and GRS; project administration GRS. All authors have read and agreed to the published version of the manuscript.

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Declarations

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Not applicable.

Consent for publication
All authors gave their consent for publication.

Competing interests
The authors declare that there are no competing interest.

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