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Arun Das, Dipanwita Bhattacharya, Annada Das, Santanu Nath, Samiran Bandyopadhyay, et al.. Current innovative approaches in reducing polycyclic aromatic hydrocarbons (PAHs) in processed meat and meat products. Chemical and Biological Technologies in Agriculture, 2023, 10 (1), pp.109. 10.1186/s40538-023-00483-8. hal-04236303

HAL Id: hal-04236303

https://hal.inrae.fr/hal-04236303

Submitted on 10 Oct 2023

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Current innovative approaches in reducing polycyclic aromatic hydrocarbons (PAHs) in processed meat and meat products

Arun K. Das¹, Dipanwita Bhattacharya², Annada Das³, Santanu Nath¹, Samiran Bandyopadhyay¹, Pramod Kumar Nanda^{1*} and Mohammed Gagaoua^{4*}

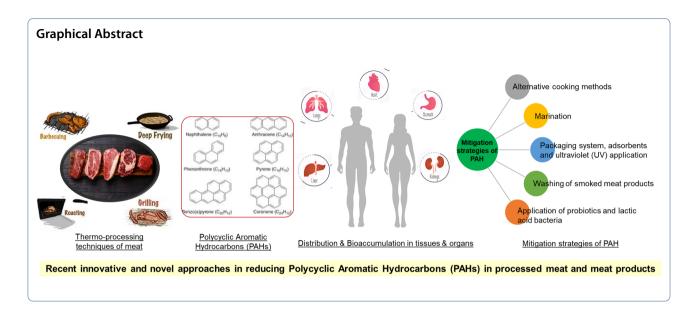
Abstract

The presence of polycyclic aromatic hydrocarbons (PAHs) in processed meat and meat products is a global concern as they are known to be carcinogenic, mutagenic, teratogenic, and genotoxic to living beings. PAHs are generated in processed meat through different thermo-processing techniques, such as smoking, grilling, barbecuing, roasting, and frying, which involve abnormal high-temperature treatments and extruded fuels. These carbonaceous compounds with two or more cyclic benzene rings are highly stable and toxic, and their generation is enhanced by faulty thermal processing techniques, contaminated raw materials, and environmental pollution. Based on their degree of toxicity, Benzo[a]pyrene (B[a]P) is recognized as the most probable human carcinogen among different fractions of PAHs by the European Commission Regulation (EC-No.1881/2006). Furthermore, the association between dietary PAHs exposures and their role as carcinogen in human beings has been reported clinically. Therefore, it is necessary to focus on prevention and control of PAHs formation in processed meat products through various strategies to avert public health concerns and safety issues. Accordingly, several approaches have been used to reduce the risk of PAHs formation by employing safe processing systems, harmless cooking methods, marination by natural plant components, use of biological methods etc. to eliminate or reduce the harmful effects of PAHs in the food system. This review provides a comprehensive insight into the occurrence and formation of PAHs in meat and meat products and their toxicological effects on human beings. Furthermore, the different cost-effective and environment friendly methods that have been employed as "green strategies" to mitigate PAHs in meat and meat products at both household and commercial levels are discussed.

Keywords Polycyclic aromatic hydrocarbons (PAHs), Thermally processed meats, Smoking, Grilling, Mitigation strategies

*Correspondence:
Pramod Kumar Nanda
npk700@gmail.com
Mohammed Gagaoua
mohammed.gagaoua@inrae.fr
Full list of author information is available at the end of the article





Introduction

Meat is one of the most significant foods in our daily diet for its valuable constituents and essential nutrients [1]. To improve the quality, safety, sensory attributes and shelflife of meat and meat products, various processing and preservation methods are employed [2]. However, the traditional and modern thermal processing of meats such as smoking, grilling, baking, barbecuing, roasting, frying etc. produce genotoxic compounds due to incomplete combustion of organic matter under insufficient oxygen [3]. Alongside these meat processing techniques, process time, temperature, distance between the food and heat source, food components, water activity, fuel, smoke used etc. are responsible for the formation of polycyclic aromatic hydrocarbons (PAHs) [4]. The formation of PAHs into food surface may be associated with different possible mechanisms, such as the pyrolysis of organic components (protein, fat or carbohydrate) of foods at high temperature (more than 150 °C), dripping of fat onto heat sources leading to incomplete combustion, incomplete burning of coal, fossil fuel, wood or other fuels under reduced oxygen levels, chemical modification of oil used as cooking medium etc., [4–6]. Akbari-Adergani et al. [7] and Yousefi et al. [8] have reported about generation of PAHs by identifying several aromatic compounds in edible cooking oils. After absorption into cell membrane and metabolism into human body, these metabolites bind with DNA and proteins by intervention of different pathways leading to formation of DNA, RNA and glutathione adducts resulting in structural disruption, DNA mutations, alteration in gene expression and carcinogenesis [6, 9].

Apart from thermal processing, PAHs can also be generated by either organic environmental pollutions, or

migratory properties of packaging materials. As far as non-smokers are concerned, carcinogenesis or mutagenesis is directly related to their unhealthy dietary habits contaminated with PAHs [10]. According to the current epidemiological surveys, prevalence of cancer among human beings is extensively attributed to dietary exposures of PAHs [6]. Overall, the most human exposures to PAHs occur by inhalation, ingestion or direct contact of these toxic substances through contaminated foods [4, 11].

Out of almost 200 organic compounds of PAHs identified in environment as particulate matter, almost sixteen PAHs are found in meat products with high carcinogenic, mutagenic and teratogenic properties, especially in human beings [11]. The International Agency for Research on Cancer (IARC) has classified processed and red meat as group 1 carcinogen to humans due to the presence of N-nitroso compounds, heterocyclic aromatic amines, and PAHs. The International Agency for Research on Cancer [12] has categorized the toxicant PAHs into the following few groups; Group 1 (carcinogenic to human), Group 2A (probably carcinogenic to human), Group 2B (possibly carcinogenic to human) and Group 3 (unclassified as carcinogenic to human) (Table 1). Based on the risk of carcinogenicity, different regulatory agencies have classified the PAHs. The European Commission has separated 4 major PAHs that exhibit carcinogenic activity, i.e., benz [a]anthracene (BaA), chrysene (Chr), benzo[b]fluoranthene (BbF), and benzo[a]pyrene (BaP) [13]. The European Food Safety Authority has also divided these toxicant PAHs into PAH2 (BaP and Chr), PAH4 (BaA, BaP, BbF, and Chr), and PAH8 (BaA, BaP, Chr, BkF, BbF, IcdP, DahA, and

 Table 1 Classification of polycyclic aromatic hydrocarbons

PAH group	Name of the PAHs compounds and groups (1, 2A, 2B and 3) ^a	Classification	References
PAH2	BaP ¹ and Chr ^{2B}	The European Commission (EU) vide Commis-	[12, 14, 107]
PAH4	BaA^{2B} , BaP^{1} , BbF^{2B} , and Chr^{2B}	sion Regulation (EC) No 835/2011	
PAH8	BaA^{2B} , BaP^{1} , Chr^{2B} , BkF^{2B} , BbF^{2B} , $IcdP^{2B}$, $DahA^{2A}$, and $BghiP^{3}$		
PAH16	Nap, Ace, Acy, Fle ³ , Phe ³ , Ant ³ , Flu, Pyr ³ , BbF ^{2B} , BkF ^{2B} , BaA ^{2B} , Chr ^{2B} , BaP ¹ , IcdP ^{2B} , DahA ^{2A} and BghiP ³	U.S. Environmental Protection Agency (EPA)	

^a Group 1 = carcinogenic to humans, group 2A = probably carcinogenic to human, group 2B = possibly carcinogenic to humans, group 3 = not classifiable as to carcinogenicity to humans

Nap Naphthalene, Ace Acenaphthene, Acy Acenaphthylene, Fle Fluorene, Phe Phenanthrene, Ant Anthracene, Flu Fluoranthene, Pyr Pyrene, BbF Benzo[b]fluoranthene, BkF Benzo[k]fluoranthene, BaA Benzo[a]anthracene, Chr Chrysene, BaP Benzo[a]pyrene, IcdP Indeno[1,2,3-cd]pyrene, DahA Dibenzo[a,h]anthracene, BghiP Benzo[ghi] perylene

BghiP) [14]. Amongst these, the Joint FAO/WHO Expert Committee has widely announced BaP as the most probable human carcinogen or an indicator of carcinogenesis, as the occurrence of PAHs and their associations with increased risk of colo-rectal cancer is primarily attributed to benzopyrene [15]. Epidemiological surveys have also found a positive correlation between higher intake of thermo-processed red meat with high fat content and colo-rectal cancers in humans. Therefore, identifying the meat-based products with high risk for PAHs contamination and detection of their carcinogenic concentrations is required from public health safety point of view.

Currently, various processing/cooking methods in combination with innovative strategies are employed by different workers as some of the PAHs reduction strategies in meat and meat products. These includes optimization of the processing conditions, including time, temperature during smoking and grilling, and selection of wood/ charcoal type. Inclusion of natural plant extracts during marination of meat, use of probiotics and lactic acid bacteria, natural cellulosic adsorbents and casings as packaging for smoked sausages etc. are also being explored as bioremediation measures for PAHs [16–19]. This review summarizes various points, providing a comprehensive insight to the occurrence and formation of PAHs in meat and meat products, and their adverse effects on human health. Furthermore, various cost effective, and environment friendly approaches to mitigate PAHs in meat and meat products are also discussed in this paper.

Mechanism of PAHs formation in processed meat

Meat is no doubt a highly recommended nutritious food and performs a significant role in human health system [20]. However, the presence of PAHs in meat may be either by different food processing mechanisms, including curing, heating, drying, smoking, grilling, barbecuing etc., or environmental pollutants contaminating air, soil and water [3, 21]. Although the exact reason is not known, three possible mechanisms for formation of

PAHs in processed meat have been conferred here [4, 22]. The formation of PAHs into meat goes through a series of radical reactions, including incomplete combustion of organic components (pyrolysis) present in meat, such as fat, protein, and carbohydrate at 200 °C and above. Dripping of fat onto flame during grilling or smoking and leaching of natural-nutrient rich juices from meat into the fuel may hasten the mechanism of pyrolysis generating volatile PAHs which are towed by smoke and get accumulated on the surface of meat [23, 24]. Another very important and common reason of PAHs formation and deposition on meat surface may be the incomplete and unstable combustion of fuels, such as charcoal, hardwood, or straw under insufficient oxygen [4, 25], and use of oils as cooking medium [7].

In fact, pyrolysis and pyrosynthesis are two typical processes, where chemical modifications of carbonaceous organic compounds are common to produce PAHs during thermal processing, though the responsible mechanism is still under investigation [22]. A favorable atmosphere for pyrolysis which is stimulated by raised temperature leads to fragmentation of large organic compounds into small molecules such as propane and ethylene along with intermediate free radicals with high reactivity. Propargyl recombination, hydrogen abstraction and acetylene addition (HACA) mechanism, Diels-Alder mechanism, and phenyl-addition-cyclization (PAC) are different elusive mechanisms involved for cyclization, benzene ring formation and ultimately ring-ring condensation producing high molecular weight polyamides [22, 24]. In addition, it has been reported that Maillard reaction between proline and reduced sugar under very high temperature and short time (600–840 °C for 1 s) may promote PAHs formation by pyrolysis of proline [26]. However, the levels of PAHs formation in thermally processed meat is influenced by the meat type and fat content, processing/cooking method employed, temperature, distance,

duration and type of fuels, food additives used etc., which have been discussed extensively later.

PAHs: the structure, metabolism, toxicity and health effects

PAHs are a group of chemicals with two or more conjugated benzene or cyclopentadiene rings composed of 5-6 carbon and hydrogen atoms [27], produced either by incomplete burning (pyrolysis) of carbon-containing materials, or organic materials, such as greasy meat. Based upon the number of benzene rings and molecular weights, PAHs show their toxicities towards human beings, animals and even bacteria [28]. Light PAHs (naphthalene, phenanthrene, fluorene, acenaphthene etc.) consist of two to three aromatic rings, and are less toxic, more unstable and volatilize immediately. On the other hand, heavy PAHs, such as pyrene, benzo[a]anthracene, chrysene, benzo[a]pyrene, having four and more rings, are more stable and toxic [29]. In general, PAHs are highly soluble in non-polar solvents and edible oils but have low solubility in water. Furthermore, they are lipophilic in nature with high melting, boiling point, vapour pressure, octanol-water partition coefficient indicating high bioaccumulation into living beings with less biodegradability [27, 29–31].

Living organisms get exposures of PAHs either by direct contact with skin or through different routes, such as inhalation, and ingestion of food, including meat and meat products. After being absorbed, these compounds are circulated to different systems and organs of body and bio-accumulated in liver, intestine, skeletal muscle system, adipose tissue, extra hepatic tissues etc. by blood capillaries and lymphatic vessels and exhibit their toxic metabolisms disturbing regular cellular functions [3, 32]. Their metabolism and biotransformation are complex processes forming different intermediates ended up with diol-epoxides and radical cations assisted by cytochrome P450 peroxidase and aldo–keto reductase enzymes. Figure 1 depicts the formation, exposure and metabolism of PAHs in human health chain.

The metabolites bind covalently to cellular macromolecules, such as proteins, DNA and RNA forming adducts [33]. If not phagocytosed by macrophages and excreted in feces and urine, they cause biochemical disruption and

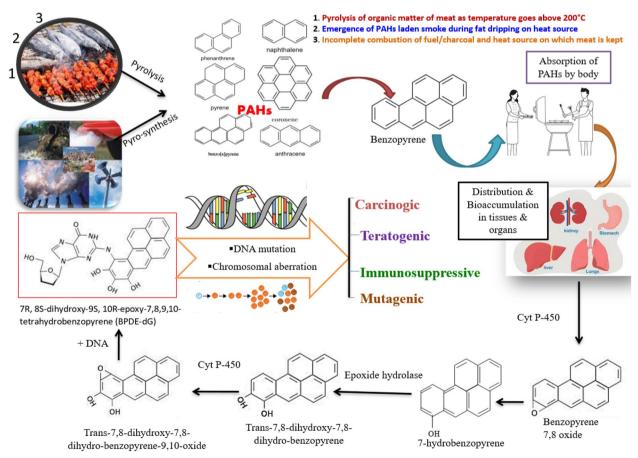


Fig. 1 Formation, exposure and metabolism of PAHs in human health chain. [Adapted from [4, 22]]

cellular damage, alter gene expression inducing carcinogenic, unpreventable mutagenic, immunosuppressive, and teratogenic damage [3, 6, 29].

Based upon the route of exposure, concentration and time, PAHs can show acute or chronic symptoms, including eye irritation, nausea, vomiting, diarrhea, skin allergies, liver and kidney damage etc. [24, 34]. Long-term exposures to unvented smokes, generated during traditional indoor smoking, might be the cause of high mortality rate in lung cancer among women [3, 35]. Besides, indoor cooking, grilling, barbecuing etc. are the significant risk factors for incidences of cancer in nasopharyngeal, blood, prostate, skin, breast etc. among exposed people [3, 22, 27]. The consumption of grilled meat has been reported to increase the risk of renal cell carcinoma [36]. Reports available suggest that, consuming smoked meat and fish more frequently, containing PAHs enhance the risk of stomach cancer in human beings [3]. PAHs, particularly increased B[a]P concentration in processed and ready-to-eat (RTE) meat samples, are capable of causing genetic changes by significantly modifying the expression of KRAS, a key gene related to colo-rectal cancers [37]. BaP is also reported to cause disrupted human endocrine system-related infertility [29, 38]. Furthermore, the risk of cancer due to consumption of foods of animal origin is reported to be 2.63/107 and 9.3/107 BaP equivalents for children/adolescents and adults, respectively [22]. Upon long-term exposure, the organs rich in adipose tissue might develop tumors due to accumulation and bioavailability of PAHs, that are lipophilic in nature [39].

Research on toxic effects of PAHs conducted at cellular levels in different animal models and case—control studies in human have highlighted its veracity [38, 40]. Deformation of mouse micronucleus red blood cells, lethargy and anxiety of fish larvae are few examples of toxicity of PAHs on cell level [24, 40]. The teratogenic effects of PAHs and their covalently attached metabolites with DNA may create congenital disabilities, such as low birth

weight, premature delivery, heart malformations and low intelligence quotient of offspring. In animal models and case control studies on human beings, the harmful effects of B(a)P and B(a)A, two leading PAHs, responsible for causing irreversible DNA damage, lung, breast and stomach tumors, esophageal, skin, colorectal and gastric cancer, papillomas, hepatomas with reported malignancy have been extensively reported [24, 35, 40].

PAHs in meat products and human health risk assessment

Over the last few years, the threat of cancer has increased ubiquitously among human beings because of modern lifestyle and daily dietary intake of PAHs [11]. Several studies have now acknowledged the positive correlation between PAHs contaminated meat intake and prevalence of cancer throughout the globe. Thus, PAHs toxicity is slowly becoming the silent epidemic. To protect the health of consumers from negative effects of PAHs intake from diet, the maximum admissible limits for PAHs compounds, especially BaP and ∑PAH4, have been set by various agencies and regulatory bodies for various raw and treated meat products which are presented in Table 2. As the consumption of PAHs contaminated meat products above permissible levels can be potentially harmful to human health, the ALARA (as low as reasonably achievable) principle is in force in the EU. However, epidemiological studies conducted in different parts of the globe on formation of PAHs in meat and meat products depict a different picture. In most of the cases, PAHs levels often surpass the established limits deemed acceptable by current legislations. Several reports are available in this regard investigating the presence of PAHs in different food matrices [41].

In a study conducted in Taiwan, Kao et al. [42] reported total PAHs formation in charcoal grilling of poultry (6.3–238.8 ng/g) and red meat (0.1–547.5 ng/g) and the highest amount of BaP (4.0 ppb) in chicken drumstick grilled at 74 °C for 20 min. It was postulated that concentration

Table 2 PAHs compounds and their maximum permissible limits in processed meat products

Meat products	PAHs compound	Permissible limits	References
Thermally processed meat and meat products	BaP	6 μg/kg	[108]
Thermally processed meat and meat products	PH4	35 μg/kg	
Bacon and meat products	BaP	2 μg/kg	
Bacon and meat products	PH4	12 μg/kg	
Smoked meat and meat products	BaP	2 μg/kg	EU. Commission regulation (EU)
Smoked meat and meat products	PAH4	12 μg/kg	no 835/2011 of 19 August 2011
Traditionally smoked meat and smoked meat products. (Applicable	BaP	5 μg/kg	(European Commission, 2014)
in Ireland, Spain, Croatia, Cyprus, Latvia, Poland, Portugal, Romania, Slovak Republic, Finland, Sweden and the UK)	PAH4	30 μg/kg	

of PAHs formation depends on duration and temperature of grilling, unsaturation of fatty acids in meat, lipid oxidation and degradation products, such as cyclohexane and hydroperoxides. Estimating BaP and PAH4 concentrations in grilled and fried pork in Shandong of China, Jiang et al. [43] stated that some samples crossed the maximum permissible limits of 2 and 12 µg/kg set for BaP and PAH4 by European Union (Commission Regulation (EC) No 1881/2006 amended by Commission Regulation (EU) No 835/2011). In Finland and Sweden, same reflections were seen for traditionally smoked meat and fish products with high BaP and PAH4 contents, especially in pork products with 5.6–13.2 μg kg⁻¹ of BaP contents, though Commission Regulation (EU) No. 1327/2014 set maximum limits in finished products should not be beyond 5.0 μg kg⁻¹ and 30.0 μg kg⁻¹ for BaP and PAH4, respectively. Likewise, Hokkanen et al. [10] made several experiments on different modified smoking processes to check the levels of PAHs formed and noticed that traditional direct smoking method could be a significant risk factor for cancerous toxicity with exceptionally high PAH4 formation, which is non-compliant with legislations. Wretling et al. [44] also reported similar findings in Sweden with increased BaP (6.6–36.9 µg kg⁻¹) in traditionally "sauna" smoked meat products. [45] investigated the effect of traditional smoking methods on the formation of BaP and PAH4 in smoked dry sausage Hercegova ckakobasica in Herzegovina, a part of Bosnia. The PAH4 concentrations in this pork-based sausage stuffed into natural casings was 24.46 µg/kg, which was almost double of legislative prescribed values (12 μg/kg).

Bogdanović et al. [46] made a survey in Croatian population on acute and chronic exposures of BaP and PAH4 through contaminated meat products and found that dry sirloin (39.0 ng/day), Kulen (32.4 ng/day) and dry homemade sausage (20.72 ng/day) were the largest contributors for daily BaP intake. In another study, potential human health risk assessment was carried out in local markets of Iran by investigating the content of PAHs in raw and cooked meat products [47]. The scientific survey report claimed that in sausages and burgers, anthracene (14.12 μ g/kg) and acenaphthylene (13.4 μ g/kg) were present in higher amount than the normal European Standard (2 μ g/kg), and fried meat products showed very high level of PAHs (23.31 μ g/kg).

Puljić et al. [48] conducted research on drycured smoked pork meat products "Hercegova ckape cenica" for different smoking procedures of traditional and industrial methods to elucidate the threatful intervention of PAHs. The researchers concluded that PAH4 was in much more than critical limit when conventional smoking method was carried out. Furthermore, as far as PAH16 is concerned, surface layer of meat was more

exposed to the risk than inner part. Supportive statements regarding these research findings were justified with probable cause of less contamination with PAHs in inner core of meat products may be due to protection from light and oxygen. However, in case of longer storage, diffusion occurs into inner part also, otherwise surface layer detection of PAHs are reported as major findings [3, 32].

From different epidemiological studies, it is evident that several factors are cognate with formation of different concentrations of PAHs in meat products. Some militating factors will be emphasized here in this review. PAHs formation depends upon the amount of fat present in meat or the type of oil present as cooking medium during different thermal degradation processes. For example, in barbecued-grilled meat the concentration of PAHs and BaP are highly correlated with presence of higher amount of unsaturated fatty acids, responsible for more benzene rings agglomeration [13]. In addition, new challenges such as oxidation of fatty acids present in oil may create new derivatives from photo-induced PAHs that may enhance their toxicities [4, 49]. In Malaysian charcoal-grilled satay from beef and chicken, significant differences were noticed for BaP concentration (7.4 mg/ kg and 2.0 mg/kg) by Mohammadi and Valizadeh-kakhki [50]. Similar kind of result was reported by Kao et al. [42] for highest BaP concentrations in lamb steak (5.8 mg/ kg) followed by chicken drumstick (4.0 mg/kg). Over and above that, this principle may vary based upon related and intermediate factors, when are interlaced with each other. Chicken and beef grilled products may show higher PAHs concentrations when they are either grilled with skin or possibly a large surface to volume ratio is plotted in barbequed minced beef burger or chicken breast etc. [51, 52].

Although thermal processes/different cooking methods such as smoking, frying, grilling, baking, boiling barbecuing, roasting etc. are employed to enhance the sensory attributes of meat products, these processes are accompanied with high temperatures, pyrolysis, fat dripping and intense smoke generation which can increase the levels of PAHs in meat [4]. In fact, the use of distinct types of fuels, i.e., gas, electric, wood, charcoal etc. have persuasive contributions for formation of different levels of PAHs in meat, as partial combustion of fuels under insufficient oxygen, result in formation, deposition, and penetration of volatile particles upon the smoked products [3, 13]. From different comparative studies on heating methods and PAHs generation, it has been found that electric and gas grilling, broiling, indirect smoking produces comparatively less concentrations of PAHs and BaP in different species of meat [22, 52]. Smoking, grilling or roasting with different wood (log fire, pinecones, ear leaf locust tree, acacia, hickory sawdust, aspen, spruce etc.) sources have significant influence on generating higher concentration of PAHs compared to charcoal grilling [52, 53]. Kim et al. [54] got positive alternatives of using wood smoking by "flameless and smokeless charcoal", coconut shells, broadleaf trees, oak etc. that decreased the PAHs noticeably in beef, chicken and salmon. Viegas et al. [21] made aware of harmful aspect of reusing same charcoal of gas for barbecuing continuously without cleaning that may produce higher molecular weight PAHs (BaP from 3.1 to 8.7 mg/kg) with more potential detrimental effects.

Liquid smoking is always beneficial and getting advantages over conventional direct smoking method, as liquid fumigants are prepared through a series of fractionation, filtration and purification that can reduce the PAHs contamination in food [24, 29]. Zachara et al. [55] reported the lowest PAHs concentration in pork ham in case of liquid smokes (0.75-2.97 µg/kg) compared to industrial (1.27–7.76 μg/kg) and traditional (8.90–24.57 μg/ kg) smoking methods. In case of traditional fermented smoked sausage preparations, it is advisable to use cellulose casings instead of natural casings, as generated BaP and PAHs cannot penetrate the hydrophilic cellulose casings easily due to their low porosity, smooth geomorphology and negligible relation with carbon particles [56]. Another well-connected information was stated by different research findings with a conclusion that distance between heat source and food components affect inversely PAHs levels [52, 57].

The extent of ventilation and smoke density have a significant impact on the levels of PAHs formation into smoked chamber. In fact, improper inflow of air into the smoke chamber rises the temperature, thereby increasing the smoke density [29, 58]. Epidemiological surveys suggest that extended period of cooking (well done) or degree of doneness and distance from the heat source have a negative influence on PAHs formation, including BaP in meat [13, 52]. Purcaro et al. [57] also revealed that higher cooking temperature (500-700 °C) always favor for higher level of PAHs formation in meat, though in lower temperature range (100-150 °C) also PAHs can be formed in longer geological timescale when carbon and hydrogen containing components are present as the precursor of PAHs. Therefore, it is always advised to reduce the cooking temperature with concomitant cooking time and increase the distance between meat and fuel sources, or opt for indirect heating process to avoid the charring or overcooking [59].

Apart from smoking as a method, different researchers have also worked on other cooking methods to find their effect on PAHs formation in meat products. Mirzazadeh et al. [60] investigated the effects of microwave, pan-frying, and grilling in smoked beef sausages and

found the microwave cooking procedure as a healthier method compared to others with decreased levels of PAHs formation. Olatunji et al. [61] reported grilling and boiling processes better, as these methods reduced the concentrations of PAH4 in smoked chicken (10.52 µg/ kg by 51% and 64.35%, respectively). Comparing the electric and charcoal grilling processes, Hamzawy et al. [62] concluded that electric grills are better and should be frequently used to reduce the B(a)P contamination in grilled chicken. Employing different cooking methods, Onwukeme et al. [63] found boiling as the safest cooking process followed by barbecued and roasted methods, whereas the concentrations of PAHs was the highest in fried chicken product. Comparing the roasting and frying cooking processes, Arfaeinia et al. [64] opined that, frying chicken is better option with 45.29% and 30.72% lower 16PAHs and B(a)P, respectively, than roasting process. The authors also suggested gas roasting for achieving reduction in PAHs concentration (1.15 fold) compared to charcoal roasting. In a study conducted by Büyükkurt et al. [65], pan frying was also adjudged as a safer cooking method for beef meat than barbecuing with regard to the formation of PAHs and its human exposure through diet, as pan fried beef meat had lower levels of BaP (1.39 versus 1.62 μg/kg) and PAH4 (5.58 versus 5.73 µg/kg) compared to barbecued ones. Ohmic heating and infrared heating are new cooking techniques that can be employed to reduce PAHs formation in meat products or keep within the safe limits [66]. For example, cooking beef-based meat ball employing these techniques produced $4.44 \mu g/kg$ of 16 PAHs, which were within the safe limits defined by EU, i.e., $5 \mu g/kg$ [67].

The type of oil is also important, as findings suggest that use of rapeseed, soybean, sesame, and sunflower oil reduce PAHs and BaP formations [68, 69]. To reduce PAHs formation during frying of meat, fresh and unused oils may be used [70]. Furthermore, shallow-pan frying or hot air-frying technique is recommended, as it is comparatively safer than deep-frying [71, 72]. However, given a choice, cooking techniques such as steaming, boiling, and bracing are better because of a lesser carcinogenic risk than frying [72].

Mitigation strategies for PAHs

So far, discussion on different processing techniques of traditional meat products and formation of life-threatening carcinogenic compounds and their toxicities has been made in this article. As the consumption of PAHs contaminated meat and meat products is becoming a subject of wide scientific concern because of public health issues, there is a need to shed light on best possible ways of PAHs reduction strategies, The strategies for reduction and control measures of PAHs formation have been

divided into two segments, i.e., (i) treatment of raw materials before thermal processing, including selection of lean masses, use of suitable fuels, filters, marinades and probiotics, heating methods etc. and (ii) treatment after thermal processing, including warm water rinsing and suitable packaging. Recent innovative strategies that are being employed by various researchers to reduce PAHs formation in meat and meat products, are also enlisted in Table 3.

Utilization of suitable resources of fuels and alternative cooking methods

Raw materials such as meat, fuels and cooking oils have the possibility of pre-contamination by PAHs prior to processing. Furthermore, most PAHs in processed meat products are generated during traditional and faulty cooking processes. Therefore, preventive measures would be beneficial to minimize the density of PAHs formation during cooking. In this regard, use of hard wood containing less lignin instead of soft wood is recommended, as the later burn out quickly at high temperature resulting more PAHs formation in traditional smoking method [24, 29]. In favor of this statement, it has been found that log fire, pine cones, mesquite wood produced high BaP and 4PAHs content in different traditional meat products during smoking [52]. Even coconut shells are used as "flameless and smokeless" charcoal reducing the pyrolysis process [21]. Essumang et al. [73] noticed that use of bagasse instead of woods as a source of smoke generator could effectively reduce the content of PAH4 in smoked fish.Hitzel et al. [74] made an investigation with different wood chips (spruce, oak, alder, poplar, beech, hickory) and found that poplar and hickory reduced PAHs contamination in frankfurters and mini-salamis made up of pork and beef by 35-55%, whereas alder and beech produced highest concentration of PAHs. Malarut and Vangnai [53] reported in their study that BaP and PAH4 concentrations were found in the range of 0.4-0.5 µg/ kg and 1.1–1.5 μg/kg, respectively, in smoked sausages, when beech, neem (Azadirachta indica), copper pod (Cassia siamea), ear leaf acacia (Acacia auriculiformis) and eucalyptus camaldulensis (Eucalyptus camaldulensis) wood were used. The noticeable fact is that both the concentrations of toxins were below the legislative limits by EU, i.e., 2 µg/kg and 12 µg/kg for BaP and sum of PAH4, respectively. Using different charcoal types, Kim et al. [54] demonstrated that white charcoal was little producer of PAH4 when compared with black and extruded charcoal in different meat grilling processes. Therefore, traditional smoked meat producers can be advised to use hard wood (maple, oak, hickory etc. or bagasse) or white charcoal as preventive measures. Pre-heating and charring the wood or charcoal to generate the smoke at high temperature range and extinguishing the flame before grilling or barbecuing the meat may be suitable physical or chemical approaches to alter the chemical profile of generated smoke specially PAHs. Furthermore, reheating of pre-burn charcoal to generate the flame, exposure to high-level temperature for longer time, fat dripping, maintaining improper distance (<25 cm) from heat sources should be prohibited to lessen PAHs concentration in meat products. These general instructions need to be internalized by small stakeholders. For example, Chaemsai et al. [75] pre-heated (650 °C) mangrove charcoal for 5 min, 20 min and 5 h before their use to grill meat and opined that charcoal should glow fully before starting grilling. From the above discussion, it is clear that several factors are intermixed for fat/wood pyrolysis and pyro-synthesis process; therefore, each possible and responsible factor needs to be remedied.

Most of the commercial setups use gas/electric ovens for roasting or barbecuing and indirect smoking methods using different filters (zeolite, granular activated carbon, and gravel filters) to reduce PAHs content in meat products. In a study, Sampaio et al. [6] could be able to show that zeolite and carbon filters reduced the BaP content and PAH4 by 90% and 85%, respectively. Thriving result was observed when chicken samples were grilled wrapping in banana leaf (34.7 µg/kg) and aluminum foil (45.4 μg/kg) to check the 3PAHs [4, 76] and these type of innovative approaches are always appreciable to terminate the current issue, especially for traditional food makers or roadside vendors. Eldaly et al. [77] also utilized this wrapping principle of meat grilling using some barriers between product and flame and a significant reduction of BaP content was observed in mutton and beef. Casings (natural/artificial) play an important role in preventing the contamination of PAHs, specially while preparing sausages, by creating a barrier layer, where surface deposition of smoke particles gradually form clogging pores [29]. In comparison with natural casings, cellulose or collagen casings are more effective to act as good blockade of PAHs penetration. It is because of the fact that natural casings have high porosity and uneven morphology that elevates the chances of PAHs contamination into food, whereas synthetic casings with smooth and compact surface get the deposition of PAHs in very little amount and their penetration ability is also less [29, 56, 78]. During smoking or barbecuing of meat, indirect heating or use of electric or gas oven instead of charcoal, grilling of leaner portion of meat, maintaining proper distance from heat source etc. should be followed by producers for lesser contamination of toxic carcinogens into food. Besides, additional precautions need to be taken by producers to decontaminate the raw materials with toxic environmental pollutants as the possible risk factors

 Table 3
 Current approaches in reducing PAHs formation in meat and meat products

Meat product	Novel and innovative strategies/processing/cooking methods employed	Effects on polycyclic aromatic hydrocarbons (PAHs) formation	References
Guinea fowl meat	Effect of gamma irradiation (GI) (irradiated at 0, 2.5, 5.0 and 7.5 kGy) on meat smoked for 1.7 h at 67 ± 3 °C using neem tree wood	 ✓ Gl significantly (p ≤ 0.05) reduced the concentrations of total PAHs, and PAH4 index in meat ✓ Reduction was dose-dependent with increasing Gl dose resulted in decreasing PAHs concentrations ✓ The BaP of samples reduced by 88.5%, 99.38% and 100% with increasing irradiation dose for 2.5, 5.0 and 7.5 kGy, respectively, compared to control 	[95]
Duck meat	Effect of marinated extracts of green tea (GT); bamboo leaves (BL); grape seed (GS) and rosemary (RE) @ 5 , 15, 25 g/kg on duck skin and meat roasted in oven for 45 –60 min at 202 – 203 °C, with the center temperature at 224 °C	✓ GT @ 25 g/kg exhibited the best inhibitory effects of 75.8% and 79.7%, on BaP and PAH4, respectively, in roasted duck skin ✓ BL @ 25 g/kg could effectively reduce PAH4 content in duck meat compared to other extracts	[110]
Dry sausages <i>Hercegova 'ckakobasica</i> (pork meat—75% and pork fat 25%)	Effect of traditional smoking of sausages (with collagen or natural casings) using hard wood (beech, hornbeam, and oak) and its sawdust @ 2-3 h for 6 days and thereafter on alternate days up to 30 days at 3.5–11.2 °C, or industrial smoking at 25 °C for 4 h a day (8-30 min) for 3 days on heating plate using beech and oak sawdust	✓ Samples with collagen casing and smoked in industrial chamber had the lowest PAH16 content (12 µg/kg) compared to sausages in natural casings smoked in the traditional manner (24.46 µg/kg) ✓ Smoking manner (industrial vs traditional) along with duration and combustion temperature played vital role in PAH4 formation	[45]
Pork meat	Effect of inclusion of natural plant extracts such as bay leaf, black pepper, turmeric, jalapeno pepper and tamarind paste in marinades and charcoal grilling (12 min) of pork between 280 and 300 °C.	✓ Jalapeno pepper marinade had statistically significant reducing effect (95% reduction) on Σ 12PAH levels (4.76 ± 0.08 µg/kg) compared to control sample (98.48 ± 0.81 µg/kg)	[111]
Traditional Portuguese dry-cured sausage (DCS) Paio	Effect of burning oak (<i>Quercus ilex L</i>) wood on smoking of DCS (20 h in 3 days, 60 h in 8.5 days), or until reaching 38–40% weight loss	 Smoking of sausage for 20 h in 3 days was effective with no PAH and comparable to control samples BaP and PAH4 were detected in products subjected to longer smoking periods 	[15]
Charcoal-grilled goat <i>satay</i> with sliced fat	Effect of marinating raw goat with shallots (<i>Allium cepa</i> var. Ascalonicum) juices @ 10%, 20%, and 30% for 60 min at 4 °C using wood charcoal for 6 min with a temperature of 531.06 \pm 30.55 °C and 0.5–1 cm distance from flame	✓ BaP and BaA formation was not detected in goat satay marinated with 10% and 20% shallots juices compared to controls (0.39±0.01 mg/kg)	[112]
Loin pork steaks	Effect of marinating meat (1:1) with pilsner beer (PB), nonal-coholic pilsner beer (POB), and black beer (BB) for 4 h at 5 °C and charcoal grilling at 200–230 °C for 10 min	✓ BB marinade was the most effective in reducing PAH8 (53%) followed by POB (25%) and PB (13%) compared with unmarinated sample (20.57 ng/g) ✓ BB marinade was the most efficient in reducing BaP formation (39.48%) compared with unmarinated sample (2.71 ± 0.82 ng/g)	[21]
Smoked pork sausage	Evaluation of cellulose aerogels from (NaOH/urea, LiBr, and LiOH/ urea) solutions under different smoking (temperature—50, 80, 110, and 140 °C) and time (2, 4, and 6 h) conditions of pork sausages	✓ The LiBr-functionalized absorbent (at 140 °C for 6 h) offered the highest adsorptive efficiency for PAH4 without causing significant differences (p < 0.05) in the physicochemical, microbiological, and sensory parameters compared to the control group (without adsorbent) ✓ Apart from being eco-friendly, cellulosic aerogels could be used to remove PAHs and effectively maintain the desired quality of smoked meat and meat products	[113]

(continued)
Table 3

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Meat product	Novel and innovative strategies/processing/cooking methods employed	Effects on polycyclic aromatic hydrocarbons (PAHs) formation	References
Meat (beef loin, pork belly, and chicken thigh)	Effect of white charcoal (from oak), black from broadleaf trees, or extruded charcoal (from coconut shell, coal, starch, barium nitrate, and sodium nitrate) on grilling of meat at 200 °C	 ✓ Meats grilled using extruded charcoal showed the highest levels of PAHs (p < 0.0001) compared to others ✓ Higher levels of 4 PAHs were found in pork belly than beef loon and chicken thigh meat, due to its high fat content (p < 0.0001) ✓ Combination of white charcoal and low-fat (beef loin and chicken thigh) meat had reduced PAHs formation 	[18]
Charcoal-grilled pork loin	Effect of spraying vinegar—white wine (WV), red wine (RV), apple cider (AV), elderberry (EV), AV with raspberry juice @30–33 mg/gm on meat and grilling at 200 °C for 10 min	 ✓ EV exhibited the highest PAH4 inhibition (5.60 ng/g, 82% less than control) in meat, followed by WV (79%), RV and AV (66%), and AV with raspberry juice (55%) compared to control samples (31.47 ng/g) ✓ Spraying meat with vinegars before grilling limited PAHs formation in grilled meat compared with other mitigation strategies involving phenolic rich products 	[84]
Chinese traditional smoked bacon	Marinade in solution containing pine needle (<i>Cedrus deodara</i>) extract-PNE @ 0.025%, 0.05%, 0.1% and 0.2%, w/w on skinless ham butt kept for 4 days at 4 °C and smoked using applewood at 80 \pm 5 °C for 2 h	 ✓ Inhibitory ability of PNE on the generation of PAHs in smoked bacon was concentration dependent ✓ PNE was effective in nullifying PAH4 of smoked bacon compared with control sample (9.19 µg/Kg) ✓ PNE could be used to improve the quality and safety of smoked bacon 	[114]
Sirloin pork	Effect of grilling pork using low-temperature refined traditional wood charcoal (LTC), high-temperature refined charcoal (HTC), charcoal briquettes (CB) at 350–400 °C for 20 min	 Grilling pork with HTC had significantly lower BaP (88%), PAH4 (81%) and PAH16 (75%) contamination levels than LTC and CB HTC-grilled meat was safer with better palatability quality in terms of juiciness and tenderness 	[115]
Sirloin pork	Effect of deep-fat frying Sirloin pork in lard or palm oil at 180 °C for 24 times or 8 h (20 min of each batch, including 5 min frying time and 15 min waiting time)	✓ BaP levels in meat fried with palm oil were much lower (0.08–0.23 µg/kg) than with lard (0.53–1.86 µg/kg) ✓ Pork fried in palm oil had lower PAH4 (1.46–2.72 µg/kg) and PAH16 levels (10.43–34.13 µg/kg) compared to PAH4 (<3.09–10.17 µg/kg) and PAH16 levels (<24.52–57.46 µg/kg) with lard fried pork	[115]
Charcoal barbecued pork patties	Effect of perilla leaves extract-PLE @ 0.2% or 0.4% on medium-cooked (barbecued for 9 min until internal temperature reached 71 °C) and well-cooked (barbecued for 16 min until internal temperature reached 80 °C) pork patties	✓ PLE @ 0.4% exhibited stronger suppressive effect (p < 0.05) on formation of PAH4 and PAH8 and BaP in well-cooked compared to 0.2%, control and medium-cooked patties	[116]
Charcoal-grilled chicken wings	Effect of marination using phenolic marinades (gallic, proto- catechuic, and ferulic acids) @ 0.1, 1, 3, and 5 mg/L of phenolic aqueous solutions for 4 h at 48 °C and grilling at 270, 240, and 210 °C for 8 min	 ✓ Concentration of BaP significantly decreased (p < 0.05) as grilling temperature decreased from 3.28, 2.57, and 1.38 ng/g at 270, 240, and 210 °C, respectively ✓ Lowest total PAHs contents were obtained with use of protocatechuic acid @ 3 mg/L marinade ✓ Grilling marinated meat at lower temperatures had greater inhibitory effects on PAHs formation 	[117]

Table 3 (continued)			
Meat product	Novel and innovative strategies/processing/cooking methods employed	Effects on polycyclic aromatic hydrocarbons (PAHs) formation	References
Cold smoked pork sausages	Effect of lactic acid bacteria (LAB) suspension viz. <i>Pediococcus acidilactici</i> KTU05-7, <i>Pediococcus pentosaceus</i> KTU05-9 and <i>Lactobacillus sakei</i> KTU05-6 strains on pork sausages before (at 18 °C for 60 min) and after (16 °C for 130 min) smoking	 ✓ LAB treatment before and after smoking of sausages significantly (p<0.05) decreased both BaP and chrysene ✓ PAHs formation in outer layers of sausages were more in comparison with inner layers ✓ Sausages treated with LAB before smoking were more acceptable than treated after smoking ✓ Samples treated with P. pentosaceus had the highest acceptablity 	[105]
Duck meat	Effect of smoking (24 h vs 48 h) period and wood types viz. beech (Fagus sylvatica), plum (Prunus domestica) and cherry (Prunus cerasus)	✓ Duck meat had highest concentrations of PAHs, irrespective of smoking period or wood type ✓ PAHs concentration was higher while using beech wood followed by cherry and plum woods ✓ 48 h of smoking contributed to higher and fastest accumulation of PAHs in duck meat	[118]
Pork sausages	Influence of smoking at 55°C, 65°C, 75°C, 85°C, and 95°C for 3 h or for 2, 3, 5, 6, 8, and 9 h at 75°C using sawdust of beech wood, apple, plum, birch, oak, walnut, alder on pork sausages	 ✓ Sausages smoked for 3 h at 55 °C, had lower BaP and PAH4 content ✓ Higher temperatures (55–95 °C) and longer smoking periods (2–9 h) showed increasing trend in PAHs concentrations ✓ Apple and walnut wood sawdust were better as yielded lower PAH concentrations compared to others 	[119]
Grilled Sirloin pork	Effect of marinade (dietary antioxidants, diallyl disulfide-DADS and quercetin @100 mg/kg or 500 mg/kg) treatment of Sirloin pork at $4^{\circ}\pm 2^{\circ}$ C for 1 h followed by charcoal grilling @ 2 min/side	✓ DADS @ 500 mg/kg meat in marinade significantly decreased BaP (100%) and heavy PAHs (84%) in charcoal-grilled pork ✓ Quercetin @ 500 mg/kg meat in marinade could reduce BaP (23%) and heavy PAHs (55%) compared to control samples	[120]
Roasted duck wings	Effect of coriander root extract (CRE) and coriander leaf extract (CLE) @ 200, 400, 600, 800, 1000 mg/L on marination of duck wings at 4 °C for 4 h and roasted for 8 min using bamboo charcoal	✓ CRE-600 exhibited greater inhibitory effect (87.4%) followed by CRE-800 (79.7%) and CRE-400 (79.0%) on PAH8 forma- tion in roasted duck wings in comparison with control group (22.84 ng/g) ✓ CRE could be considered as a kind of natural source to miti- gate PAHs in heat-processed meat products	[121]
Chicken breasts, thighs and wings	Effect of thawing (microwave at 310 W for 3 min, refrigerator at 4 °C for 24 h or immersion in distilled water at 20 °C for 1 h followed by deep-fat frying at 180 °C for 10 or 35 min at minutes) or hot air frying (at 180 °C for 35 min)	2.60–3.17 µg/kg) 96–2.71 µg/kg) igher (p<0.05) total t (p>0.05) on PAH ered (p<0.05) total han by other means	[1/2]
Beef burgers	Effect of replacement of tallow in burgers with vegetable oil (VO) and milk fat (MF) followed by flipping (every 3 min) during charcoal grilling at 280 and 300 °C	 Partial replacement of beef tallow with vegetable oils and milk fat in burgers reduced the formation of PAHs Replacing beef tallow with vegetable oils better approach in designing meat products with controlled PAHs content 	[122]

Table 3 (continued)			
Meat product	Novel and innovative strategies/processing/cooking methods employed	Effects on polycyclic aromatic hydrocarbons (PAHs) formation	References
Raw ground pork	Effect of marinating solution using cinnamon or green tea powder @ 0.5% over 2, 4, 8, 12 or 24 h	✓ Marinating raw pork either with cinnamon or green tea solution for 2–12 h was more effective in inhibiting PAHs formation as compared untreated samples ✓ Extending time length of marination (24 h) increased the PAHs content in pork due to elevation of PAHs precursors, such as benzaldehyde, 2-cyclohexene-1-one and trans, trans-2,4-decadienal	[123]
Ground pork meat fried pork balls	Effect of ginger or rosemary @ 0.25% and 0.75% on pork meat balls fried using soybean oil at 180 °C for 3 min	 Ginger had no significant effect on the PAH4 content in fried pork balls Rosemary @ 0.25% and 0.75% reduced the PAHs content in fried pork balls by 30% and 35% compared to control (9.34 ng/g). This could be due to the high temperature resistance of its active ingredients 	[124]
Charcoal-grilled beef steak	Effect of in-package cold plasma (ICP) treatment (at 85 kV for 180 s) of charcoal-grilled (180–220 °C for 12 min) beef steak pretreated with vegetable oil (colza, soyabean, sunflower seed) or solid animal oil (pork fat, butter) @ 50 g/kg sample	✓ ICP technique to pre-treat raw meat before grilling could significantly reduce PAHs production in the grilling process ✓ Sunflower seed oil had an inhibitory effect on PAHs formation; the groups with ICP pretreatment showed a significant decrease in PAHs content (p < 0.05)	[125]
Charcoal-grilled chicken drumsticks	Effect of charcoal grilling chicken drumsticks (without skin, with skin, and skin removal after processing) at about 400 ℃ for 10, 20 or 40 min	 ✓ Presence of skin and long-term grilling (40 min) had a greater impact on PAHs levels (35.71 ng/g) compared to short grilling time for 10 min (14.28 ng/g) ✓ Skin of chicken drumsticks should be removed to reduce the PAHs content ✓Longer the grilling time, the higher the content of PAHs was noted 	[126]
Grilled beef satay	Effect of honey (bee honey-Apis mellifera and "kelulut" honey (<i>Trigona</i> sp.)-spices marination and gas-grilling beef satay samples at temperatures (150 °C, 250 °C, and 350 °C)	✓ PAH15 level in beef satay increased significantly (p < 0.05) with increasing grilling temperatures, with the highest concentrations detected at 350 °C ✓ Inverse quantitative profiles of PAHs formation observed in marinated gas-grilled beef satay	[127]
Barbecued pork sausages	Effect of marination with distilled (white vinegar—WV) or fermented vinegar (aromatic—AV; vinegar—MV; sun—SV) @ 2%, w/w for 6 h and grilling pork sausages at 280 °C (core 75 °C)	✓ Vinegars significantly (p < 0.05) inhibited the formation of BaP (26.8–82.3%) in barbecued pork sausages ✓ Fermented vinegars were effective in enhancing the sensory (tenderness, flavor, juiciness and overall acceptability) attributes of sausages ✓ Among vinegars, SV (82.3%) inhibited BaP more than AV (65.0%) and MV (79.2%) compared to WV (26.8%) and control group	[128]

Table 3 (continued)			
Meat product	Novel and innovative strategies/processing/cooking methods employed	Effects on polycyclic aromatic hydrocarbons (PAHs) formation	References
Pork jerky	Effect of flavoring materials (20% sugar and 10% soy sauce; 10% sugar and 10% soy sauce; 20% sugar and 5% soy sauce; 10% sugar and 5% soy sauce; standard formula and 0.5% curcuma powder; standard formula and 0.5% cinnamon powder) and roasting temperatures (180 °C and 220 °C) for a time of 10 min	 ✓ 20% sugar and 10% soy sauce were more effective in inhibiting formation of PAHs (52.639 ng/g) compared to control (72.609 ng/g) in pork jerky ✓ Pork jerky roasted at 220 °C generated a higher PAHs content than 180 °C ✓ Sugar had a more pronouncing effect in inhibiting PAHs formation than soy sauce 	[129]
Beef patties	Effect of nutmeg and ginger essential oils (0.02% and 0.04%) and their nanoemulsions on beef patties grilled at internal temperature of 75 °C for 5 min and stored for 45 and 90 days at $-18^{\circ}\mathrm{C}$	 ✓ Increase in the storage time significantly increased PAHs levels of the beef patties ✓ Nanoemulsion-based nutmeg oil @0.04% had a great impact in preventing carcinogenic compound formation in grilled patties 	[130]
Charcoal-grilled pork sausages	Effect of garlic powder @ 0.05%, 0.1%, or 0.15% (w/w), or garlic essential oil-GEO @ 0.002%, 0.004%, or 0.006% (w/w) to meat marinated at 4 °C for 6 h and grilling the sausage at surface and core temperature of 280 °C and 75 °C, respectively, for 20 min	 ✓ Garlic was more efficient in reducing BaP formation (37.2–62.3%) than GEO (29.1–57.1%) compared to control (1.99 µg/kg) ✓ BaP content decreased significantly (ρ < 0.001) with increasing concentrations of garlic in sausages ✓ BaP content decreased significantly with the increasing concentration of GEO (ρ < 0.001) in the charcoal-grilled pork sausages 	[131]
Beef meat balls	Effect of animal fat types (10% beef intramuscular fat, 10% sheep tail fat, 5% beef intramuscular fat + 5% sheep tail fat)on meatball formulations charcoal barbecued at 200 °C for 8 min kept at +4 °C for 2 h	 ✓ Animal fat significantly affected amount of BaP and PAH4, and the levels of BaP and PAH4 were ranged between 2.33–4.30 and 8.41–15.48 ng/g, respectively ✓ Meatballs formulated with 10% sheep tail fat had significantly (p < 0.05) higher levels of PAH4 (15.48 ng/g) ✓ Use of a mixture of intermuscular fat and sheep tail fat helped in reducing PAHs formation (about 46%) compared to meatballs using only sheep tail fat 	[132]
Pork sausages	Effect of y-irradiation at doses of 0, 1, 4, and 8 kGy, on pork sausages baked at 220 °C for 20 min	✓ The concentration of PAH4 in irradiated sausage was notably reduced by 16.89% (1 kGy), 36.79% (4 kGy), and 51.68% (8 kGy) compared with unirradiated sausages (46.83 µg/kg) ✓ γ-ray irradiation could be used as a powerful food preservation method to reduce chemical hazards, thereby improving food safety	[133]
Grilled pork sausage	Effect of grape seed extract-GSE (10%) on pork sausage roasted using an electric oven for 20 min at 240 °C (core temperature about 90 °C) and stored for 2, 4, 6, and 8 days	✓ PAH12 and PAH4 in GSE treated sausages were significantly lower (p < 0.05) up to 4 days, but notably increased on 8th day compared to control group ✓ Sausages should be processed and consumed early, when GSE is used for preservation	[134]

Table 3 (continued)			
Meat product	Novel and innovative strategies/processing/cooking methods employed	Effects on polycyclic aromatic hydrocarbons (PAHs) formation	References
Charcoal-grilled pork belly	Effect of marination with $Gochujang$ (fermented red-pepper paste) and storing (10 days at 9 °C) of pork belly under vacuum followed by charcoal grilling at internal temperatures of 71 °C for 3.5 min or 81 °C for 5 min	 ✓ PAHs formation was higher at 81 °C than at 71 °C in charcoalgrilled pork belly (ρ < 0.05) ✓ Storage time increased the inhibitory effects of Gochyjang marinede with maximum reduction in total 16 PAHs (63.06%) observed with moderate cooking (71 °C) on day 10 (ρ < 0.05) ✓ PAH4 reduced to 39.86 and 121.26 µg/kg in 71 °C and 81 °C treated samples, respectively, compared with control (ρ < 0.05) 	[54]
Barbequed chicken wings	Effect of marinating chicken wings with curcumin @ 0.01, 0.05, 0.25 or 0.3 mg/kg at 4 °C for 4 h and grilling at 220 °C	✓ Curcumin @ 0.3 g/kg was effective in inhibiting PAHs by 65% compared to untreated sample (3.18 ng/g) ✓ Addition of curcumin before grilling is a feasible strategy to limit PAHs levels.	[135]
Chicken satay	Effect of chicken meat marinated for 10 min with lemon @ 0%, 5%, 10% and 15% on PAHs	✓ Chicken satay marinated with 15% lemon marinates had reduced content of PAH4 compared to other treatments and control	[136]
Charcoal grilled pork	Effect of apple polyphenol (AP) @ 0.02%, 0.05%, 0.10% or 0.20% on PAH formation in meat marinated at 4 °C for 4 h, and charcoal grilled at 200 °C	✓ AP @ 0.20% completely inhibited BaP formation, whereas the rate of PAH4, PAH8 and PAH16 inhibition were 52.68%, 53.10% and 37.36%, respectively ✓ Addition of 0.20% AP also retained the functional properties of the grilled pork	[137]
Grilled beef patties	Beef patities fortified@0.5% and 1% with raw, or black garlic (aged at different combinations of temperature and duration) and cooked on a hot plate at 200 °C for 7 min on the polycyclic aromatic hydrocarbons (PAHs) content and toxic potency	✓ Beef patties fortified with black garlic@1% (aged at 70 °C for 45 days) had reduced PAH8 content of up to 94.12% compared to raw garlic ✓ Patties fortified with black garlic had a lower incremental lifetime cancer risk than raw garlic fortified patties	[138]
Roasted pork loin	Pork loin roasted with dried fruits (prunes, apricots or cranberries) at 180°C for 60 min	✓ Pork loin roasted with cranberries had strongest inhibitory effect on total PAHs content (58%) compared to apricots (35%) and prunes (48%) ✓ Cranberries had the strongest inhibitory effect with reduction in BaP content by 100% ✓ Use of dried fruits in thermally processed meat is an effective way in reducing the PAHs content and the risk of cancer	[139]

of PAHs contamination into food. Therefore, extensive investigation is required for thorough monitoring of the raw materials during transportation, processing, storage, and possibility of environmental exposures (e.g., air, soil, and surface water quality) from safety point of view. Furthermore, successful industrial PAHs reduction strategies need to be adapted by traditional smoked meat manufacturers, irrespective of the consumer preferences and sensory attributes.

Use of marinades to decrease PAHs

Marination is a universal practice used specially for improving the texture and quality attributes (tenderness, juiciness, flavour) of thermo-processed meat products. Selection of appropriate marinade ingredients can reduce the level of PAHs in the end product by influencing the physico-chemical properties of the processed meat. The reason being, pretreatment of meat with a mixture of spices, fruits or vegetables or their extracts containing inimitable active principles, such as citric acid, ascorbic acid, antioxidants, phenolic components etc. before thermal processing can inhibit the formation of carcinogenic components or may accelerate the process by the faulty practices [41].

Research findings of several workers are available in this aspect. For example, addition of onion (30 g/100 g of meat) or garlic (15 g/100 g of meat) during meat processing resulted in a 60% and 54% decrease in 6PAHs levels [79]. Pretreatment with tomato juice, garlic paste, onion, salt and spices (cumin, coriander, and black pepper) reduced down the PAHs level significantly in chicken after thermal processing compared to using of garlic alone and that may be due to antioxidative properties of spice mixtures [80]. Sinaga et al. [81] marinated duck meat before charcoal grilling for 60 min with juice of pepper (Zanthoxylum acanthopodium) and observed a 2.6 times reduction of BaP in treatment (295 µg/kg) compared to control group (787 µg/kg).In a recent study, Onopiuk et al. [29] incorporated different plant extracts individually or as mixture (bay leaf, black pepper, turmeric, jalapeno pepper and tamarind paste) of marinates for pre-treatment of pork neck before grilling. The authors reported that although plant extracts significantly reduced the concentration of PAHs formation in pork products, phenolic components, especially of jalapeno pepper reduced almost 95% of 12PAHs $(4.76 \pm 0.08 \mu g/kg)$. The authors concluded that biologically active substance capsaicin present in jalapeno acted as scavenger of produced free radicals, which prevented the cyclisation and oxidation reactions thereby enhancing the safety and shelf life of grilled meat products. Similarly, effect of different beers (Pilsner beer, non-alcoholic Pilsner beer, and dark beer) for marinating pork before heat processing showed noticeable changes to diminish BaP concentration up to 1 μ g/kg [82].

Vinegar, which is a fermented product, possesses active components such as phenolic compounds and is often used to improve the microbiological quality, safety, and shelf-life of food products [83]. In a study conducted by Cordeiro et al. [84], vinegars with different levels of antioxidative performances showed significant differences in inhibiting the PAHs concentration in smoked meat products. It was reported that use of acidic substances in marinate could reduce remarkable levels of PAHs during thermal processing of meat, in lieu of alkaline ingredients or oil in marination that enhance the level of heavy PAHs formation in meat. In this regard, lemon juice or tamarind juice showed fruitful results to inhibit PAHs formation in grilled meat products other than their role in improving textural and physico-chemical properties of meat [6, 76, 85]. Eldaly et al. [77] studied the effect of yoghurt in combination with different spice mixtures on marination of beef for preparation of kabab and kofta. Marinating beef before grilling reduced PAHs levels to 57.93 µg/kg in grilled kebabs and 30.2 µg/kg in grilled kofta compared to 119.8 μg/kg of PAHs in *kabab* and 59.2 μg/ kg of PAHs in kofta of untreated samples. Marinating with vinegars brought positive result reducing 4PAH content of charcoal grilled pork loin [84]. The highest PAHs reduction was with Elderberry vinegar (82%) followed by white wine vinegar (79%), red wine and cider vinegars (66%), and fruit vinegar with raspberry juice (55%). Natural resources of food with effective phenolic components and their free radical scavenging capacity may act as potential inhibitors for carcinogen formation in smoked meat products. Still then, more advanced research is needed in this field to unwind the exact mysterious mechanisms of application of acidic marinades and their restricting actions on PAHs formation upon severe heat treatment. Likewise, marinades containing different antioxidants (epigallocatechin gallate, gallocatechin, catechin, epicatechingallate, catechingallate, eriodictyol, naringenin, quinic acid) extracted from green tea made notable droppings in PAHs concentrations during grilling or roasting of chicken wings or pork meat [86–88] possibly due to presence of polyphenols as active key components. Darwish et al. [89] studied the antioxidative effects of micronutrients rosmaric and ascorbic acids against heat-treated meat and concluded that such molecules protected human colon (CaCo-2) cells from BaP induced mutagenicity and oxidative stress. From the above findings, it can be deduced that marination with different phytochemicals containing polyphenols and flavonoids, spice mixes, curd etc. together with associated precautions might be

used successfully to reduce PAHs content in thermally processed meat products.

Packaging systems, adsorbents and ultraviolet (UV) applications

The purpose of packaging is to safeguard quality, reduce losses, and extend shelf-life, till the processed food products reaches to the end user. With technological advancements, packaging materials now offer new prospects in eliminating hazardous compounds, such as PAHs content from various foods, including smoked meat products. Kuzmicz and Ciemniak [90] reported that different kinds of packaging materials, including high density polyethylene (HDPE), low density polyethylene (LDPE), polypropylene, oxo-degradable, and polyethylene terephthalate etc. have good PAHs adsorbent properties, to where the PAHs can migrate from food. In separate studies, BaP reduction has been reported by application of LDPE packaging system for smoked sausages and roasted meat products, respectively, by different researchers [91, 92]. Likewise, Chen and Chen [92] have reported that packaging of roasted duck meat into LDPE at 25 °C reduced almost 73% of BaP level after 24 h of storage. Besides, application of UV (2–3 h) to LDPE film also reduced BaP content specifically up to 13.5-29.2%.

In recent years, a great attention is being paid on the development of novel aerogels as an ideal alternative to various adsorbents because of their desirable quality properties, such as low density, high porosity, and large surface area [93]. Conducting a study, Kim et al. [54] used natural, renewable and environment friendly cellulosic aerogels (NaOH/urea, LiBr, and LiOH/urea) as adsorbent layer to remove PAHs from smoked meat and meat products. Based upon surface structure and pore size distribution of the cellulosic aerogels, the LiBr-functionalized absorbent exhibited the highest adsorptive efficiency for total PAHs. Even chlorinated polyethylene (CPE), which is microplastics polymer obtained through structural or surface modification of conventional polyethylene, is reported to adsorb many organic compounds, including PAHs and benzene derivatives faster, present in freshwater [94]. The efficacy or adsorption behavior of CPE may be tested in different food matrices, including PAHs contaminated meat and products. In a recent study, gamma irradiation of smoked guinea fowl (Numida meleagris) meat at a dose of 5 kilo grey (kGy) was reported to have the potential to reduce the concentrations of PAHs and their carcinogenic derivatives compared to non-irradiated meat [95]. Therefore, it can be assumed that progressive advanced research on smart or active packaging system and application of ultraviolet rays may be the great reduction tools of PAHs for ensuring safety of meat products.

Washing of smoked meat products

Washing of smoked meat products refers to the removal of skin or outer layer of the product, rinsing with lukewarm water, cleaning under running tap water etc. [96, 97]. Few reports are available indicating that washing as a procedure is effective in reducing the PAHs content in smoked meat products. Mahugija and Njale [98] observed the effect of washing of smoked products with purified lukewarm water (60 °C, 2-3 min) to decrease the total PAHs content significantly. Though this technique is quite helpful to mitigate the problem, sometimes may not pass the organoleptic tests. Furthermore, washed products showed lesser shelf-life suggesting future studies to focus on storage and preservation of the washed smoked products. In another study, pork loins rinsed immediately after smoking error had reduced PAHs content (BaP = $3.43 \pm 0.21 \, \mu g/kg$; PAH4 = $25.33 \pm 1.88 \, \mu g/kg$) in the surface part of samples, compared to PAHs content (BaP= $7.33\pm0.77 \mu g/kg$; PAH4= $61.14\pm1.72 \mu g/kg$) in smoked but unrinsed surface samples [97]. The PAHs (BaP and PAH4) values obtained after rinsing smoked dry-cured pork loin be considered safe, as are within the permissible values (BaP < 5 μ g/kg; PAH4 < 30 μ g/ kg) of the EU Regulation for traditional meat and meat products.

Application of probiotics and LAB

Biological methods are now becoming promising alternatives over physical and chemical decontamination methods to eliminate carcinogenic and mutagenic compounds from foods. It is because of their GRAS (Generally Recognized as Safe) status, inexpensive nature, and nature-friendly properties. The reason being many microorganisms (bacteria, algae, and fungi) have the ability to utilize PAHs as carbon source, required for their growth and development. In recent years, a novel environment friendly mitigation strategy to reduce the PAHs contamination in food has drawn the attention by biological degradation procedure with lactic acid bacteria (LAB) and probiotics [99, 100]. Various literatures have also mentioned the mechanisms involved to deactivate the toxicity of food-borne carcinogens, especially PAHs and acrylamides. The steps involved in PAHs breakdown include activation of antioxidative enzymes, such as oxidase, manganese peroxidases, lipases etc., conversion of organic and stable carcinogens into less toxic degradable hydrophilic metabolites, and above all, the binding of heat generated carcinogen to the cell wall and peptidoglycans of these bacteria may detoxify them biologically [99, 101, 102].

An elaborative description about the probable decontamination mechanisms of lactic acid bacteria against food carcinogens, specially BaP has been described by Shoukat [17]. The physical binding of DBP (Di-n-butyl phthalate) of organic toxins with C-O, OH and/or NH functionals groups of peptidoglycan layer of cell wall of probiotics and lactic acid bacteria by formation of hydrogen bonds has been illustrated by FTIR (Fourier transform infrared spectroscopy) and MD (molecular dynamics) techniques in laboratory [17, 103]. However, the binding efficiency of individual organism varies based upon their different specificities, such as pH, incubation temperature and time, stability with acid and heat treatment, nutritional availability, concentration of bacterial cells etc. For instance, heat treated dead LAB showed similar kind of BaP binding ability of live viable pellets in a study conducted by Zhao et al. [104]. Likewise, the binding rate of the viable cell of Lactobacillus pentosus CICC 23163 and Lactobacillus plantarum CICC 22135 with toxins were 64.36% and 66.73%, respectively, and showed no significant differences compared to the binding ability (67.83% and 62.18%) of heat-treated (121 °C for 15 min) pellets from the same strain [103].

As mentioned earlier, biological detoxifications of PAHs are a promising alternative to chemical methods and challenging task with highest efficacy in near future. Lactobacillus delbrueckii subsp. lactisATCC 4797, Lactobacillus plantarum ATCC 8014, Lactobacillus casei ATCC11578, Lactobacillus sakei 23 K, and Lactobacillus plantarum WCFS1, Bifidobacterium adoleascentis ATCC 15703, B. infantis, B. bifidum, B. adolescentis, B. longum, B. lactis, and B. breve, Streptococcus thermophilus are different probiotics and LABs detoxified different PAHs from food system, specially the smoked meat products successfully either in-vivo or in-vitro [17, 102]. It was reported that acidic pH, higher incubation time and temperature and concentrations are linearly correlated with PAHs binding ability of probiotics and LAB [17]. Yousefi et al. [102] made an experiment with artificially contaminated PAH4 (BaA, BaP, BbF and Chr) phosphate buffer saline to check the detoxification efficiency of Lactobacillus brevis TD4 and observed that maximum binding rate was obtained at initial concentration of 10 ppm, pH 5, bacterial population of 109 CFU/mL and 24 h of incubation time.

In yet another study, fermented potato juices containing metabolites (bacteriocin, pediocin etc.) of different strains of lactic acid bacteria (*Pediococcus acidilactici* KTU05-7, *Pediococcus pentosaceus* KTU05-9 and *Lactobacillus sakei* KTU05-6) isolated from spontaneous rye sour dough were applied over the surface of pork

sausages at 18 °C and for 60 min before or after smoking [105]. It was interesting to note that not only BaP and chrysene, the toxic carcinogens from surface of sausages, but also different biogenic amines (cadaverine, spermidine and putrescine) from either surface or core of the sausages were reduced simultaneously. Lactic acid bacteria and their fermented metabolites are also reported to significantly inhibit the growth of different pathogenic and food spoilage organisms, including *Pseudomonas aeruginosa* and *Escherichia coli*, enhancing the shelf stability of products. Different experiments in gastrointestinal digestion cell lines and animal model have also proved the role of LAB for removing the BaP and other dietary toxins [106].

Although the role of probiotics detoxifying diet contaminated with BaP or other PAHs is known, introduction of genetically modified organisms (GMO) for better binding ability with toxins may lead to a new possible hope for dietary PAHs induced cancer. Furthermore, synthesis of biosurfactants by suitable and safe microorganisms also could be helpful in bioremediation of toxins [19], as the microbial biosurfactants have the ability to break down and disperse the toxins and PAHs. In this regard, the probiotic bacteria bind to PAHs on the meat surface. The bound PAHs along with the probiotics can be eliminated from meat surface using further processing methods, such as slicing, washing, marination etc. As limited studies have been conducted on role of the potentials of probiotics to remove PAHs as compared to other bioremediation strategies, further research is needed.

Conclusion

In this review, we conducted a critical discussion about the formation, occurrence, mechanisms of toxicity and different reduction strategies of PAHs in heat processed meat products. It is worth to mention that the fatal effects of PAHs such as toxicity, carcinogenicity, teratogenicity, and mutagenicity on living organisms have been recognized and reported by several epidemiological studies and government organizations. Furthermore, the association between dietary PAHs exposures and their role as carcinogens in human beings has been established. Therefore, it is necessary to focus on prevention and control of PAHs formation in processed meat products through various strategies to avert public health concerns and safety issues. However, the task is truly challenging because of the diversity of regional and type of foods, consumer preferences and end-point cooking temperatures [140] and meat processing/preparation systems practiced throughout the globe [141]. To reduce the PAHs content, efforts should be made in terms of the optimization of the cooking and processing conditions during thermal processing of meat products.

This is possible through innovative approaches such as right combination of heating treatments, cooking conditions, and technical processes (wood types, time-temperature combination, smoking filters and adsorbents, plant-derived antioxidants and phenolic components as marinades, bio-elimination processes by applying LAB and probiotics) to remove the already existing PAHs from meat products. While doing so, it should be kept in mind that strategies adopted to reduce PAHs during grilling or smoking process should not drastically alter/ affect sensory characteristics of meat products. Above all, food safety policies and regulations must be revised, reinforced and monitored by legislative authorities from time to time. This is required to ensure that the meat products contain PAHs within permissible limits and are safe for human consumption.

Acknowledgements

Not applicable.

Author contributions

AKD: conceptualization, methodology, software, data curation, visualization, supervision, writing—original draft, writing—review and editing. DB: resources, writing—original draft. AD: resources, writing—original draft. SN: writing—review and editing. SB: resources, writing—review and editing. PKN: conceptualization, methodology, resources, writing—original draft, writing—review and editing. MG: conceptualization, methodology, resources, data curation, writing—original draft, writing—review and editing.

Funding

Not applicable.

Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that there are no competing interests.

Author details

¹Eastern Regional Station, ICAR-Indian Veterinary Research Institute, Kolkata 700 037, India. ²Department of Livestock Products Technology, Faculty of Veterinary and Animal Sciences, Banaras Hindu University, Varanasi 221 005, India. ³Department of Livestock Products Technology, West Bengal University of Animal and Fishery Sciences, Kolkata 700 037, India. ⁴PEGASE, INRAE, Institut Agro, 35590 Saint-Gilles, France.

Received: 9 July 2023 Accepted: 22 September 2023 Published online: 10 October 2023

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