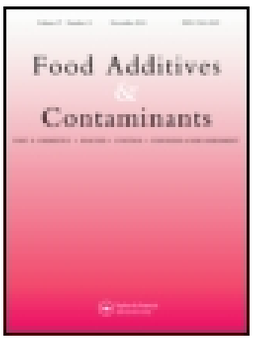

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Polycyclic aromatic hydrocarbons contamination of traditionally grilled pork marketed in South Benin and health risk assessment for the Beninese consumer

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) contamination was monitored in grilled pork sold in Beninese street restaurants, as well as in grilled pork from a well-controlled experiment replicating traditional grilling using *Acacia auriculiformis* wood as fuel. Fifteen PAHs were analysed using a high-performance liquid chromatography method coupled with fluorescence detection. To assess the risk for the consumer, the margins of exposure (MOEs) were calculated, as the ratio between benchmark PAHs levels and consumer intakes. A MOE below 10,000 indicates a concern for human health for carcinogenic compounds such as PAHs. Benzo(a)pyrene (BaP) levels up to 17.9 and 53.6 µg/kg were found in grilled pork sampled in restaurants and from the controlled experiment, respectively. When considering both median estimated daily intake and median PAHs contamination levels, MOEs calculated for Benzo(a)pyrene (BaP) alone, or for the sum of 2, 4 or 8 PAHs were above 10,000, meaning no risk in these cases. However, for the same PAHs contamination level, MOE for consumers having large amounts of grilled pork (97.5th percentile and maximum level of pork consumption) were well below 10,000. When considering the maximum level of PAHs contamination, MOEs ranged between 257 and 2,757 for the high and median levels of consumption, indicating a safety concern for these consumers. This study reveals that Beninese grilled pork consumers from South Benin can be exposed to high levels of PAHs, which might result in public health issues.

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Traditional grilling; benzo(a) pyrene; margin of exposure; *Acacia auriculiformis*

Introduction

In developing countries, meat and fishery products are often preserved from spoilage by smoking, curing, roasting or grilling (Akpambang et al. 2009; Anihouvi et al. 2013; Kpoclou et al. 2013; FAO 2015a, 2015b). These processes improve both shelf life and organoleptic properties of the end products. However, these processes, which are performed in a traditional way, can result in food contamination by toxic compounds such as polycyclic aromatic hydrocarbons (PAHs), which can have adverse effects on consumers' health (EFSA 2008; CAC 2009; Agodokpessi et al. 2011; Ledesma et al. 2016). PAHs are chemical contaminants produced from the incomplete combustion of organic matter during heat processing of food (including grilling) and may be transported through smoke (EFSA 2008; Lee et al. 2016; Singh et al. 2016; Lu et al. 2017). Due to their genotoxic properties, 16 PAHs were initially included in a priority list from European Union (EU) (SCF 2002). Later, the European Food Safety Authority (EFSA) showed that four PAHs were particularly relevant according to both their occurrence in food and their toxicity, and considered them as suitable indicators for PAH risk assessment in food (EFSA 2008). These four PAHs are benzo[a]pyrene

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(BaP), chrysene (CHR), benzo[b]fluoranthene (BbF) and benz[a]anthracene (BaA). This group is named PAH4 in this paper. Among PAH4, BaP is the only compound recognised as carcinogenic for humans (Group 1) by the International Agency for Research on Cancer (IARC) (WHO/IARC 2012). The three others are classified as Group 2B or "possibly carcinogenic to humans" (WHO/IARC 2012). Before the 1 September 2014, there was a maximal limit in food for BaP only, which was 5 µg/kg in both European (EC, 2006) and Beninese (MAEP 2007) regulations. Since the 1 September 2014, EU maximal limits for BaP and PAH4 were lowered to 2 µg/kg and 12 µg/kg, respectively, while the Beninese regulation remained unchanged.

Several factors such as temperature and duration of smoking, distance between heat source and product, fat content and drainage or type of wood, can influence the amount of PAHs contaminating the food after smoking or grilling (Stołyhwo and Sikorski 2005; Akpambang et al. 2009; CAC 2009; Farhadian et al. 2010; Kpoclou et al. 2014; Lee et al. 2016). PAHs contamination of grilled meat, including pork, has been previously reported (Poligné et al. 2001; Chung et al. 2011; Chaber and Cunningham 2015; Ledesma et al. 2015). Specifically for pork, several studies have reported the presence of PAHs after grilling (Rose et al. 2015; Lee et al. 2016; Lu et al. 2017) and after smoking (Ledesma et al. 2016; Zachara et al. 2017). For example, Duedahl-Olesen et al. (2015) reported high levels of BaP (63 µg/kg) and PAH4 (195 µg/kg) in barbecued samples of Danish pork.

In Benin, traditionally produced grilled pork may contain PAHs due either to smoke generated from different wood species identified as PAHs producers (Kpoclou et al. 2014), and/or to fat combustion occurring when fat drips into embers during processing. Traditional direct grilling is a process during which pork is directly laid on embers, while during indirect grilling, embers are produced in a combustion chamber and pork is placed in a grilling chamber, both chambers connected by a hole (Iko Afé 2017). According to the Codex Alimentarius Commission, the use of the indirect process results in lower PAHs content than for the direct process (CAC 2009). The present study aimed to evaluate the potential PAH contamination in grilled pork sold in Beninese street restaurants, and to monitor this contamination in a well-controlled experiment replicating traditional pork grilling using *Acacia auriculiformis* wood as fuel. Since grilled pork is widely consumed by the Beninese population, another aim of this study was to assess the exposure of consumers to PAHs through the consumption of grilled pork, and to assess the risk linked to this exposure.

Material and methods

PAH contamination of grilled pork

Sampling of grilled pork in street restaurants

Twenty-four samples of ready-to-eat grilled pork were randomly collected in street restaurants in a 131-km radius centred in Cotonou, Republic of Benin. They included samples with different traditional grilling methods (direct and indirect) and types (skewer, slice, piece and wrapped).

Experimental design and sampling

Six pigs of indigenous breed, from the same genitor, fed and bred in the same farm of Porto-Novo (Southern of Benin), were used for this experiment. Three processors were selected according to their skill and experience (minimum of 10 years) in pork grilling practice to conduct the experiments. Each processor received two pigs. Each pig, after slaughter and fur removal (singeing), was divided into two parts, so each processor worked with four half-pig carcases. Two half carcases were used for direct grilling and the other two half carcases were used for indirect grilling. Each grilling process (direct and indirect) was conducted in three independent experiments in duplicate. So, in total, each grilling experiment (direct or indirect) was repeated 6 times. In addition, a sample of raw meat was kept from each pig carcase, as a control of PAH contamination before grilling.

The direct grilling was performed using a vertical grill made with a recycled metallic barrel. The barrel grill has only one chamber where both combustion and grilling occur. To be processed, pork was laid on a grid at the top of the barrel grill. The indirect grilling was performed with a locally made clay grill composed of two adjacent compartments (combustion chamber and grilling chamber) separated by a perforated partition. For all experiments, pork was

processed according to the same method. Briefly, after slaughtering, bristles were removed by putting slaughtered pigs on embers made from wood and the processors used knives to remove bristles from pigskin. The carcase was deboned, sliced and seasoned before grilling according to direct or indirect technique. The end of processing was based on the colour and the texture of grilled pork. The mean (and standard deviation) temperature measured in the sliced pork and duration of grilling of the six experiments were 66°C (± 7.5°C) and 42.2 min (± 13.1 min) for direct grilling, and 62.3°C (± 11°C) and 49 min (± 6.3 min) for indirect grilling. *A. auriculiformis*, which is the main firewood used for traditional pork grilling (Iko Afé 2017), was used for all experiments.

At the end of the process, the 18 samples (12 grilled samples and 6 raw meat samples) of pork were transported to the laboratory in cool bags (dry ice), and then kept at −20°C until the analysis.

Analytical procedure for PAHs determination

PAHs were determined according to the analytical method described by Kpoclou et al. (2014).

Standards and reagents. Fifteen PAHs (Dibenzo[a,e]pyrene (DeP), Dibenzo[a,l]pyrene (DlP), Dibenz[a,h]anthracene (DhA), Benzo[a]Pyrene (BaP), Benz[a]anthracene (BaA), Benzo[j]fluoranthene (BjF), Benzo[k]fluoranthene (BkF), Benzo[ghi]perylene (BgP), Chrysene (CHR), Dibenzo[a,h]pyrene (DhP), Benzo[b]fluoranthene (BbF), Indeno[1,2,3-cd]pyrene (IcP), Dibenzo[a,i]pyrene (DiP), 5-Methylchrysene (5MC), Benzo[c]fluorene (BcL)) were used in this study. Standard solutions of individual PAHs and a certified mix PAHs solution 183 were purchased from Dr Ehrenstorfer GmbH (Germany). The injection standard deuterated DiP-D14 (in toluene, purity: 99.7%), was purchased from LGC Promochem (France). Commercial solutions purchased were dissolved in acetonitrile to obtain working solutions which were stored at 4°C in dark vials sealed with polytetrafluoroethylene (PTFE)/silicone caps. Solvents acetonitrile and methanol were of HPLC grade and were supplied by Biosolve (Valkenswaard, The Netherlands). Solvents dichloromethane and water (HPLC grade) were purchased from VWR (Leuven, Belgium). Solvents cyclohexane and n-hexane in Picograde quality were purchased from Sigma-Aldrich (Bornem, Belgium) and Promochem (Wesel, Germany), respectively. Solid Phase Extraction (SPE) cartridges columns (Chromabond HR-X 6 ml/500 mg) were purchased from Macherey-Nagel (Eupen, Belgium).

Extraction and purification of grilled pork samples. Grilled pork samples were ground in meat mincer (Kenwood, Pro 1600, Model MG510, UK). About 75 g sample was lyophilised for 48 h (Freezemobile Virtis, INC. Gardiner, New York) and 1 g of freeze-dried sample was used for PAH analysis. The weight difference before and after lyophilisation enables estimation of the sample moisture content. Samples were extracted using an accelerated solvent extraction (ASE 200, Dionex Corporation). One gram of freeze-dried sample was weighted in pre-washed ASE cell and then extracted with hexane/acetone (50:50, v/v) according to the method described by Veyrand et al. (2007). Sample extracts were evaporated using a TurboVap II Evaporator (Zymark, Germany), before the addition of 5 ml of cyclohexane. Purification was performed using SPE cartridges. After conditioning the SPE column using 15 ml of ethyl acetate and 10 ml of cyclohexane, the extract was loaded, and the column was rinsed with 6 ml of cyclohexane/ethanol (70:30, v/v). PAHs were eluted using 12 ml of cyclohexane/ethyl acetate (40:60, v/v), according to Veyrand et al. (2007). After evaporation of the solvent to dryness, using a TurboVap, 90 µl of acetonitrile were added together with 10 µl of deuterated DiP-D14 (250 pg/µl in acetonitrile), used as injection standard. For the calculation of the extraction recovery, four fortified samples were prepared from blank raw pork bought in a Belgian supermarket (where no PAH was detected) by adding, prior the extraction step, a solution containing the 15 PAHs in acetonitrile, to reach a final concentration of 1 µg/kg (corresponding to the third point of the calibration curve), except for IcP and BjF, for which the spiking concentration was 2 µg/kg (corresponding to the second point of the calibration curve). In the same injection series, a procedure blank and a spiked blank matrix used as an internal quality control sample were used as controls of both extraction and purification steps.

HPLC analysis. HPLC analysis was carried out using a Model 600 E solvent delivery system, equipped with a Model 717 automatic injector, a Mistral ^{TM} oven and a 2475 Fluorescence detector (all from Waters, Milford, MA, USA). A C18 Pursuit 3 PAH (100 mm × 4.6 mm, 3 µm) equipped with a ChromGuard (10 × 3 nmm) pre-column, both from VARIAN (Agilent technologies, Santa Clara, USA), were used to separate the PAHs. Five microlitres of extract were injected on the HPLC column. Separation and fluorescence detection were performed according to the methods described by Brasseur et al. (2007) and Danyi et al. (2009). Seven calibration solutions containing the PAHs in increasing concentrations from 10 to 1600 pg/µL for BjF and IcP (for which the detection limit is higher than for the other 13 PAHs) and from 2.5 to 400 pg/µL for the 13 other PAHs, were injected with each series of sample extracts. An independent reference solution containing the 15 PAHs at a level corresponding to the blank spiked samples (see above) was used to calculate the recovery for each PAH as the ratio between both calculated reference solution and spiked blank matrix concentrations. The deuterated internal standard DiP-D14 was spiked at a constant concentration (i.e. 250 pg/µL in acetonitrile) in each calibration and reference level. The response (ratio between native and internal standard PAHs peak areas) was plotted against standard concentrations. Quadratic regression was used for curve fitting and calculation of native PAHs. For each detected PAH, the relative retention time (i.e. the ratio between the retention time of the PAH found in the sample and the retention time of the deuterated DIP injection standard) was checked. This relative retention time should not differ from the relative retention time of the PAH of the reference solution by more than ± 2.5% (EC 2002). As an additional quality control, a certified solution containing the 15 PAHs (each a concentration of 20 pg/µl) was injected with each series of samples. The software Empower (Waters, Milford, MA, USA) was used to control the gradient, and to process the data. The first point of the calibration curve was set arbitrarily as the limit of quantification (LOQ), as it is not possible to give a quantitative result if the response is not included in the working range of the calibration curve. For each sample, the individual moisture content was taken into account to express PAH concentration in µg/kg of wet weight (the moisture content of pork before grilling ranged between 55.9% and 73.1%).

Estimation of daily consumption of pork by Beninese consumers

The daily consumption frequency of grilled pork (how many times the consumer eats grilled pork per day) was estimated during a face-to-face survey conducted with 300 consumers of grilled pork, aged between 18 and 78 years, composed of 17.3% of women, and with a body weight ranging from 33 to 120 kg with a mean of 70 ± 13 kg (Iko Afé 2017). To estimate the individual daily intake amount of grilled pork, each interviewed consumer was asked how much money they spent on consumption of grilled pork. A quantity of grilled pork corresponding to the price mentioned by interviewed consumer was then purchased immediately in the same restaurant and weighed to estimate the quantity of grilled pork consumed. Three hundred data points of grilled pork daily consumption, expressed in grams of grilled pork per day, were then obtained by multiplying the individual daily frequency of consumption and the weighted quantities of grilled pork. The minimum, median, 97, 5th percentile and maximum daily consumptions of grilled pork were 0.7, 28.6, 214.3 and 342.9 g/day, respectively.

Methodology of risk assessment

Exposure assessment was realised following a deterministic approach (AFSCA 2005). The PAHs Estimated Daily Intakes (EDI), expressed in ng PAH per kg body weight (b.w.) per day, were calculated on the basis of the median and maximum contamination data of grilled pork (n = 36 grilled pork samples) (all grilled pork contamination data recorded in this study were considered together (i.e. commercial samples and samples from the control experiment) as samples from the control experiment were consumed as well). The EDI was calculated for each of the 300 consumers, taking into account their body weight and grilled pork consumption, both recorded during the consumption survey described above. The EDI were calculated according to the following formula:

For risk characterisation, the margin of exposure (MOE) (EFSA 2005, 2008; Constable and Barlow 2009) was calculated as followed, for the minimum, median, 97.5 percentile and maximum EDI:

$$\mathrm{MOE} = \frac{\mathrm{BMDL_{10}(ng/kg\ b.w./day)}}{\mathrm{EDI(ng/kg\ b.w./day)}}$$

where the $BMDL_{10}$ (benchmark dose lower confidence limit) is the 95% lower confidence limit of a benchmark leading to 10% extra risk of hepatocellular adenomas in laboratory animals (EFSA 2008). EFSA (2008) has determined $BMDL_{10}$ for BaP and various groups of PAHs, i.e. PAH2 (sum of BaP and BaA), PAH4 (sum of BaP, Chr, BaA and BbF) and PAH8 (sum of PAH4, BkF, BgP, DhA and IcP). The $BMDL_{10}$ of BaP, PAH2, PAH4 and PAH8 used in this study is 0.07 mg/kg b.w./day, 0.17 mg/kg b.w./day, 0.34 mg/kg b.w./day and 0.49 mg/kg b.w./day, respectively (EFSA 2008). According to EFSA, for carcinogenic compounds such as PAH, an MOE above 10,000 means a low concern for consumer's health (EFSA 2005, 2008; Constable and Barlow 2009).

Data analysis

Descriptive statistics (minimum, median, maximum, percentile and mean ± standard deviation) were performed using Microsoft Excel 2013 software. One-way analysis of variance (ANOVA) was used to compare means of several groups (control, direct grilled samples and indirect grilled samples) with Statistica 7 (Version 7.1, StatSoft France, 2006). Dunnett post hoc test was carried out to compare PAH contamination in grilled pork from different processing methods (direct and indirect grilling) to the one in a control raw pork sample. A significant difference was accepted at $p < .05$.

Results and discussion

Validation of the analytical method

Recovery (extraction yield) was calculated from four replicates and ranged between 50.2% and 110.3% for the 15 PAHs analysed (Supplementary data 1) and between 77.0% and 105.9% for the PAHs of the PAH4 group, which is in agreement with the performance criteria described in the European Commission Regulation (EC) N° 333/2007 (EC 2007) (PAH4 recovery must be between 50% and 120%). PAH LOQs, on basis of 50% moisture content, were 0.1 µg/kg except for IcP and BjF, which displayed a LOQ of 0.5 µg/kg (Supplementary data 1). The relative retention time of all PAHs detected in samples complied with the criteria of variation of ± 2.5% compared to the relative retention time of the standard PAHs (EC 2002).

PAHs contents of commercial samples of grilled pork randomly collected

Table 1 shows minimum, maximum and median PAHs contents found in 24 commercial samples of grilled pork, and Supplementary Data 2 shows the individual concentrations of PAH. No significant difference ($p > .05$) was recorded from the comparison of BaP and PAH4 average concentrations of the four types of grilled pork (piece, slice, skewer and wrapped grilled pork) using the Kruskal–Wallis test. BaP concentrations varied between 0.4 and 17.9 µg/kg. For PAH4 and PAH8, concentrations ranged from 3.7 to 129.6 µg/kg and from 4.1 to 157.7 µg/kg, respectively. Most samples (70.8% and 95.8%, respectively) exceeded the EU maximum limit (EC 2006) of 2 µg/kg for BaP and 12 µg/kg for PAH4 (data not shown). Such high-level contamination in grilled pork can be explained by the processing practices; as recorded in a previous survey with stakeholders (Iko Afé 2017), pork is in direct contact with smoke during processing, which is a PAHs food contamination factor (Stołyhwo and Sikorski 2005). Moreover, there is no possibility to set the temperature of fuel (wood or charcoal) combustion to a constant value for processing and there is no standard size for pieces of pork to be spread on grill for processing. These conditions constitute important sources of variability in contamination levels of grilled pork. This variability can explain the high standard deviation recorded for BaP (3.5 ± 2.6 µg/kg; 8.7 ± 6.0 µg/kg; 2.4 ± 1.5 µg/kg; 5.4 ± 6.1 µg/kg for piece, slice, skewer and wrapped grilled pork, respectively) and PAH4 (35.6 ± 17.3 µg/kg; 72.1 ± 42.9 µg/kg; 23.0 ± 6.6 µg/kg; 52.2 ± 48.2 µg/kg, respectively) concentrations (data not shown).

Table 1. Minimum, median and maximum PAHs (µg/kg, wet weight) content in 24 commercial grilled pork as marketed to consumers in South Benin.

PAHs	Minimum	Maximum	Median
BbF	**0.5**	**23.0**	**5.3**
DlP	0.2	2.2	0.4
DhA	0.1	2.2	0.5
BgP	0.4	14.1	3.6
DeP	0.3	5.8	1.2
BjF	1.1	14.5	3.5
BcL	0.5	31.4	9.1
BaA	**1.5**	**46.8**	**9.5**
CHR	**1.3**	**53.1**	**11.0**
5MC	0.2	8.3	1.4
BkF	0.2	8.0	1.7
BaP	**0.4**	**17.9**	**3.5**
IcP	0.8	10.9	2.3
DiP	0.4	0.9	0.5
DhP	<0.1	<0.1	<0.1
∑PAH2	1.7	71.0	14.7
∑PAH4	**3.7**	**129.6**	**30.1**
∑PAH8	4.1	157.7	37.9
∑PAH15	16.8	548.6	133.2

Legend. PAH2: benzo[a]pyrene; chrysene; PAH4: PAH2, BaA and BbF; PAH8: PAH4, BkF, BgP, DhA and IcP; PAHs in bold refer to individual and sum of PAH4.

Effect of grilling process on pork contamination with PAHs

PAHs content in raw pork

As shown in Figure 1, BaP levels in raw meat ranged between 0.9 and 3.4 µg/kg while PAH4 levels varied between 3.9 and 18 µg/kg. Two samples out of six were non-compliant with both the 2 µg/kg (BaP) and 12 µg/kg (PAH4) EU maximal limits (EC 2006). However, the highest level of BaP recorded in raw pork (3.4 µg/kg) is below the maximum limit in smoked meat (5 µg/kg) set by Benin regulation (MAEP 2007).

This contamination is probably due to singeing used for pig hair removal, as recorded by Nnaji et al. (2017) with PAHs in cattle meat from Nigeria. These authors showed levels of BaP of 5.70 ± 1.30 µg/kg in singed meat before washing and 2.10 ± 0.80 µg/kg after washing. Also, this contamination of raw pork could result from an indirect exposure to wood smoke before grilling, as previously reported by Onyango et al. (2012) in Kenya, where PAH contaminations were observed at concentrations below 1 µg/kg (wet weight) in raw pork, goat meat and beef exposed to smoke on processing sites.

PAHs content in pork after grilling

PAH concentrations in grilled pork are shown in Table 2, Figure 2 and Supplementary data 3. The concentrations of BaP ranged between 2.4 and 53.6 µg/kg for direct grilled pork and between 4.8 and 34.8 µg/kg for indirect grilled pork (Table 2). For PAH4, the concentration recorded ranged between 53.8 and 300.6 g/kg for direct grilled pork and between 59.9 and 182.5 µg/kg for indirect grilled pork. The maximum concentrations of BaP and PAH4 recorded during the controlled experiment exceeded their maximum concentrations observed in collected commercial samples.

This can be explained by the fact that commercial samples were from different processing sites using different fuels (Iko Afé 2017) including charcoal of low PAH production and these commercial samples were also composed of several types of pork (skewer, slice, piece and wrapped). Moreover, during the

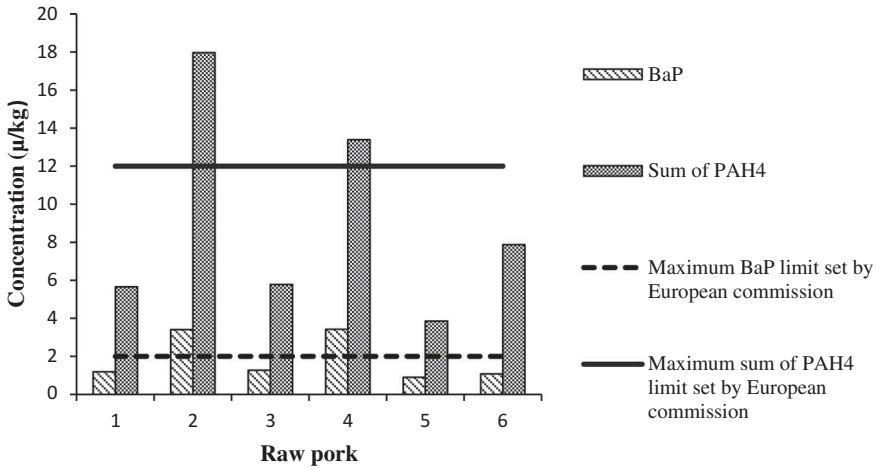

Figure 1. Individual concentration of BaP and the sum of PAH4 in raw pork samples used for direct grilling (1–3) or indirect grilling (4–6).

Table 2. PAHs content (µg/kg, wet weight) in direct and indirect grilled pork obtained from the controlled experiment.

	Direct grilled pork (n = 6)			Indirect grilled pork (n = 6)		
PAHs	Minimum	Maximum	Median	Minimum	Maximum	Median
BbF	**11.5**	**48.7**	**22.5**	**8.7**	**22.0**	**19.9**
DlP	0.7	2.5	1.1	0.2	0.3	0.3
DhA	0.5	10.3	4.8	0.6	3.5	1.8
BgP	9.7	29.7	16.6	5.2	22.4	17.1
DeP	0.9	7.2	3.6	2.3	3.7	3.0
BjF	7.2	36.1	17.0	5.6	20.6	14.4
BcL	8.0	62.7	26.9	12.7	30.8	21.7
BaA	**18.4**	**105.6**	**53.8**	**22.5**	**43.3**	**39.1**
CHR	**21.5**	**92.7**	**47.6**	**20.8**	**82.4**	**27.5**
5MC	1.1	2.2	1.6	0.4	0.6	0.5
BkF	4.9	17.6	10.2	3.5	14.4	9.6
BaP	**2.4**	**53.6**	**28.3**	**4.8**	**34.8**	**16.4**
IcP	6.1	26.2	14.7	3.6	17.4	12.1
DiP	1.5	4.2	2.3	0.2	1.3	0.8
DhP	0.4	0.8	0.6	<0.1	<0.1	<0.1
∑PAH4	53.8	300.6	154.3	59.9	182.5	104.0

Legend. PAHs in bold refer to individual and sum of PAH4.

controlled experiment, only hard wood from *Acacia auriculiformis* was used for all experiments, as it was shown to be the most commonly used for traditional pork grilling (Iko Afé 2017). Several reports show that Acacia wood produces large amounts of PAH in foodstuffs during grilling and smoking (Essumang et al. 2013; Kpoclou et al. 2014).

All 12 grilled samples from the controlled experiment showed that the BaP and PAH4 levels are above 2 (BaP) and 12 µg/kg, (PAH4) maximum limits set by European Commission (EC) regulation n° 1881/2006 (EC 2006).

The significant differences ($p < .05$) recorded between PAHs concentration in raw pork and grilled pork (Figure 2) suggest the grilling process as the main source of grilled pork contamination. Chung et al. (2011), Viegas et al. (2012) and Oz and Yuzer (2016) have also recorded contamination of meat products including pork due to grilling processes. Moreover, no significant difference ($p < .05$) in sum of PAH4 (Figure 2(a)) and individual PAHs (Figure 2 (b)) concentrations was recorded between grilled pork samples from both direct and indirect grilling processes. This shows that both grilling methods (direct or indirect) lead to contamination of grilled pork with PAHs. This could be explained by the functioning of two grills used in our study. Indeed, the two compartments (combustion chamber and grilling chamber) of locally made clay grill are separated by a perforated partition leading to direct contact of smoke with pork throughout the process. However, they differ from each other by the fact that during indirect grilling fat cannot drop into embers whereas in direct grilling this is possible. This factor of difference seems not to be sufficient to create any real difference in contamination with PAHs in this study. These results differ from those of Lee et al. (2016) who reported significant reduction in PAHs concentrations when a device preventing fat dropping onto fire was implemented.

Risk assessment results according deterministic approach

Exposure assessment

Table 3 shows PAHs EDI of grilled pork by consumers, estimated using median and maximum BaP, PAH2, PAH4 and PAH8 levels in the 36 samples

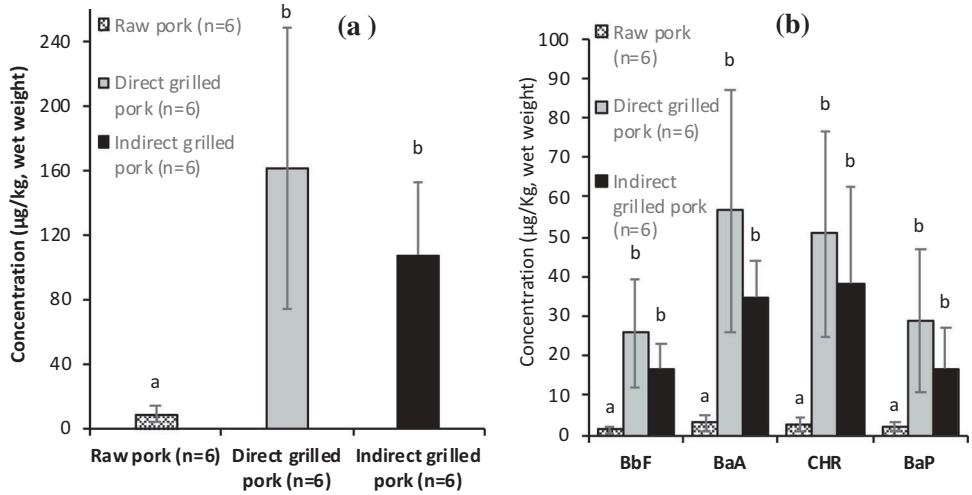

Figure 2. Comparison of sum of PAH4 (a) and individual PAHs (b) concentrations in raw and grilled pork according to grilling process. Raw pork = Control; Mean ± standard deviation; value with the same letter is not significantly different ($p = .05$).

Table 3. EDI and MOE of grilled pork consumer using median (scenario 1) and maximum (scenario 2) concentrations of BaP, PAH2, PAH4 and PAH8 recorded in 36 grilled pork samples.

	EDI (ng/kg b.w/day)				MOE			
	BaP	PAH2	PAH4	PAH8	BaP	PAH2	PAH4	PAH8
Scenario 1*								
Minimum	0.1	0.4	0.7	1.0	941,826	481,018	467,503	514,300
Median	2.6	12.4	25.6	33.5	26,803	13,689	13,304	14,636
97.5 Percentile	17.8	84.7	174.2	228.2	3,932	2,008	1,952	2,147
Maximum	24.2	115.3	237.2	310.7	2,888	1,475	1,433	1,577
Scenario 2**								
Minimum	0.7	2.0	4.1	5.2	96,871	86,202	83,925	94,592
Median	25.4	69.3	142.4	182.0	2,757	2,453	2,388	2,692
97.5 Percentile	173.1	472.4	970.4	1240.8	404	360	350	395
Maximum	235.7	643.2	1321.3	1689.5	297	264	257	290

Legend. BMDL10 (BaP): 0.07 mg/kg b.w/day; BMDL10 (PAH2): 0.17 mg/kg b.w/day; BMDL10 (PAH4): 0.34 mg/kg b.w/day and BMDL10 (PAH8): 0.49 mg/kg b.w/day (EFSA, 2008).
*: Median PAHs levels: 5.5 µg/kg, 26.2 µg/kg, 54.0 µg/kg and 70.7 µg/kg for BaP, PAH2, PAH4 and PAH8, respectively.
**: Maximum PAHs levels: 53.6 µg/kg, 146.3 µg/kg, 300.6 µg/kg and 384.3 µg/kg for BaP, PAH2, PAH4 and PAH8 respectively.

analysed. The EDI (expressed as ng/kg b.w/day), considering median contamination levels, ranged from 0.1 to 24.2 for BaP, 0.4 to 115.3 for PAH2, 0.7 to 237.2 for PAH4 and from 1.0 to 310.7 for PAH8. In a worst-case scenario, using maximum PAHs contamination levels, the EDI ranged from 0.7 to 235.7 for BaP, 2.0 to 643.2 for PAH2, 4.1 to 1321.3 for PAH4, and 5.2 to 1689.5 ng/kg b.w/day for PAH8.

Risk characterisation

The MOEs calculated using median and maximum PAHs concentrations are shown in Table 3. When using median PAHs levels, MOE ranged from 2,888 to 941,826 for BaP, 1,475 to 481,018 for PAH2, 1,433 to 467,503 for PAH4 and 1,577 to 514,300 for PAH8, depending on the grilled pork consumption level. For high consumers (97.5 percentile and maximum level of consumption), as well as when the maximum concentrations of PAHs were considered for MOE calculation, these MOEs were well below 10,000, indicating a potential concern for their health.

In Nigeria, Akpambang et al. (2009) also reported MOE well below 10,000 for BaP and PAH8 EDI from consumption of traditionally smoked-dried bush meat. In EU, the EFSA (2008) calculated MOEs above 10,000 (15,900–17,900) for median consumers, using mean EDI of PAHs from various food sources. However, when 97.5 percentile EDI was used, the MOEs (9,500–9,900) were below 10,000 for PAH2, PAH4 and PAH8 but not BaP (10,800). In this study, MOE values are much lower than the values recorded by EFSA. The further the MOE is below 10,000, the highest is the risk, meaning that there is a real concern for the Beninese grilled pork consumer health. Even if data exist on risk assessment on food consumption contaminated with PAHs conducted in European, Asian and American countries, very few studies have been recorded in case of African countries (EFSA 2008; Domingo and Nadal 2015; Ingenbleek et al. 2019) to enable comparisons. Furthermore, the probabilistic approach of risk assessment that involves use of distribution has not been explored in this study and should be performed in the future when more data become available.

Also, the present study did not consider the consumption of other grilled or smoked products or any other source of PAH exposure, which means that the risk due to PAH intake is probably underestimated. Beside the risk associated with meat consumption, consumers may also be exposed to PAHs through inhalation in grilled pork eating-places. Boström et al. (2002) showed that PAHs exposure via ambient air could be responsible for lung cancer, and Zhang et al. (2009) reported that the death rate due to lung cancer among non-smoking Chinese people in rural areas might arise from exposure to smoke produced from biomass and coal combustion in kitchen. To manage the risk linked to PAHs exposure, it seems important to decrease their levels in grilled pork through improvement of grilling processes and awareness campaign. The same recommendation was made by Akpambang et al. (2009) who showed that when bush meat was smoked under controlled condition, MOEs were above 10,000, indicative of a lower risk for consumers.

Conclusion

Grilled pork as marketed in South Benin is highly contaminated with BaP and PAH4, with levels exceeding nine times (BaP) and eleven times (PAH4) the limits set in European regulation (EC 2006) and four times the maximal limit of BaP set in regulation of Benin (MAEP 2007). None of the direct and indirect grilled pork samples were compliant to this European regulation. Additionally, no significant difference ($p > .05$) was recorded between PAHs concentrations of direct grilled pork and indirect grilled pork as well as for commercial samples and for samples from the grilling processing. From this study, it appears that grilled pork consumption presents risk for consumers' health due to PAHs ingestion as MOE below 10,000 were recorded for higher consumers considering a median PAHs meat contamination and also when median EDI was used in the worst scenario using maximum PAHs concentrations. Improvement of traditional pork grilling is required as a risk management action to preserve consumers' health.

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No potential conflict of interest was reported by the authors.

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References

[AFSCA] Agence fédérale pour la Sécurité de la Chaîne alimentaire. 2005. Terminologie en matière d'analyse des dangers et des risques selon le codex alimentarius [*Terminology as regards analysis of the dangers and risks according to Codex Alimentarius*]. Bruxelles. [accessed 2019 Aug 28]. http://www.afsca.be/comitescientifique/publications/brochures/_documents/2005-09_SciCom_Term_Fr.pdf.

Agodokpessi G, Ade G, Hinson V, Ade S, Okoumassou C-X, Fayomi B, Gninafon M. 2011. Prévalence des troubles respiratoires chez les femmes exerçant sur un site artisanal de fumage de poisson à Cotonou au Bénin [*Prevalence of respiratory disorders among women working on an artisanal site of fish smoking at Cotonou in Benin*]. Mali Méd. 26:34–38.

Akpambang VOE, Purcaro G, Lajide L, Amoo IA, Conte LS, Moret S. 2009. Determination of polycyclic aromatic hydrocarbons (PAHs) in commonly consumed Nigerian smoked/grilled fish and meat. Food Addit Contam A. 26:1096–1103.

Anihouvi DGH, Kayodé APP, Anihouvi VB, Azokpota P, Kotchoni SO, Hounhouigan DJ. 2013. Microbial contamination associated with the processing of tchachanga, a roasted meat product. Afr J Biotechnol. 12:2449–2455.

Boström C-E, Gerde P, Hanberg A, Jernström B, Johansson C, Kyrklund T, Rannug A, Törnqvist M, Victorin K, Westerholm R. 2002. Cancer risk assessment, indicators, and guidelines for polycyclic aromatic hydrocarbons in the ambient air. Environ Health Persp. 110:451–489.

Brasseur C, Brose F, Pirlot A, Douny C, Eppe G, Maghuin-Rogister G, Scippo M-L. 2007. Validation of the analytical procedure for the determination of polyaromatic hydrocarbons in smoke flavourings using high performance liquid chromatography coupled to an ultraviolet, diode array or fluorescence detector. Accredit Qual Assur. 12:535–542.

[CAC/RCP 68-2009] Codex Alimentarius Commission/ Recommended international code of practice. 2009. Code of practice for the reduction of contamination of food with polycyclic aromatic hydrocarbons (PAH) from smoking and direct drying processes. 1st ed. [accessed 2019 Aug 28]. http://www.fao.org/input/download/standards/11257/CXP_068e.pdf.

Chaber A-L, Cunningham A. 2016. Public health risks from illegally imported african bushmeat and smoked fish. EcoHealth. Short communication. Ecohealth. 13(1):135–138. doi: 10.1007/s10393-015-1065-9.

Chung SY, Yettella RR, Kim JS, Kwon K, Kim MC, Min DB. 2011. Effects of grilling and roasting on the levels of polycyclic aromatic hydrocarbons in beef and pork. Food Chem. 129:1420–1426.

Constable A, Barlow S. 2009. Application of the margin of exposure approach to compounds in food which are both genotoxic and carcinogenic. In Summary report of a workshop held in October 2008, Organised by the International Life Sciences Institute. Europe Risk Assessment of Genotoxic Carcinogens in Food Task Force. [accessed 2019 Aug 28]. https://ilsi.eu/wp-content/uploads/sites/3/2016/06/MOE-WS-Report.pdf.

Danyi S, Brose F, Brasseur C, Schneider Y-J, Larondelle Y, Pussemier L, Robbens J, Saeger SD, Maghuin-Rogister G, Scippo M-L. 2009. Analysis of EU priority polycyclic aromatic hydrocarbons in food supplements using high performance liquid chromatography coupled to an ultraviolet,

diode array or fluorescence detector. Analyt Chim Acta. 633:293–299.

Domingo JL, Nadal M. 2015. Human dietary exposure to polycyclic aromatic hydrocarbons: A review of the scientific literature. Food Chem Toxicol. 86:144–153.

Duedahl-Olesen L, Aaslyng M, Meinert L, Christensen T, Jensen AH, Binderup M-L. 2015. Polycyclic aromatic hydrocarbons (PAH) in Danish barbecued meat. Food Control. 57:169–176.

[EC] European Commission. 2002. Commission decision of 12 August 2002 implementing council directive 96/23/EC concerning the performance of analytical methods and the interpretation of results (Text with EEA relevance) (notified under document number C(2002) 3044). [accessed 2019 Aug 28]. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32002D0657&from=EN.

[EC] European Commission. 2006. Regulation No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. [accessed 2019 Aug 28]. https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:364:0005:0024:EN:PDF.

[EC] European Commission. 2007. Amending Regulation (EC) No 333/2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a) pyrene in foodstuffs. Off J Eur Union L. 215:9–16. [accessed 2019 Aug 28]. https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2007:088:0029:0038:EN:PDF.

[EFSA] European Food Safety Authority. 2005. Opinion of the Scientific Committee on a request from EFSA related to A harmonised approach for risk assessment of substances which are both genotoxic and carcinogenic. EFSA J. 282: 1–31. [accessed 2019 Aug 28]. https://efsa.onlinelibrary.wiley.com/doi/pdf/10.2903/j.efsa.2005.282.

[EFSA] European Food Safety Authority. 2008. Polycyclic aromatic hydrocarbons in food: scientific opinion of the panel on contaminants in the food chain. EFSA J. 724: 1–114. [accessed 2019 Aug 28]. https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2008.724.

Essumang DK, Dodoo DK, Adjei JK. 2013. Effect of smoke generation sources and smoke curing duration on the levels of polycyclic aromatic hydrocarbon (PAH) in different suites of fish. Food Chem Toxicol. 58:86–94.

[FAO] Food and Agriculture Organization. 2015a. Guide for developing and using the FAO-Thiaroye Processing Technique (FTT-Thiaroye). In Ndiaye O, Sodoke KB, Diei-Ouadi Y, editors. Rome, Italy: Food and Agriculture Organization of the United Nations. [accessed 2019 Aug 28]. http://www.fao.org/3/a-i4174e.pdf.

[FAO] Food and Agriculture Organization. 2015b. Strengthening the performance of post-harvest systems and regional trade in small-scale fisheries – case study of post-harvest loss reduction in the Volta Basin riparian countries. In: Diei-Ouadi Y, Sodoké BK, Oduro FA, Ouedraogo Y, Bokobosso K, Rosenthal I, editors. Fisheries and aquaculture circular No. 1105. Rome, Italy. [accessed 2019 Aug 28]. http://www.fao.org/3/a-i5141e.pdf.

Farhadian A, Jinap S, Abas F, Sakar ZI. 2010. Determination of polycyclic aromatic hydrocarbons in grilled meat. Food Control. 21:606–610.

Iko Afé OH 2017. Assessment of the risk related to polycyclic aromatic hydrocarbons exposure, in grilled pork, smoked fish and dried/smoked fish processed in Benin [Doctorate training dissertation]. Liege, Belgium: University of Liege.

Ingenbleek L, Veyrand B, Adegboye A, Hossou SE, Koné AZ, Oyedele AD, Kisito CSKJ, Dembélé YK, Eyangoh S, Verger P, et al. 2019. Polycyclic aromatic hydrocarbons in foods from the first regional total diet study in Sub-Saharan Africa: contamination profile and occurrence data. Food Control. 103:133–144.

Kpoclou EY, Anihouvi BV, Azokpota P, Soumanou MM, Daube G, Douny C, Brose F, Scippo M-L, Hounhouigan DJ. 2013. Microbiological and physico-chemical quality of smoked shrimp, an expanding food condiment in beninese local markets. Food Public Health. 3:277–283.

Kpoclou EY, Anihouvi VB, Azokpota P, Soumanou MM, Douny C, Brose F, Hounhouigan DJ, Scippo M-L. 2014. Effect of fuel and kiln type on the polycyclic aromatic hydrocarbon (PAH) levels in smoked shrimp, a Beninese food condiment. Food Addit Contam A. 31:1212–1218.

Ledesma E, Rendueles M, Díaz M. 2015. Characterization of natural and synthetic casings and mechanism of BaP penetration in smoked meat products. Food Control. 51:195–205.

Ledesma E, Rendueles M, Díaz M. 2016. Contamination of meat products during smoking by polycyclic aromatic hydrocarbons: processes and prevention. Food Control. 60:64–87.

Lee J-G, Kim S-Y, Moon J-S, Kim S-H, Kang D-H, Yoon H-J. 2016. Effects of grilling procedures on levels of polycyclic aromatic hydrocarbons in grilled meats. Food Chemi. 199:632–638.

Lu F, Kuhnle GK, Cheng Q. 2017. Heterocyclic amines and polycyclic aromatic hydrocarbons in commercial ready-to-eat meat products on UK market. Food Control. 73:306–315.

[MAEP] Ministère de l'Agriculture, de l'Elevage et de la Pêche. 2007. Arrêté 2007 N°0362/MAEP/D-CAB/SGM/DRH/DP/SA du 30 Octobre 2007 portant fixation des teneurs maximales pour certains contaminants dans les denrées alimentaires en République du Bénin, publié au journal officiel n°9 du 01 Mai 2008 [Decree 2007 N°0362/MAEP/D-CAB/SGM/DRH/DP/SA of 30 October 2007 fixing maximum limits for some contaminants in foods of Republic of Benin, published in the Official Journal n°9 of May 01, 2008]. [accessed 2019 Aug 28]. http://extwprlegs1.fao.org/docs/pdf/ben146450.pdf.

Nnaji J, Madu E, Chukwuemeka-Okorie H. 2017. Polycyclic Aromatic Hydrocarbons (PAHs) content in cattle hides and meat singed with scrap rubber tyres. J Appl Sci Environ Manag. 21:1105–1110.

Onyango AA, Lalah JO, Wandiga SO. 2012. The effect of local cooking methods on Polycyclic Aromatic Hydrocarbons (PAHs) contents in beef, goat meat, and

pork as potential sources of human exposure in Kisumu City, Kenya. Polycycl Aromat Comp. 32:656–668.

Oz F, Yuzer MO. 2016. The effects of cooking on wire and stone barbecue at different cooking levels on the formation of heterocyclic aromatic amines and polycyclic aromatic hydrocarbons in beef steak. Food Chem. 203:59–66.

Poligné I, Collignan A, Trystram G. 2001. Characterization of traditional processing of pork meat into boucané. Meat Sci. 59:377–389.

Rose M, Holland J, Dowding A, Petch SRG, White S, Fernandes A, Mortimer D. 2015. Investigation into the formation of PAHs in foods prepared in the home to determine the effects of frying, grilling, barbecuing, toasting and roasting. Food Chem Toxicol. 78:1–9.

[SCF] Scientific Committee on Food. 2002. Opinion of the Scientific Committee on Food on the risks to human health of polycyclic aromatic hydrocarbons in food expressed on 4 December 2002. [accessed 2019 Aug 28]. https://ec.europa.eu/food/sites/food/files/safety/docs/sci-com_scf_out153_en.pdf.

Singh L, Varshney JG, Agarwal T. 2016. Polycyclic aromatic hydrocarbons' formation and occurrence in processed food. Food Chem. 199:768–781.

Stołyhwo A, Sikorski ZE. 2005. Polycyclic aromatic hydrocarbons in smoked fish – a critical review. Food Chem. 91:303–311.

Veyrand B, Brosseaud A, Sarcher L, Varlet V, Monteau F, Marchand P, Andre F, Bizec BL. 2007. Innovative method for determination of 19 polycyclic aromatic hydrocarbons in food and oil samples using gas chromatography coupled to tandem mass spectrometry based on an isotope dilution approach. J Chromatogr A. 1149:333–344.

Viegas O, Novo P, Pinto E, Pinho O, Ferreira IMPLVO. 2012. Effect of charcoal types and grilling conditions on formation of heterocyclic aromatic amines (HAs) and polycyclic aromatic hydrocarbons (PAHs) in grilled muscle foods. Food Chem Toxicol. 50:2128–2134.

[WHO/IARC] World Health Organization/International Agency for Research on Cancer. 2012. International Agency for Research on Cancer Monographs on the evaluation of carcinogenic risks to humans. 100 F. Lyon. [accessed 2019 Aug 28]. https://monographs.iarc.fr/wp-content/uploads/2018/06/mono100F.pdf.

Zachara A, Gałkowska D, Juszczak L. 2017. Contamination of smoked meat and fish products from Polish market with polycyclic aromatic hydrocarbons. Food Control. 80:45–51.

Zhang Y, Tao S, Shen H, Ma J. 2009. Inhalation exposure to ambient polycyclic aromatic hydrocarbons and lung cancer risk of Chinese population. PNAS. 106:21063–21067.