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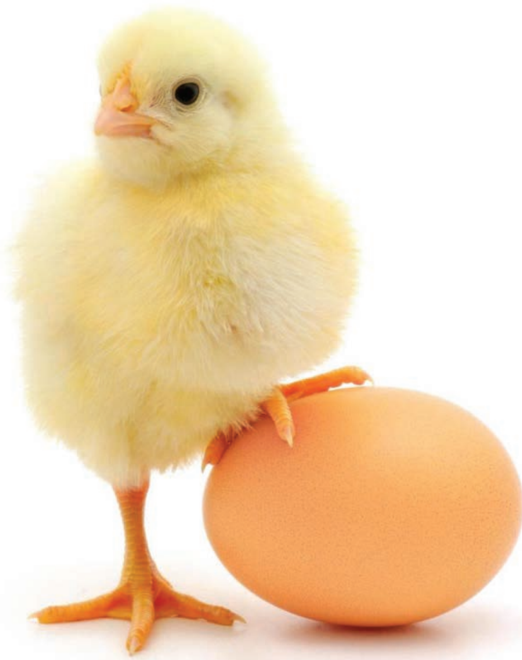

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Research Article
Health Risk Assessment of Polycyclic Aromatic Hydrocarbons (PAHs) and Heavy Metals In Grilled Chicken Meats From Benin, West Africa

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Abstract
Background and Objective: There is concern about the safety of food, especially grilled foods in Africa. This study aimed to assess polycyclic aromatic hydrocarbons (PAH) and heavy metals content in grilled chicken meat and health risk for consumers in Benin. **Materials and Methods:** A total of 35 samples of grilled chicken meat were collected from Cotonou and Abomey-Calavi following various grilling processes and devices. PAHs were analyzed by gas chromatography coupled with mass spectrometry. Heavy metals were analyzed by atomic absorption spectrophotometer and mercury by spectrophotometer coupled with VP100 hydride generator. Estimated Daily Intake (EDI) and Hazard Quotient (HQ) were used to evaluate the risk of exposure to benzo(a)pyrene, lead and cadmium. **Results:** Whole grilled chicken meat (2.761-4.062 μg kg^{-1}) did not meet the maximum limit of 2 μg kg^{-1} for benzo(a)pyrene set by European Union except whole pre-cooked grilled chicken meat (0.333±0.033 μg kg^{-1}) and whole grilled chicken meats with wood-device (1.493±0.055 μg kg^{-1}). Regardless of the device, grilled chicken meat skewers contained a tolerable level of benzo(a)pyrene (1.216-1.892 μg kg^{-1}). Lead level in whole (223.3±36 μg kg^{-1}) and skewers (153.3±18 μg kg^{-1}) chicken grilled on cabinet type charcoal-device exceeded the tolerated limit (100 μg kg^{-1}). However, levels of cadmium, mercury and arsenic were in accordance with the regulation in all analyzed samples. Only HQ of benzo(a)pyrene was above 1 (HQ>1) for whole grilled chicken meats consumed daily. Regardless of consumption frequency, HQ of lead and cadmium were less than 1 (HQ<1) for all types of grilled chicken meats. **Conclusion:** No single metal poses health risk to consumers of grilled chicken meat in Benin. However, there is a potential health risk associated with PAHs in the product, which should be managed.

Key words: Benin, benzo(a)pyrene, benzo(b)fluoranthene, chicken meat, public health

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INTRODUCTION

Chicken meat is not only an important source of protein but also the cheapest in the world. Its global consumption has grown rapidly from 5.95-10.87 kg per capita per year from 1995 to 2015. It is projected to increase to 12.79 kg per capita per year by 2025, more than double in 30 years[1,2]. It is often consumed in fried, grilled, boiled, roasted, or braised forms[3,4]. Grilling is a cooking method that submits the surface of food to dry heat, usually above, below or from the side[5]. With respect to meat, grilling increases protein digestibility, provides a distinctive aroma and flavor, while retaining the texture and shape of the meat when the temperature is between 200 and 260°C[6-9]. However, Grilling is among the main cooking methods responsible for the formation of Polycyclic Aromatic Hydrocarbons (PAHs) in foodstuffs. There is a multitude of PAHs but only 15-16 have been recognized as potentially harmful to animals and humans[10,11]. Of these, benzo(a)pyrene is the main indicator of PAH toxicity, with chrysene, benzo(a)anthracene, benzo(b)fluoranthene and benzo(a)pyrene added to form a group of four priority indicators (PAH 4)[12]. Due to their high toxicity, limits have been set in foods by different standard organizations in various countries and international institutions[13-15]. In Europe, the maximum tolerated levels for benzo(a)pyrene and the PAH4 group in food are 2 and 12 μg kg^{-1}, respectively, whereas in Benin they are 5 and 30 μg kg^{-1}, respectively[16,17]. In Benin, there is a lot of interest in PAH content in foods, especially in smoked and grilled ones. Kpoclou *et al.*[18] reported that benzo(a)pyrene (91 μg kg^{-1}) and PAH4 (490 μg kg^{-1}) levels in smoked shrimp exceeded the limits mentioned above. Similar results were obtained by Iko Afé *et al.*[19] who pointed out that smoked fish and smoked-dried fish also failed to meet the standards.

Apart from PAHs, toxic heavy metals are also contaminants that can also be found in chicken meat. Thus, limit values have been set in food products especially for lead (100 μg kg^{-1}), cadmium (50 μg kg^{-1}), mercury (500 μg kg^{-1}) and arsenic (100 μg kg^{-1})[16,20]. Iwegbue *et al.*[21] found that the levels of cadmium, nickel, chromium and lead in some chicken meat collected in northern Nigeria exceeded the tolerated limits. In Egypt, in the state of Qalyubia, the content of lead and cadmium in fried and grilled chicken meat exceeded the tolerated limits[22]. Indeed, in humans, chronic consumption of heavy metals can cause neurological, hepatic etc. and other health problems[23-25].

Nowadays, grilled chicken meat is widely consumed mostly as street food in Benin. A recent study carried out in Abomey-Calavi and Cotonou found that grilling chicken meat may promote the appearance of PAH and heavy metals in the final product[26]. Thus, this study aimed to assess PAH and heavy metal contamination in grilled chicken meat and risk exposure to consumers.

MATERIALS AND METHODS

Sample collection: Samples was collected at Cotonou and Abomey-Calavi, the two biggest and crowded cities in South of Benin. The samples were collected using various process devices such as barrel device using charcoal, cabinet type device using charcoal, cabinet type device using gas and grilling device using wood[26]. Five samples of chicken meat skewer grilled with charcoal-barrel device (BGB), chicken meat skewer grilled with cabinet type charcoal-device (BGP), whole chicken meat grilled with charcoal-barrel device (PGB), whole chicken meat grilled with cabinet type charcoal-device (PGP), whole precooked and grilled chicken meat grilled with charcoal-barrel (PpGB), whole chicken meat grilled with cabinet type gas-device (PGG), indigenous chicken meat grilled with wood (PLG) were collected and analyzed.

Analysis of PAHs content in chicken meat: Chrysene, benzo(a)anthracene, benzo(b)fluoranthene and benzo(a)pyrène were analyzed in this study. They were analyzed using the following three steps:

Extraction: Extraction was carried out according to the 3540C method described by US-EPA[27] with some modifications. Only 10 g of $MgSO_4$ was added to 10 g of ground chicken meat and the mixture was homogenized and left to stand for 15 min at room temperature. To the ground material, 50 μL of 10 ng μL^{-1} recovery standard solution were added along with 300 mL of dichloromethane and then extraction was carried out by Soxhlet at a rate of 2-5 cycles hr^{-1} overnight. With the addition of 20 mL of hexane, the extract was evaporated to 1 mL.

Purification: The purification was carried out according to the 3630C method described by US-EPA[28] with slight modifications. Prior to purification, the column was filled (prepared) with 3 g of silica soaked in enough dichloromethane. Then, 1 mL of Na_2SO_4 and 4 mL of dichloromethane were added. Finally, the column was conditioned with three portions of 6 mL of hexane. The purified extract was placed on the purification column and then eluted with 8 mL of hexane. A first fraction was collected. Then, a second elution was carried out with 14 mL of dichloromethane and a second fraction containing the PAHs was collected and filtered. The purified extract was concentrated under a nitrogen until a volume of less than 300 µL was obtained, then 50 µL of the 10 ng μL^{-1} volumetric solution and 500 µL of isooctane were added. The purified extract was analyzed on a gas chromatograph coupled to a mass spectrophotometer (GC/MS).

Analysis: The analysis was carried out according to the 8270D method described by U.S. EPA[29]. About 1 µL of the aliquot was injected in high splitless mode into the GC (Thermo Scientific) equipped with a DB-EUPAH type chromatographic column (30 m×0.25 mm) with a stationary phase of 0.25 µm at a flow rate of 1.4 mL min^{-1}. The carrier gas was helium. The initial temperature was 80°C (for 1 min), then increased from 35°C min^{-1} to 320°C and finally 3°C min^{-1} to 335°C, maintained for 10 min. The mass spectrophotometer (Thermo Scientific) was configured for electron impact in ionization mode (70 eV), selective ions in acquisition mode and the transfer line was set at 300°C with a source of 340°C. The limits of detection and quantification were respectively of the order of 0.01 and 0.03 µg kg^{-1} for each of the PAHs. Each extract was analyzed three times. The result was expressed in µg kg^{-1} of meat.

Analysis of heavy metals in grilled chicken meat: The samples were previously dried at 70°C for approximately 4 days before being ground for heavy metal determination. The assay was performed following the method described by Joyce[30] with some modifications. Afterward, 1 g of dried and ground sample, were previously added to 10 mL of H_20_2 (9%) and then left for 24 hrs. Then the sample was digested with 4 mL of HNO_3 and heated at 150°C until it became colorless. After evaporation and cooling, it was filtered through a wattman paper. Mercury was determined using cold mineralization of the sample. Pb, Cd, As, were determined in the filtrate using an atomic absorption spectrophotometer (brand SOLAAR S2, THERMO FISCHER). Mercury (Hg) was determined with the same spectrophotometer coupled with VP100 hydride generator. The heavy metal content was expressed in µg kg^{-1} of meat.

Assessment of the risk of exposure to benzo(a)pyrene, lead and cadmium: The Estimated daily intake (EDI) for traces and the hazard quotient (HQ) were calculated as described by Adamou *et al.*[31]. Previous study found that skewers were preferred by children and consumed once a week by adolescents, whereas whole chickens were consumed twice a week by adults[26]. The amount ingested per intake for skewers was 70 g (in excess) and 300 g (in excess) for whole chickens. The maximum ingested amount of whole chicken was determined by dividing the maximum meat mass of boneless grilled whole chicken by 2, because a whole grilled chicken was often shared by at least two adult individuals. The average bone mass was subtracted due to the absence of the elements of interest.

The calculation was based on an average body weight of 28 kg for children (0-15 years) and 70 kg for an adult according to the US Environmental Protection Agency[32]. The EDIs and HQs were calculated for three consumption frequencies (once a week, twice a week and every day). HQ 1, indicated that the risk of toxicity is very low, while HQ >1 indicated a high probability of toxicity:

$$EDI = \frac{C \times Q \times F}{P}$$

$$HQ = \frac{EDI}{ADI}$$

HQ = Hazard quotient
EDI = Estimated daily intake of trace elements (µg kg^{-1} b. wt. day^{-1})
C = Trace element concentration (µg kg^{-1})
Q = Quantity of grilled meat ingested per day (kg day^{-1})
F = Frequency of exposure (F = 1), it is without unit
P = Body weight of the target (kg)
ADI = Acceptable daily intake

According to the report of the Institut National de l' Environnement Industriel et des Risques (INERIS) for chronic threshold effects, the ATSDR (Agency for Toxic Substances and Disease Registry) recommends the following maximum limits for the Acceptable Daily Intake[33]:

- **Benzo(a)pyrene:** 8.10-3 µg kg^{-1} b. wt. day^{-1} for a 70 kg adult
- **Lead:** 3.6 µg kg^{-1} b. wt. day^{-1}
- **Cadmium:** 2.10-4 mg kg^{-1} b. wt. day^{-1}

RESULTS

PAH levels in grilled chicken meat: PAHs content in grilled chicken meat are shown in Table 1.

Gas-grilled chicken meat had the highest values of chrysene, benzo(a)anthracene, benzo(b)fluoranthene and benzo(a)pyrene compared to other types of grilled chicken meats. It was followed by chicken meat grilled with the

Table 1: PAHs content (µg kg^{-1}) in grilled chicken meat

Products	Chrysene	Benzo(a)anthracene	Benzo(b)fluoranthene	Benzo(a)pyrene	Sum of 4 PAHs
BGB	0.0040±0.002^{d}	0.455±0.037^{d}	0.027±0.002^{d}	1.216±0.028^{f}	1.720±0.070^{e}
BGP	0.0780±0.02^{bc}	0.650±0.020^{c}	0.524±0.010^{c}	1.892±0.030^{d}	3.140±0.080^{d}
PGB	0.0930±0.008^{b}	1.532±0.063^{b}	0.893±0.059^{b}	2.761±0.064^{c}	5.270±0.196^{c}
PpGB	0.0170±0.004^{cd}	0.058±0.007^{e}	0.065±0.017^{d}	0.333±0.033^{g}	0.480±0.060^{f}
PGP	0.1350±0.005^{b}	1.870±0.024^{a}	1.227±0.109^{a}	3.768±0.058^{b}	7.000±0.196^{b}
PGG	0.642±0.033^{a}	1.779±0.033^{a}	1.316±0.040^{a}	4.062±0.030^{a}	7.800±0.136^{a}
PLG	0.0040±0.002^{d}	0.532±0.019^{cd}	0.027±0.007^{d}	1.493±0.055^{e}	2.060±0.086^{e}

BGB: Chicken meat skewer grilled with barrel using charcoal, BGP: Chicken meat skewer grilled with cabinet type equipment using charcoal, PGB: Whole chicken meat grilled with barrel using charcoal, PGP: Whole chicken meat grilled with cabinet type equipment using charcoal, PpGB: Whole precooked chicken meat grilled with barrel using charcoal, PGG: Whole chicken meat grilled with cabinet type equipment using gas, PLG: Indigenous chicken meat grilled with wood and Values with different letter(s) within the column are statistically different

Table 2: PAHs Heavy metals content (µg kg^{-1}) in grilled chicken meats

	Lead	Cadmium	Mercury	Arsenic
BGB	73.33±36^{bc}	3.330±1.6^{b}	0.22±0.1^{d}	24.41±1.6^{b}
BGP	153.3±18^{ab}	6.667±3.3^{b}	1.49±0.010^{b}	29.47±7.2^{b}
PGB	66.67±22^{bc}	20.000±5^{ab}	2.54±0.23^{a}	24.65±1.4^{b}
PpGB	36.67±9^{c}	36.670±6^{a}	1.42±0.07^{bc}	23.77±1.9^{b}
PGP	223.3±36^{a}	3.330±1.6^{b}	0.21±0.085^{d}	44.90±2.5^{a}
PGG	36.67±18^{c}	3.330±1.6^{b}	0.85±0.12^{c}	18.53±1.7^{b}
PLG	96.67±25.8^{bc}	20.000±10^{ab}	0.74±0.33^{cd}	18.84±5.7^{b}
Standard	100*	50*	500*	100**

BGB: Chicken meat skewer grilled with barrel using charcoal, BGP: Chicken meat skewer grilled with cabinet type equipment using charcoal, PGB: Whole chicken meat grilled with barrel using charcoal, PGP: Whole chicken meat grilled with cabinet type equipment using charcoal, PpGB: Whole precooked chicken meat grilled with barrel using charcoal, PGG: Whole chicken meat grilled with cabinet type equipment using gas, PLG: Indigenous chicken meat grilled with wood. Values with different letter(s) within the column are statistically different, *Ordinance N°0362/MAEP/D-CAB/SGM/DRH/DP/SA, MAEP[16] and **Codex Alimentarius[20]

charcoal cabinet device which overtook chrysene and benzo(a)pyrene. There was no significant difference between these two grilled chicken meats with respect to benzo(a)anthracene and benzo(b)fluoranthene. Chrysene content of skewers grilled with the charcoal cabinet device was higher than that found in skewers and pre-cooked whole chicken meat grilled with the barrel device and indigenous chicken meats grilled with the wood. On the other hand, there was no significant difference in chrysene between the pre-cooked chicken and skewers grilled with the barrel device and the indigenous chickens. Likewise, there was no significant difference in the benzo(a)anthracene content of indigenous grilled chickens and grilled skewers. Pre-cooked grilled chickens contained the lowest value for benzo(a)anthracene. With regard to benzo(b)fluoranthene, the lowest values were found in indigenous grilled chicken meats, precooked chicken and skewers grilled with barrel device. They were followed by chicken meat skewers grilled with charcoal-cabinet device and the pre-cooked grilled chickens. There was a significant difference in benzo(a)pyrene content between all types of grilled chicken. In decreasing order, we have gas grilled chicken meat, grilled chicken meat with charcoal cabinet type kiln, grilled chicken with barrel kiln, grilled skewers with charcoal cabinet type kiln, indigenous chicken meat grilled with the wood, grilled skewers with barrel kiln and pre-cooked grilled chicken meats. In addition, all whole grilled chicken meat exceeded the maximum limit of 2 µg kg^{-1} for the benzo(a)pyrene content set by the European Union[17] with the exception of whole pre-cooked and grilled chicken meat with barrel device. However, the grilled chicken skewers met this limit. But, according to Beninese regulations[16], all grilled chicken meats complied with the maximum limit of 5 µg kg^{-1}. Likewise, all the grilled chicken meats complied with the respective limits of 12 and 30 µg kg^{-1} for the sum of the 4 PAHs set by the European Union and Beninese regulations.

Heavy metals levels in grilled chicken meat: Heavy metals content in grilled chicken meat are shown in Table 2.

Lead was the most abundant component in grilled chicken meats, regardless of the process considered. Lead levels in grilled skewers (153.3±18 µg kg^{-1}) and whole chicken meat (223.3±36 µg kg^{-1}) collected from operators using charcoal cabinet device were significantly highest and exceeded the tolerated limits. They were followed by indigenous grilled chicken meat (96.67±25.8 µg kg^{-1}) and grilled chicken meat skewers with the barrel device (73.33±36 µg kg^{-1}). Finally, pre-cooked and charcoal-grilled whole chicken meat (36.67±9 µg kg^{-1}) and gas-grilled chicken meat (36.67 µg kg^{-1}) contained the lowest levels. For cadmium, whole pre-cooked chicken meat grilled by charcoal-barrel device (PpGB) contained the highest level (36.67±6 µg kg^{-1}). In contrast, there was no significant

Table 3: EDI (µg kg^{-1} b. wt. day^{-1}) and HQ of benzo(a)pyrene for a child and adult

	BGB	BGP	PpGB	PGB	PGP	PGG	PLG
Child of 28 kg							
EDI 1	4.34×10^{-4}	6.76×10^{-4}	1.19×10^{-4}	9.86×10^{-4}	1.35×10^{-3}	1.45×10^{-3}	5.33×10^{-4}
EDI 2	8.69×10^{-4}	1.35×10^{-3}	2.38×10^{-4}	1.97×10^{-3}	2.69×10^{-3}	2.90×10^{-3}	1.07×10^{-3}
EDI 3	3.04×10^{-3}	4.73×10^{-3}	8.33×10^{-4}	6.90×10^{-3}	9.42×10^{-3}	1.02×10^{-2}	3.73×10^{-3}
HQ 1	1.36×10^{-1}	2.11×10^{-1}	3.72×10^{-2}	3.08×10^{-1}	4.21×10^{-1}	4.53×10^{-1}	1.67×10^{-1}
HQ 2	2.71×10^{-1}	4.22×10^{-1}	7.43×10^{-2}	6.16×10^{-1}	8.41×10^{-1}	9.07×10^{-1}	3.33×10^{-1}
HQ 3	9.50×10^{-1}	1.48	2.60×10^{-1}	2.16	2.94	3.17	1.17
Adult of 70 kg							
EDI 1	7.44×10^{-4}	1.16×10^{-3}	2.04×10^{-4}	1.69×10^{-3}	2.31×10^{-3}	2.49×10^{-3}	9.14×10^{-4}
EDI 2	1.49×10^{-3}	2.32×10^{-3}	4.08×10^{-4}	3.38×10^{-3}	4.61×10^{-3}	4.97×10^{-3}	1.83×10^{-3}
EDI 3	5.21×10^{-3}	8.11×10^{-3}	1.43×10^{-3}	1.18×10^{-2}	1.61×10^{-2}	1.74×10^{-2}	6.40×10^{-3}
HQ 1	9.31×10^{-2}	1.45×10^{-1}	2.55×10^{-2}	2.11×10^{-1}	2.88×10^{-1}	3.11×10^{-1}	1.14×10^{-1}
HQ 2	1.86×10^{-1}	2.90×10^{-1}	5.10×10^{-2}	4.23×10^{-1}	5.77×10^{-1}	6.22×10^{-1}	2.29×10^{-1}
HQ 3	6.51×10^{-1}	1.01	1.78×10^{-1}	1.48	2.02	2.18	8×10^{-1}

EDI 1: Estimated daily intake for weekly consumption, EDI 2: Estimated daily intake for bi-weekly consumption, EDI 3: Estimated daily intake for daily consumption, HQ 1: Hazard quotient for weekly consumption, HQ 2: Hazard quotient for bi-weekly consumption, HQ 3: Hazard quotient for daily consumption, BGB: Chicken meat skewer grilled with barrel using charcoal, BGP: Chicken meat skewer grilled with cabinet type equipment using charcoal, PGB: Whole chicken meat grilled with barrel using charcoal, PGP: Whole chicken meat grilled with cabinet type equipment using charcoal, PpGB: Whole precooked chicken meat grilled with barrel using charcoal, PGG: Whole chicken meat grilled with cabinet type equipment using gas and PLG: Indigenous chicken meat grilled with wood

Table 4: EDI (µg kg^{-1} b. wt. day^{-1}) and HQ of lead and cadmium for child of 28 kg

	BGB	BGP	PGB	PpGB	PGP	PGG	PLG
Lead							
DJE 1	2.61×10^{-5}	5.46×10^{-5}	2.39×10^{-5}	1.32×10^{-5}	7.96×10^{-5}	1.32×10^{-5}	3.46×10^{-5}
DJE 2	5.21×10^{-5}	1.09×10^{-4}	4.79×10^{-5}	2.64×10^{-5}	1.59×10^{-4}	2.64×10^{-5}	6.93×10^{-5}
DJE 3	1.83×10^{-4}	3.83×10^{-4}	1.68×10^{-4}	9.25×10^{-5}	5.58×10^{-4}	9.25×10^{-5}	2.43×10^{-4}
QD 1	7.24×10^{-3}	1.52×10^{-2}	6.65×10^{-3}	3.67×10^{-3}	2.21×10^{-2}	3.67×10^{-3}	9.62×10^{-3}
QD 2	1.45×10^{-2}	3.04×10^{-2}	1.33×10^{-2}	7.34×10^{-3}	4.42×10^{-2}	7.34×10^{-3}	1.92×10^{-2}
QD 3	5.07×10^{-2}	1.06×10^{-1}	4.65×10^{-2}	2.57×10^{-2}	1.55×10^{-1}	2.57×10^{-2}	6.74×10^{-2}
Cadmium							
DJE 1	1.07×10^{-6}	2.5×10^{-6}	7.14×10^{-6}	1.32×10^{-5}	1.07×10^{-6}	1.07×10^{-6}	7.14×10^{-6}
DJE 2	2.14×10^{-6}	5×10^{-6}	1.43×10^{-5}	2.64×10^{-5}	2.14×10^{-6}	2.14×10^{-6}	1.43×10^{-5}
DJE 3	7.5×10^{-6}	17.5×10^{-6}	5×10^{-5}	9.25×10^{-5}	7.5×10^{-6}	7.5×10^{-6}	5×10^{-5}
QD 1	5.36×10^{-3}	1.25×10^{-2}	3.57×10^{-2}	6.61×10^{-2}	5.36×10^{-3}	5.36×10^{-3}	3.57×10^{-2}
QD 2	1.07×10^{-2}	2.50×10^{-2}	7.14×10^{-2}	1.32×10^{-1}	1.07×10^{-2}	1.07×10^{-2}	7.14×10^{-2}
QD 3	3.75×10^{-2}	8.75×10^{-2}	2.50×10^{-1}	4.63×10^{-1}	3.75×10^{-2}	3.75×10^{-2}	2.50×10^{-1}

EDI 1: Estimated daily intake for weekly consumption, EDI 2: Estimated daily intake for bi-weekly consumption, EDI 3: Estimated daily intake for daily consumption, HQ 1: Hazard quotient for weekly consumption, HQ 2: Hazard Quotient for bi-weekly consumption, HQ 3: Hazard Quotient for daily consumption, BGB: Chicken meat skewer grilled with barrel using charcoal, BGP: Chicken meat skewer grilled with cabinet type equipment using charcoal, PGB: Whole chicken meat grilled with barrel using charcoal, PGP: Whole chicken meat grilled with cabinet type equipment using charcoal, PpGB: Whole precooked chicken meat grilled with barrel using charcoal, PGG: Whole chicken meat grilled with cabinet type equipment using gas and PLG: Indigenous chicken meat grilled with wood

difference in this level in the other grilled chicken meats. However, the average cadmium levels in all these grilled chicken meats were below the limit tolerated by the regulations in force in Benin. The mercury and arsenic levels of the grilled chicken meats analyzed were well below the maximum limits.

EDI and HQ of benzo(a)pyrene: Table 3 presents EDI and HQ of benzo(a)pyrene for a child (28 kg) and adult (70 kg).

Benzo(a)pyrene EDI levels were lower than the accepted daily intake when grilled chicken meat was consumed weekly and biweekly and did not present any health risk (HQ<1) with regard to the benzo(a)pyrene levels obtained in these meats. On the other hand, the health risk exists for both children and adults when skewers and whole chicken meats grilled with both a charcoal and gas kiln (HQ>1) was consumed daily, except for pre-cooked whole chicken grilled with charcoal barrel device (HQ<1).

EDI and HQ of lead and cadmium for a child of 20 kg: Table 4 shows EDI and HQ of lead and cadmium for a child of 28 kg.

For a 28 kg child, EDI of lead and cadmium was lower than the tolerable daily intake for these two components respectively when 70 g of grilled chicken meat skewers and whole was consumed on daily basis and therefore the hazard quotient was less than 1. The lead and cadmium levels in grilled chicken meat (skewer, whole) did not pose the risk of toxicity.

Table 5 : EDI (µg kg^{-1} b.wt. day^{-1}) and HQ of lead and cadmium for adult of 70 Kg

	BGB	BGP	PGB	PpGB	PGP	PGG	PLG
Lead							
DJE 1	4.47×10^{-5}	9.37×10^{-5}	4.10×10^{-5}	2.27×10^{-5}	1.37×10^{-4}	2.27×10^{-5}	5.94×10^{-5}
DJE 2	8.94×10^{-5}	1.87×10^{-4}	8.20×10^{-5}	4.53×10^{-5}	2.73×10^{-4}	4.53×10^{-5}	1.19×10^{-4}
DJE 3	3.13×10^{-4}	6.56×10^{-4}	2.87×10^{-4}	1.59×10^{-4}	9.56×10^{-4}	1.59×10^{-4}	4.16×10^{-4}
QD 1	1.24×10^{-2}	2.60×10^{-2}	1.14×10^{-2}	6.29×10^{-3}	3.79×10^{-2}	6.29×10^{-3}	1.65×10^{-2}
QD 2	2.48×10^{-2}	5.20×10^{-2}	2.28×10^{-2}	1.26×10^{-2}	7.59×10^{-2}	1.26×10^{-2}	3.30×10^{-2}
QD3	8.69×10^{-2}	1.82×10^{-1}	7.98×10^{-2}	4.40×10^{-2}	2.65×10^{-2}	4.40×10^{-2}	1.15×10^{-2}
Cadmium							
DJE 1	1.84×10^{-6}	4.28×10^{-6}	1.22×10^{-5}	2.26×10^{-5}	1.84×10^{-6}	1.84×10^{-6}	1.22×10^{-5}
DJE 2	3.67×10^{-6}	8.57×10^{-6}	2.45×10^{-5}	4.53×10^{-5}	3.67×10^{-6}	3.67×10^{-6}	2.45×10^{-5}
DJE 3	1.29×10^{-5}	3×10^{-5}	8.57×10^{-5}	1.58×10^{-4}	1.28×10^{-5}	1.28×10^{-5}	8.57×10^{-5}
QD 1	9.18×10^{-3}	2.14×10^{-2}	6.12×10^{-2}	1.13×10^{-1}	9.18×10^{-3}	9.18×10^{-3}	6.12×10^{-2}
QD 2	1.84×10^{-2}	4.29×10^{-2}	1.22×10^{-1}	2.27×10^{-1}	1.84×10^{-2}	1.84×10^{-2}	1.22×10^{-1}
QD3	6.43×10^{-2}	1.50×10^{-1}	4.29×10^{-1}	7.93×10^{-1}	6.43×10^{-2}	6.43×10^{-2}	4.29×10^{-1}

EDI 1: Estimated daily intake for weekly consumption, EDI 2: Estimated daily intake for bi-weekly consumption, EDI 3: Estimated daily intake for daily consumption, HQ 1: Hazard Quotient for weekly consumption, HQ 2: Hazard Quotient for bi-weekly consumption, HQ 3: Hazard Quotient for daily consumption, BGB: Chicken meat skewer grilled with barrel using charcoal, BGP: Chicken meat skewer grilled with cabinet type equipment using charcoal, PGB: Whole chicken meat grilled with barrel using charcoal, PGP: Whole chicken meat grilled with cabinet type equipment using charcoal, PpGB: Whole precooked chicken meat grilled with barrel using charcoal, PGG: Whole chicken meat grilled with cabinet type equipment using gas and PLG: Indigenous chicken meat grilled with wood

EDI and HQ of lead and cadmium for adult of 70 kg: Table 5 shows EDI and HQ of lead and cadmium for adult of 70 kg.

For an adult of 70 kg, EDI of lead and cadmium were also below the tolerable daily intake for these two elements respectively when 300 g of grilled chicken meat (skewers or whole) was consumed on daily basis and therefore, the hazard quotient was less than 1 (Table 5). It means that with the levels of lead and cadmium in grilled chicken meat, the risk of toxicity is unlikely.

DISCUSSION

Whole non-precooked chickens meat (PGB, PGP) contained higher levels of PAHs than skewers (BGB, BGP) and whole precooked chicken meat (PpGP) except gas grilled chicken meat (PGG). It should be noted that the skewers were marinated before grilling. Indeed, El Badry[3] and Farhadian *et al.*[34] showed that marinade made it possible to reduce the formation of PAHs in chicken meats during grilling. This effect of the marinade was due to the antioxidant activity of spices, which are its main constituents (the formation of PAHs starting with the oxidation of lipids) and their lack of lipids (main factor in the formation of PAHs)[35-37]. Edikou *et al.*[26] reported that the marinade used for chicken meat grilling mainly consisted of chili, garlic, ginger, flavor enhancer, salt, curry and roasted peanut flour. Likewise, Farhadian *et al.*[38] and Onwukeme *et al.*[4] also showed that pre-cooking reduced the formation of PAHs in beef and grilled chicken. This was explained by the fact that the pre-cooking causes a weak pyrolysis of the lipids that reduces the dripping of the oil on the embers and consequently a small amount of smoke compounds deposited on the meat[39]. On the other hand, the present study indicated that the levels of PAHs in the chicken meats grilled with gas was higher than those grilled with charcoal and with wood. These results are contrary to those of Farhadian *et al.*[40] who observed that gas-grilled chickens contained less PAHs compared to those grilled with charcoal. According to Hamzawy *et al.*[41], charcoal-grilled chicken meats contained 0.49-7.20 µg kg^{-1} of benzo(a)pyrene. The levels of benzo(a)pyrene, obtained in this study was found in this range except whole pre-cooked and grilled chicken meat which had a lower content (0.33 µg kg^{-1}). In addition, several other factors also influence the formation of PAHs during grilling, such as the level of lipids in the meat, the cooking time, the type of fuel, the proximity and direct contact with the heat source[34,42]. For example, the distance between the chicken meat and the heat source was smaller in the gas grilling device (9$\pm$4 cm) compared to the charcoal grill (23-31 cm)[26]. Furthermore, the benzo(a)pyrene content (2.44$\pm$0.26 µg kg^{-1}) in charcoal grilled (not precooked) chicken meats, found by Farhadian *et al.*[38] was lower than that obtained (2.761-3.768 µg kg^{-1}) in the present study. The same authors also showed that precooked and grilled chicken meat were free from benzo(a)pyrene and benzo(b)fluoranthene, unlike the precooked and grilled chickens in the current study, while Onwukeme *et al.*[4] found only benzo(a)pyrene with a content of 0.0352 µg kg^{-1} in precooked and grilled chickens. It should be noted that the nature of the roasted product and the composition of the charcoal also affect the production of PAHs. This composition depends, among other factors, on the nature of the raw material and the temperature at which the coal is made (carbonized). Roasting beef with charcoal from wood and coconut shells, respectively, did not affect PAH formation in the meat differently, while roasting with coconut charcoal produced less PAHs in the meat[43]. Coal produced from a low carbonization temperature (500°C) is likely to produce more PAHs than that obtained from a higher carbonization temperature (750°C and 1000°C)[44]. El-Badry[3] found up to 5.3 μg kg^{-1} of benzo(a)pyrene in gas-grilled chicken meats. In the present study, this value is higher than the content obtained in gas-grilled chicken meats (4.062±0.030 μg kg^{-1}). Compared to the grilled chicken meats analyzed in this study, the smoked shrimps in Benin contained 62-1020 times more PAHs [benzo(a)pyrene, chrysene, benzo(a)anthracene and benzo(b) fluoranthene][18]. Lee *et al.*[45] obtained 48-89% and 41-74% reduction in PAHs, respectively, when the oil from pork and beef does not fall on the embers and when the smoke from the embers is deflected therefore does not rise to the surface of the meat during grilling.

Cooking methods have varying effects on heavy metals in meat. According to Joyce *et al.*[30], cooking with water (boiling) and frying increased lead content in game meat while grilling (after pre-cooking) reduced it. Manganese content was also reduced by boiling, frying and grilling[30]. Copper content increased after cooking with water or roasting, while frying reduced it. On the other hand, cadmium was not affected by any of these cooking methods[30]. Our results also showed low levels of heavy metals in chicken meats that were pre-cooked and grilled compared to those grilled directly, which showed higher lead levels. Authors even recommended grilling for lead reduction for bushmeat consumers[30]. Diaconescu *et al.*[46] made similar recommendations after showing reduction of nickel and chromium by grilling and microwave cooking but found no impact on lead reduction in fish. Perelló *et al.*[47] showed that cooking methods such as boiling, frying, grilling and smoking did not have a significant impact on lead, cadmium, mercury and arsenic contents in fish but reduced arsenic content in meat (chicken, pork, veal). These authors considered time, temperature and fuel as important parameters when evaluated whether heavy metals were reduced by cooking methods. Indeed, cooking time in the above-mentioned studies varied between 5 and 30 min with a temperature between 170 and 200°C using electric, gas and coal. In the present study, the grilled chicken meats were obtained after about 1h of grilling with a grilling temperature ranging from 130-145°C. In Tamale (Ghana), there was no significant difference in heavy metals (Zinc, lead, cadmium, copper, manganese) between raw and grilled beef and between raw and grilled guinea fowl meat[48], while lead and cadmium were not found in raw and grilled beef meat in East Lagon (Accra Suburbs)[49].

In Estonia, Reinik *et al.*[50] reported similar results about grilled chicken meats consumed by both children and adults. In Catalonia (Spain), meats were the most common source of PAH intake but it was noted that the daily intake of PAHs by an adult (70 kg) had decreased in 2008 (6.72 μg day^{-1}) compared to the years 2000 (8.42 μg day^{-1}) and 2006 (12.04 μg day^{-1})[51]. These values are well below the values obtained in our study.

PAHs can reach human body through several ways (air, skin contact etc.) but the main access (88-98% of the cases) is food[4,52,53]. Reactive metabolites produced by PAHs can interfere with the function of targeted organ (liver, intestine and other extrahepatic tissues), in which they are activated, posing a health threat to humans resulting in fatal and lethal toxicity. In addition, once absorbed, PAHs are distributed through the blood to several tissues, especially those with a high fat content and some are metabolized into DNA active mutagens or genotoxic carcinogenic compounds (diol epoxides)[54]. Then, these bind to cellular macromolecules, including DNA causing DNA replication errors and mutations that will trigger the cancer process[34,40,50].

Human health risk from contaminants based on their amount measured in the food is increasingly questioned in favor of bioaccessibility because this method would overestimate the amount of contaminants absorbed orally[55]. By definition, the bioaccessibility of a contaminant in food is defined as the fraction of the contaminant that is released from the food matrix into the gastrointestinal tract and thus becomes available for intestinal absorption[56]. This is achieved by a simulation model of digestion and intestinal absorption *in vitro*. Very few studies have looked into this avenue for PAHs. A study by Wang *et al.*[57] showed that only 24.3 and 31.1% of PAHs in fish were bioaccessible under gastric and intestinal conditions, respectively.

Assessment of heavy metal toxicity risks associated with the consumption of chicken meat has also been the subject of several studies around the world. The results obtained in the present study are similar to those obtained by Mohamed *et al.*[58] who found that grilled chicken meats did not present a health hazard risk through lead, cadmium, arsenic and chromium. Bortey-Sam *et al.*[59] showed that lead, cadmium, mercury and arsenic in chicken meat did not pose the health risk to the consumers living near the gold mines of Tarkwa (Ghana), although the levels of these elements were high and even higher than the limits set by the USDA[60] and the Commission of the European Union[17] in some organs. Nevertheless, the consumption of gizzard and liver contributed significantly to the toxic exposure to these heavy metals[60]. Similar results were also observed for cadmium, lead, manganese, zinc and nickel in Port-Harcourt, Nigeria[61].

However, in considering bio-accessibility in the analysis of toxicity risk of heavy metals, Maulvault *et al.*[62] found that cadmium and arsenic from the digestion of black scabbardfish and edible crab were highly bio-accessible (up to 100%), unlike mercury which was only 40% bio-accessible. Furthermore, this bio-accessibility depend on the cooking method to which they were submitted. For example, the bioaccessibility of mercury was 32, 40 and 80% in fried, steamed and grilled black sheaths, respectively. Thus, roasting contributed more to the bioaccessibility of mercury from the black sheath. Similar study was conducted by He *et al.*[63] on two other fish species (*L. japonicus* and *P. major*) and showed that cadmium and arsenic were more than 45% bioaccessible and grilling and frying contributed more to this bioaccessibility than steaming and boiling. Similar findings were also made on arsenic in a larger study of marine fish species available on the European market[64].

CONCLUSION

Grilled chicken meat contained high level of benzo(a)pyrene that exceeded Benin and European tolerable limits except precooked grilled whole chicken meat and chicken meat skewers. Benzo(a)pyrene was the most abundant in gas-grilled chicken meat. According to Beninese regulations, only chicken meat grilled on charcoal-cabinet type device had non-compliant lead levels whereas the levels of cadmium, mercury and arsenic were low in all samples. Benzo(a)pyrene poses a health risk to children and adults who consume 70 and 300 g of grilled chicken meat daily, respectively. There are no health risks associated with lead and cadmium from grilled chicken meat, regardless of the amount consumed. Knowledge and capacity of processors should be reinforced in order to apply improved grilling methods for PAHs reduction in chicken meat.

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