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Contamination of smoked fish and smoked-dried fish with polycyclic aromatic hydrocarbons and biogenic amines and risk assessment for the Beninese consumers

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ABSTRACT

The present study aims to assess the contamination of smoked and smoked-dried fish sampled on Beninese market with biogenic amines and polycyclic aromatic hydrocarbons (PAHs). Fifteen PAHs, ten biogenic amines and nineteen amino acids were analysed in thirty-six fish samples, using liquid chromatography techniques. The assessment of consumer exposure was carried out by calculating the estimated daily intake (EDI) which was compared to a toxicological reference value. The exposure to histamine, the most toxic biogenic amine, was calculated and compared with an acute reference dose (ARfD) of 50 mg/meal. The margin of exposure (MOE) to PAHs was calculated as the ratio between benchmark PAH levels and EDI. MOE to carcinogenic compounds below 10,000 indicates a potential concern for human health. Amino-acid profile varied as a function of fish species with a high content of histidine (the precursor of histamine) recorded in *Cypselurus cyanopterus*, the Atlantic flying fish (2.9 g/100 g, dry weight) followed by *Scomber scombrus*, the Atlantic mackerel (1.9 g/100 g, dry weight). High histamine concentration (4,384.2 mg/kg) was recorded in one sample of *C. cyanopterus*, a non-scombroid fish, exceeding 44 times the maximal limit of 100 mg/kg, set by the EU and Benin regulations. Histamine intake calculated using the maximum measured histamine concentration exceeded the ARfD. Concerning the PAH contamination, none of the smoked and smoked-dried fish samples were compliant with the EU regulation, and the MOE of the consumers were below 10,000 (for both median and maximum PAH contamination levels). In conclusion, the consumption of smoked and smoked-dried fish could represent a major concern for the Beninese consumer health because of both histamine and PAHs contamination.

1. Introduction

Fish is the most available protein source commonly consumed in developing countries. In African countries like Benin, for preservation purpose, fish is traditionally smoked according to two main methods: hot smoking and smoke-drying (Assogba et al., 2019). During hot smoking, the fish is laid on a mesh tray directly above the smoke source (e; g. barrel kilns, Chorkor) and is cooked but not dried while during smoke-drying, the fish is firstly hot smoked and then dried using different types of hard woods from acacia or mango trees (Assogba et al., 2019).

In Benin, the smoking equipment and the type of fuel used for

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smoking were previously reported to favour the production of PAHs in smoked shrimp (Kpoclou et al., 2014). The maximal limit of Benzo [a] Pyrene (BaP), a compound recognized as carcinogenic to humans (Group 1) by the International Agency for Research on Cancer (IARC) (WHO/IARC, 2012), is 2 μg/kg in the EU (EC, 2006) and 5 μg/kg in Benin (MAEP, 2007). In addition to BaP, there is an EU maximal limit of 12 μg/kg for the sum of four PAHs (BaP, Chrysene – CHR –, Benzo [b] fluoranthene – BbF – and Benzo [a] anthracene – BaA) also called "PAH4" (EC, 2006). After processing, smoked and smoked-dried fishes are kept at ambient temperature (28 ± 2 °C) on mesh tray laid on smoking equipment or packed, and often stored, in vegetable basket covered with cement paper, board paper or with unused old clothes. These practices result in microbial contamination with spoilage bacteria, yeast and moulds (Anihouvi et al., 2019) and can cause the production of biogenic amines.

Biogenic amines are non-volatile amines found in rich-protein foods (fish and meat) or fermented food (i.e. cheese, beer or wine), as a result of microbial contamination leading to enzymatic decarboxylation of free amino acids (Chong et al., 2011; EFSA, 2011; Latorre-Moratalla et al., 2017; Sagratini et al., 2012). Histamine and tyramine are both biogenic amines which presence in food is undesirable due to their adverse effects on consumer health such as hypertension, headache and allergic reactions (EFSA, 2011; Latorre-Moratalla et al., 2017; Marissiaux et al., 2018). Histamine poisoning is frequently misdiagnosed because its symptoms are similar to those caused by allergy. Histamine formation in fish tissue results from the histidine decarboxylase activity of spoilage bacteria including *Morganella morganii* (Enterobacteriaceae), which optimum temperature for histamine production is 25 °C (Chong et al., 2011; Lehane & Olley, 2000). This production can result from post-catching contamination, bad handling and packaging practices and/or abusive storage temperature conditions (Chong et al., 2011; Kim et al., 2002; Sagratini et al., 2012; Taylor, 1986).

Among biogenic amines, only histamine has a maximal limit in the EU (EC, 2005) and Benin (MAEP, 2009) of 100 mg/kg in fish flesh. Although histamine formation and the presence of PAHs have been reported in fish after drying, frying, grilling or smoking (Chung et al., 2017; Tongo et al., 2017; Zachara et al., 2017), to our best knowledge, only few studies focused on the consumer exposure assessment to biogenic amines (Latorre-Moratalla et al., 2017; Rauscher-Gabernig et al., 2009) and PAHs through smoked fish consumption (Akpambang et al., 2009; Asamoah et al., 2021; Bogdanović et al., 2019; Domingo & Nadal, 2015; Racovita et al., 2021). The present study aims to assess PAHs and biogenic amines contamination of smoked and smoked-dried fish and quantitatively assess the corresponding risk for the South Beninese consumers.

2. Material and methods

2.1. Sampling

A total of 36 samples were randomly collected in South Benin among which 18 were sampled from the manufacturers (processing sites) and 18 from markets. Six different fish species were represented, three for smoked fish: Atlantic mackerel (*Scomber scombrus*), Nile tilapia (*Oreochromis noliticus*) and Benguela hake (*Merluccius polli*), and three for smoked-dried fish: Atlantic flying fish (*Cypselurus cyanopterus*), great barracuda (*Sphyraena barracuda*) and Bonga shad (*Ethmalosa fimbriata*). Six samples per species were collected including three samples on processing sites and three in markets (Iko Afé et al., 2020a). The fish was traditionally smoked by female processors using kilns and firewood in open space, as reported by Assogba et al. (2019). After collection, samples were first wrapped in aluminium foil and then packed in sterile stomacher bags, kept at 4 °C and transported to the laboratory. Samples were ground with a laboratory blender (model 38BL40, Waring, New Hartford, Connecticut, USA) and approximately 75 g were lyophilized (Freezemobile, Virtis Inc., Gardiner, NY, USA).

2.2. Laboratory analyses

2.2.1. Amino acid analysis

The profile of total amino acids (except tryptophan) was carried out using ion exchange chromatography with the BIOCHROM20 Plus amino acid analyser (BIOCHROM Limited, the UK), according to Paul et al. (2016). Approximately 100 mg of each sample were weighted and 10 ml of hydrochloric acid (HCl, 6N) were added and the mix was heated at 110 °C for 24 h in an oven (hydrolysis). After this hydrolysis, the mix was cooled on crushed ice. Then, 40 ml of citrate buffer at pH 2.2 was added (with continuous stirring) while the samples were still on ice. After this, the pH was adjusted between 0.5 and 1 using 7.5 N NaOH and then readjusted to 2.2 using 1 N NaOH. This solution was transferred in a flask containing 1 ml of 500 μM Norleucine (N8513 SIGMA) internal standard in citrate buffer solution. The volume of this flask was made 100 ml by adding citrate buffer at 2.2 pH and 1 ml of this solution was filtered through a 0.2 μm of filter and injected in the BIOCHROM 20 Plus amino acid analyser (BIOCHROM Limited, UK). All the analyses were performed in duplicate and results were presented as means of two repetitions (g/100 g of dry matter – DM).

2.2.2. Biogenic amine analysis

Ten biogenic amines were analysed according to Douny et al. (2019). Analytical standards of biogenic amines (tryptamine hydrochloride, tyramine hydrochloride, cadaverine dihydrochloride, spermine tetrahydrochloride, spermidine trihydrochloride, 2- phenylethylamine hydrochloride, putrescine dihydrochloride, histamine dihydrochloride, serotonin hydrochloride, and methylamine hydrochloride), glycine, dansyl chloride, and 1,7- diaminoheptane were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium hydroxide, Normapur quality trichloroacetic acid (TCA), ethanol, and perchloric acid 60% were provided by VWR International (West Chester, PA, USA). Proanalysis quality sodium hydrogen carbonate was from Merck (Darmstadt, Germany). HPLC quality acetone, LC-MS quality acetonitrile, methanol, and UPLC quality water were from Biosolve (Valkenswaard, The Netherlands). Two grams of ground fresh sample were weighted into a glass tube to which was added 200 μl of a 100 ng/μL solution of 1,7-diaminoheptane (internal standard) in 5% trichloroacetic acid (TCA). After the addition of 2.3 ml of 0.4 M of perchloric acid, the mixture was shaken for 15 min and centrifuged (3700 g, 10 min, at room temperature). This extraction was performed twice, and both supernatants were combined. One milliliter of the extract was used for dansylation, by the addition of 2 mL of dansyl chloride (10 mg/mL in acetone) and incubation at 70 °C for 15 min. After dansylation, 100 μL of glycine (150 mg/mL in water) was added to bind to the dansyl chloride in excess. After centrifugation (3700 g, 10 min, at room temperature) and filtration, 5 μl of the final extract were injected on UPLC Acquity system integrated autosampler (Acquity Sample Manager FTN), solvent delivery system (Acquity QSM H Class), and column heater coupled to an Acquity Fluorescence detector (FLD), all from Waters Corporation (Milford, MA, USA). The column used was an Acquity UPLC BEH C18 (2.1 × 100 mm, 1.7 μm), with a UPLC BEH C18 VanGuard pre-column (2.1 × 5 mm, 1.7 μm), both from Waters Corporation. The limit of quantification (LOQ) ranged between 1.3 and 10 mg/kg depending on the biogenic amines.

2.2.3. PAHs analytical procedure

Standard solutions of individual PAHs were purchased from Dr Ehrenstorfer GmbH (Germany). The injection standard (IS) 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 cyclohexane and n-hexane in Picograde quality were purchased from Sigma-Aldrich (Bornem, Belgium) and Promochem (Wesel, Germany),

Table 1
Amino acid profile of smoked fish and smoked-dried fish species in South Benin.

Amino acids (g per 100 g product, dry weight)	*Scomber scombrus*	*Oreochromis niloticus*	*Merluccius polli*	*Cypselurus cyanopterus*	*Ethmalosa fimbriata*	*Sphyraena barracuda*
Amino acids precursor of biogenic amines						
Glycine	2.7 ± 0.1	5.4 ± 0.0	4.3 ± 0.1	5.0 ± 0.1	4.4 ± 0.1	4.9 ± 0.1
Tyrosine	1.7 ± 0.0	2.4 ± 0.0	2.9 ± 0.1	2.8 ± 0.1	2.0 ± 0.1	2.9 ± 0.1
Histidine[a]	1.9 ± 0.0	1.7 ± 0.0	1.6 ± 0.0	2.9 ± 0.0	1.7 ± 0.0	1.7 ± 0.1
Arginine	2.9 ± 0.1	4.7 ± 0.0	5.0 ± 0.0	5.1 ± 0.2	3.9 ± 0.1	5.3 ± 0.1
Phenylalanine[a]	2.0 ± 0.0	3.1 ± 0.0	3.3 ± 0.1	3.2 ± 0.1	2.6 ± 0.0	3.5 ± 0.1
Lysine[a]	4.0 ± 0.1	6.4 ± 0.0	7.1 ± 0.0	7.0 ± 0.2	5.3 ± 0.1	7.5 ± 0.3
Tryptophan[a]	nd	nd	nd	nd	nd	nd
Other amino acids						
Threonine[a]	2.3 ± 0.0	3.4 ± 0.0	3.7 ± 0.0	3.8 ± 0.1	2.8 ± 0.1	3.9 ± 0.1
Valine[a]	2.5 ± 0.0	3.6 ± 0.0	3.9 ± 0.0	4.0 ± 0.2	3.0 ± 0.0	4.2 ± 0.1
Methionine[a]	1.5 ± 0.0	2.2 ± 0.0	2.6 ± 0.0	2.4 ± 0.1	1.9 ± 0.1	2.7 ± 0.1
Isoleucine[a]	2.2 ± 0.0	3.2 ± 0.0	3.5 ± 0.0	3.5 ± 0.1	2.6 ± 0.1	3.7 ± 0.1
Leucine[a]	3.8 ± 0.0	5.8 ± 0.1	6.5 ± 0.0	6.4 ± 0.2	4.8 ± 0.2	6.8 ± 0.2
Alanine	3.0 ± 0.0	5.1 ± 0.0	5.1 ± 0.0	5.1 ± 0.1	4.1 ± 0.1	5.6 ± 0.1
Cystine-cysteine	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
Aspartic acid[b]	4.9 ± 0.1	7.9 ± 0.1	8.7 ± 0.0	8.3 ± 0.4	6.4 ± 0.3	9.0 ± 0.2
Serine	2.1 ± 0.0	3.1 ± 0.0	3.8 ± 0.0	3.5 ± 0.1	2.7 ± 0.1	3.5 ± 0.1
Glutamic acid[c]	7.6 ± 0.1	12.6 ± 0.1	14.0 ± 0.1	13.2 ± 0.4	10.3 ± 0.3	14.2 ± 0.1
Proline	1.8 ± 0.0	3.1 ± 0.4	2.9 ± 0.0	3.3 ± 0.0	2.7 ± 0.0	3.2 ± 0.1
Total	47.1	74.0	79.2	79.8	61.5	82.9

nd = non-determined.
[a] Essential amino acids.
[b] Sum of aspartic acid and asparagine.
[c] Sum of glutamic acid and glutamine.

respectively. Solvents dichloromethane and water (HPLC grade) were purchased from VWR (Leuven, Belgium). Solid Phase Extraction (SPE) cartridges columns (Chromabond HR-X 6 ml/500 mg) were purchased from Macherey-Nagel (Eupen, Belgium).

Fifteen PAHs were analysed in one gram of lyophilized fish sample. Samples were extracted using accelerated solvent extractor (ASE 200, Dionex Corporation) and purified using solid phase extraction (SPE) cartridges (Chromabond HR-X 6 ml/500 mg). Injection and fluorescence detection were performed using a HPLC system including 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). The extraction and fluorescence detection methods were described in details earlier in Brasseur et al. (2007), Kpoclou et al. (2014) and Iko Afé et al. (2020b). For each series of injection, seven calibration solutions containing the 15 PAHs in increasing concentrations from 2.5 to 400 pg/μL, except for BjF and IcP (from 10 to 1600 pg/μL) were injected. The deuterated injection standard was spiked at a constant concentration (i.e. 250 pg/μL in acetonitrile) in each calibration solution. The response (ratio between native and internal standard PAHs peak areas) was plotted against calibration 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 IS) was checked. The PAHs LOQ were 0.1 μg/kg fresh weight, except for IcP and BjF for which the LOQ was 0.5 μg/kg fresh weight.

2.3. Estimation of consumption of smoked and smoked dried fish by Beninese consumers

The daily consumption frequency of smoked fish and smoked-dried fish was estimated during a face-to-face survey conducted with 250 consumers of smoked fish and/or smoked-dried fish (Iko Afé et al., 2020a). Among the 250 consumers, only 186 consumers having smoked fish and/or smoked-dried fish at least once per month were included in this study. They were aged between 18 and 89 years, composed of 62.9% of women, and with body weight ranging from 32 to 120 kg with an average of 63.1 ± 13.1 kg (Iko Afé et al., 2020a).

Among these 186 consumers, 59% (n = 110) consumed only smoked fish while 16% (n = 30) consumed only smoked-dried fish and 25% (n = 46) consumed both smoked and dried smoked fish. Each consumer was asked how much she/he spent for her/his consumption of smoked fish or smoked-dried fish at the moment of the interview. A quantity of smoked fish or smoked-dried fish corresponding to the price mentioned by the interviewed consumer was then purchased and weighted to estimate the quantity of smoked fish or smoked-dried fish consumed daily. By multiplying the individual daily frequency of consumption declared by the interviewee and the weighted quantities of fish, 110, 30 and 46 data of daily consumption (expressed in grams of fish per day) were then obtained for smoked fish, smoked-dried fish and for both, respectively.

2.4. Methodology of risk assessment

2.4.1. Exposure assessment

The exposure assessment was calculated following a deterministic approach (AFSCA, 2005). For each consumer, the estimated daily intake (EDI) of PAHs expressed in ng PAH per kilogram body weight (kg b.w.) per day were calculated using the median and maximum (worst-case scenario) contamination data of smoked fish and smoked-dried fish while the histamine intake, expressed in mg/meal, was calculated using the maximum contamination data as follows:

$$\text{Histamine intake (mg/meal)} = [\text{Histamine}](\text{mg/kg}) \times \text{Daily consumption (kg/meal)}$$

Table 2
Minimum, maximum and median concentrations of biogenic amines (mg/kg wet weight) in commercial smoked and smoked-dried fish samples (n = 36), respective precursor amino acid (AA) and number of samples above the LOQ.

Biogenic amines.	Precursor AA	Minimum[a]	Maximum	Median	Number of samples > LOQ
Methylamine	Glycine	<1.5	89.1	<1.5	9
Tryptamine	Tryptophan	<3	111.6	<3	10
2-phenylethylamine	Phenylalanine	<1.5	30.1	<1.5	4
Putrescine	Arginine, Ornithine	<1.5	113.6	<1.5	15
Cadaverine	Lysine	<0.75	632.2	<0.75	12
Histamine	Histidine	<10	4384.2	<10	8
Serotonine	Hydroxytryptophane	<1.5	56.2	3.2	22
Tyramine	Tyrosine	<3.5	700.9	<3.5	10
Spermidine	Arginine, Ornithine	<0.75	138.0	3.3	19
Spermine	Arginine, Ornithine	<1.25	281.9	<1.25	15

LOQ: limit of quantification.
[a] All minimum concentrations were below the respective LOQ of each biogenic amine.

$$\text{PAHs EDI (ng/kg b.w./day)} = [\text{PAH}]\text{(ng/g)} \times \frac{\text{Daily consumption (g/day)}}{\text{Body weight (kg b.w.)}}$$

The EDI for each of the 46 consumers having both smoked fish and smoked-dried fish was calculated as the sum of the EDI for smoked fish consumption and the EDI for smoked-dried fish consumption.

2.4.2. Risk characterization

The margin of exposure (MOE) for PAHs was calculated for each consumer. The MOE is the ratio between a benchmark dose lower confidence limit ($BMDL_{10}$) determined in laboratory animals and the EDI of the consumer (Benford et al., 2010; EFSA, 2005, 2008). In case of carcinogenic compounds such as PAHs, a MOE above 10,000 means a low concern for the human health. EFSA (2008) has determined $BMDL_{10}$ for BaP and 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 are 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).

The acute exposure to histamine is more relevant than chronic exposure, contrary to PAHs, and thus for each consumer, the histamine intake per meal was compared with an acute reference dose (ARfD) of 50 mg/meal (EFSA, 2011).

2.5. Statistical analysis

Microsoft Excel was used for basic statistics (i.e. mean, standard deviation, minimum, maximum, percentiles). All data were first analysed for normality (Shapiro-Wilk) and homogeneity (Leven) tests using R 3.5.1 (R core Team, 2018). Means of two groups were compared using Mann and Whitney *U* test or Student's T-Test (R 3.5.1, R core Team, 2018). A significant difference was accepted at $p < 0.05$.

3. Results and discussion

3.1. Consumption of smoked and smoked-dried fish

From the 110 consumers of smoked fish only, the minimum, median, 97.5th percentile and maximum daily consumption of smoked fish were 2.4, 59.0, 446.7 and 539.5 g/day, respectively, while, for the 30 consumers of smoked-dried fish only, these data were 0.9, 10.0, 316.2 and 592.1 g/day. For the 46 consumers having both smoked and smoked-dried fish, the minimum, median, 97.5th percentile and maximum daily consumption were 6.4, 62.2, 188.6 and 192.7 g/day and 0.5, 23.5, 124.7 and 380.7 g/day, for smoked fish and smoked-dried fish respectively. These data were used to estimate PAH exposure.

The fish consumption for biogenic amines intake calculation was expressed in g/meal, the day of consumption. The minimum, median, 97.5th percentile and maximum consumption of smoked fish were 24.1, 96.3, 337.2 and 674.3 g/meal, respectively, while the minimum, median, 97.5th percentile and maximum consumption of smoked-dried fish were 7.9, 26.4, 232.6 and 282.0 g/meal, respectively.

3.2. Amino acids profiles of fish species

The eight essential amino acids (AA) for human needs were present in all the six fish species (Table 1), which suggests that these fish species are good nutritional sources as reported by several authors (FAO, 1999). Six of the investigated AA are precursors of biogenic amines when they are present in the product as free AA, and subjected to decarboxylation under microbial activity (see Table 2 for the names of the biogenic amines and their precursors). Histamine is known as the most toxic biogenic amine and is formed from the decarboxylation of histidine. As shown in Table 1, the highest level of histidine (2.9 g/100 g DM) was recorded in *C. cyanopterus* (Exocoetidae).

The general amino acid profile of the six fish species showed that the AA contents ranged between 0.2 ± 0.0 g/100 g DM (cysteine) and 14.2 ± 0.1 g/100 g DM (sum of glutamic acid and glutamine). The low level observed for cysteine can result from its partial destruction due to acid hydrolysis during the analysis, as reported by Barbeau and Hilu (1993). Salma and Nizar (2015) also reported cysteine as the least present AA in *S. scombrus*. Histidine and tyrosine contents (1.9 and 1.7 g/100 g DM, respectively) recorded for *S. scombrus* (Atlantic mackerel) in this study were lower than those reported by Toppe et al. (2007) (2.4 and 2.3 g/100 g DM, respectively) for the same species. On the contrary, Usydus et al. (2009) found less histidine (0.7 g/100 g) in smoked mackerel than the present study.

The amino acid profiles of all fish species were dominated by glutamic and aspartic acid (7.6–14.2 and 4.9–9 g/100 g DM, respectively). These two AA were also predominant in different smoked fishes (mackerel, sprat, herring, salmon Baltic, Norwegian salmon and trout) collected in the Polish market (Usydus et al., 2009). Among essential AA analysed, lysine and leucine (4–7.5 and 3.8–6.8 g/100 DM, respectively) were the predominant. This is nutritionally important since in humans, leucine participates in skin and bone wound healing, while lysine, a limited AA in cereal products, contributes to calcium absorption (Paul et al., 2016; WHO, 2007). This study is the first which showed the amino acid profile of *Cypselurus cyanopterus*, *Merluccius polli*, *Sphyraena barracuda* and *Ethmalosa fimbriata*.

3.3. Biogenic amine content in fish samples and health risk for the Beninese consumer

3.3.1. Biogenic amines in the collected samples of smoked and smoked-dried fish

All the thirty-six samples contained at least one biogenic amine (supplementary data 1 and 2). The concentrations of histamine and

Table 3
Histamine intake of smoked fish consumers (n = 110) and smoked-dried fish consumers (n = 30) using maximum concentration of histamine (worst-case scenario), depending on the level of consumption (Minimum, P50, P97.5 and maximum) in South Benin.

	Histamine exposure (mg/meal)	
	Smoked fish[a]	Smoked-dried fish[b]
Minimum	36.4	34.8
P50	145.6	115.9
P97.5	509.6	1019.9
Maximum	1019.1	1236.2

The minimum, median (P50), 97.5th percentile and maximum consumptions of smoked fish were 24.1, 96.3, 337.2 and 674.3 g/meal, respectively.
The minimum, median (P50), 97.5th percentile and maximum consumptions of smoked-dried fish were 7.9, 26.4, 232.6 and 282.8 g/meal, respectively.
[a] Maximum concentration of histamine = 1511.3 mg/kg for smoked fish.
[b] Maximum concentration of histamine = 4384.2 mg/kg for smoked-dried fish.

tyramine which are two biogenic amines with adverse health effects (EFSA, 2011), ranged between <10 and 4384.2 mg/kg and between <3.5 and 701 mg/kg, respectively (Table 2).

Among the eighteen smoked fish samples, only one sample (*S. scombrus*) was shown to contain a measurable level of histamine (1511.3 mg/kg) (supplementary data 1). On the contrary, seven of the eighteen smoked-dried fish samples showed a histamine content above the LOQ: one from *S. barracuda* (65.1 mg/kg) and six from *C. cyanopterus* samples (171–4384.2 mg/kg) (supplementary data 2). This contamination could be due to inappropriate packaging and/or long periods of storage (up to one year), as reported by Assogba et al. (2019).

The highest histamine level (4384.2 mg/kg), corresponding to almost 44 times the EU and Benin limit of 100 mg/kg, was found in the non-scombroid species *C. cyanopterus*. A similar level of histamine was recently reported to cause histamine fish poisoning in Belgium, in a 50-year-old woman after tuna fish consumption, with symptoms such as flushing, palpitations, headache, dizziness, and diffuse thoracic oppression (Marissiaux et al., 2018). The second highest histamine level (1511.3 mg/kg) was found in the *S. scombrus* species. These two species (*S. scombrus* and *C. cyanopterus*) with the highest histamine levels are also those showing the highest histidine (its precursor) content (Table 1) (EFSA, 2011).

Microorganisms producing histidine decarboxylase are mainly Enterobacteriaceae including *Morganella morganii* which optimal temperature for histamine production is 25 °C (Chong et al., 2011; Lehane & Olley, 2000). Anihouvi et al. (2019) reported the presence of Enterobacteriaceae in smoked and smoked-dried fish marketed in Benin, generally stored at ambient temperature, i.e. between 25 °C and 32 °C (Dossou et al., 2016; Onzo et al., 2014). EFSA (2011) reported that the optimum temperature for biogenic amine formation by mesophilic bacteria is between 20 °C and 37 °C. Indeed, beside histamine, cadaverine and putrescine were also found in the same sample in which 4384 mg/kg of histamine was measured (supplementary data 2). These two biogenic amines could increase histamine toxicity due to their inhibitory effect on diamine oxidase and histamine N-methyl transferase, two histamine-degrading enzymes present in the human intestinal tract (den Brinker et al., 1996; Lehane & Olley, 2000; Zaman et al., 2010).

Among the thirty-six collected samples, only one sample displayed a concentration of tyramine above 600 mg/kg (701 mg/kg, supplementary data 1). Prester et al. (2011) and scientists from FAO/WHO (2013) reported that tyramine presence in food containing histamine can also potentiate the negative effect of histamine due to its competition with histamine-metabolizing enzymes substrates. The biogenic amine index (BAI) calculated for each sample as the sum of concentrations of tyramine, histamine, putrescine and cadaverine was presented in supplementary data 1 and 2. Five samples of smoked fish (supplementary data 1) and eight samples of smoked-dried fish (supplementary data 2) had a BAI above 90 mg/kg, the threshold suggested by Du et al. (2002) to indicate an advanced decomposition of fish.

Table 4
Comparison of PAHs concentration (μg/kg wet weight) according to the type of fish (A) and collection places (B).

A		
PAHs	Fish type	
	Smoked fish (n = 18)	Smoked-dried fish (n = 17)
BaP	21.8 ± 21.2[a]	78.5 ± 53.8[b]
∑PAH4	119.3 ± 107.5[a]	484.2 ± 305.6[b]
∑PAH15	204.5 ± 187.4[a]	802.8 ± 489.8[b]
B		
PAHs	Collection places	
	Processing sites (n = 18)	Market (n = 17)
BaP	39.0 ± 44.7[a]	60.2 ± 52.6[a]
∑PAH4	246.7 ± 249.1[a]	349.6 ± 326.4[a]
∑PAH15	403.3 ± 404.1[a]	592.3 ± 529.0[a]

Mean ± standard deviation; different letters in the same row indicate a significant difference ($p < 0.05$).

3.3.2. Histamine risk assessment

Histamine contamination was found in only one sample of smoked fish samples (supplementary data 1) and seven samples of smoked-dried fish (supplementary data 2), so only a "worst-case scenario" of histamine intake has been considered, based, respectively, on the level found in the contaminated smoked fish sample and on the maximum level found in smoked-dried fish. These intakes were calculated for each consumer. According to the fish consumption level, the histamine intake ranged from 36.4 to 1019.1 mg/meal for smoked fish consumers and from 34.8 to 1236.2 mg/meal for smoked-dried fish consumers (Table 3).

Histamine intakes were compared to the acute reference dose (ARfD) of 50 mg histamine/meal suggested by EFSA (2011). From reported studies, it was found that healthy volunteers showed no symptoms after ingestion of 25–50 mg histamine with solid food like fish (EFSA, 2011). The same threshold of 50 mg was also suggested as no-observed-adverse-effect-level (NOAEL) for the headache and flushing symptoms (EFSA, 2011). All the calculated intakes (except for the minimum level of fish consumption) exceeded the ARfD of 50 mg/kg, showing a high concern for these consumers who could have been subjected to histamine fish poisoning. Considering the individual levels of consumption and the maximum histamine level found in smoked or smoked-dried fish, about 95% of smoked fish consumers and 90% of smoked-dried fish consumers could be exposed to histamine levels exceeding the ARfD (data not shown). Moreover, the risk is probably underestimated as consumers also eat other foods which may contain histamine.

Even though this study was carried out on the assumption of healthy consumers, the presence of biogenic amines such as cadaverine, putrescine and even tyramine which can potentiate histamine effect can decrease the threshold of histamine dose needed to provoke an adverse reaction in fish, as mentioned by FAO/WHO (2013). To our best knowledge, only one published study dealt with histamine risk assessment, which was carried out on Spanish fermented sausages (Latorre-Moratalla et al., 2017). In the study reported by Latorre-Moratalla et al. (2017), the mean dietary exposure to histamine was 1.4 mg/meal showing a very low risk to have histamine intoxication. Due to the fact that formation of biogenic amines including histamine is related to microbial contamination, management strategies to monitor histamine in smoked fish and smoked-dried fish will be difficult even if it was required. Different strategies which can limit microbial contamination of fish since capture till sale of processed fish can be addressed with special focus on good hygiene practices.

Table 5
Minimum, median and maximum PAHs content (μg/kg wet weight) in the 35 samples of smoked and smoked dried fish used for risk assessment.

PAHs	Smoked fish (n = 18)			Smoked-dried fish (n = 17)		
	Minimum	Maximum	Median	Minimum	Maximum	Median
Benzo[b]fluoranthene (BbF)	**2.5**	**59.9**	**12.3**	**7.6**	**166.7**	**65.8**
Dibenzo[a,l]pyrene (DlP)	<0.1	1.5	0.7	<0.1	7.5	1.7
Dibenz[a,h]anthracene (DhA)	0.2	13.7	2.7	1.0	32.1	9.3
Benzo[ghi]perylene (BgP)	1.8	43.2	8.5	5.5	103.7	36.8
Dibenzo[a,e]pyrene (DeP)	0.3	36.2	2.3	1.4	65.8	11.3
Benzo[j]fluoranthene (BjF)	2.0	47.6	9.1	6.7	128.9	40.3
Benzo[c]fluorene (BcL)	5.9	108.6	18.7	21.2	315.8	94.2
Benz[a]anthracene (BaA)	**4.4**	**126.5**	**26.4**	**31.9**	**403.7**	**138.9**
Chrysene (CHR)	**7.0**	**142.3**	**31.0**	**32.1**	**603.3**	**165.5**
5-Methylchrysene (5 MC)	<0.1	28.0	2.5	<0.1	133.1	10.4
Benzo[k]fluoranthene (BkF)	1.2	27.3	5.4	3.0	75.9	27.2
Benzo[a]Pyrene (BaP)	**2.1**	**75.1**	**14.5**	**10.1**	**183.4**	**69.7**
Indeno[1,2,3-cd]pyrene (IcP)	1.4	37.3	5.8	4.5	83.6	27.6
Dibenzo[a,i]pyrene (DiP)	<0.1	3.8	0.7	<0.1	7.6	2.0
Dibenzo[a,h]pyrene (DhP)	<0.1	1.5	0.4	<0.1	2.6	1.0
PAH4	**15.9**	**399.9**	**88.9**	**81.7**	**1265.7**	**509.1**

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

3.4. Polycyclic aromatic hydrocarbons in smoked and smoked-dried fish and health risk for the Beninese consumer

3.4.1. PAHs content of the samples of smoked and smoked-dried fish

The 36 samples of smoked and smoked-dried fish of this study displayed quantifiable levels of BaP and PAH4 (supplementary data 3 and 4). BaP concentration ranged between 2.1 and 1403.4 μg/kg while the sum of PAH4 ranged between 15.9 and 10,966.4 μg/kg, indicating that all samples were non-compliant with the maximum levels of EU regulation for BaP (2 μg/kg) and the sum of PAH4 (12 μg/kg) (EC, 2006). Similarly, 34 out of the 36 samples exceeded 5 μg/kg, the maximal limit for BaP in Benin. One sample of smoked-dried fish displayed a very high concentration for all PAHs including BaP (1403.4 μg/kg), PAH2 (6695.6 μg/kg), PAH4 (10,966.4 μg/kg) and PAH8 (11, 701.54 μg/kg) (sample n° 3, supplementary data 4). This sample was, however, considered as an outlier and was not used further. Indeed, the use of kernel density estimation (using R 3.5.1) to visualise the shape of the data showed that this sample influenced a lot the data distribution.

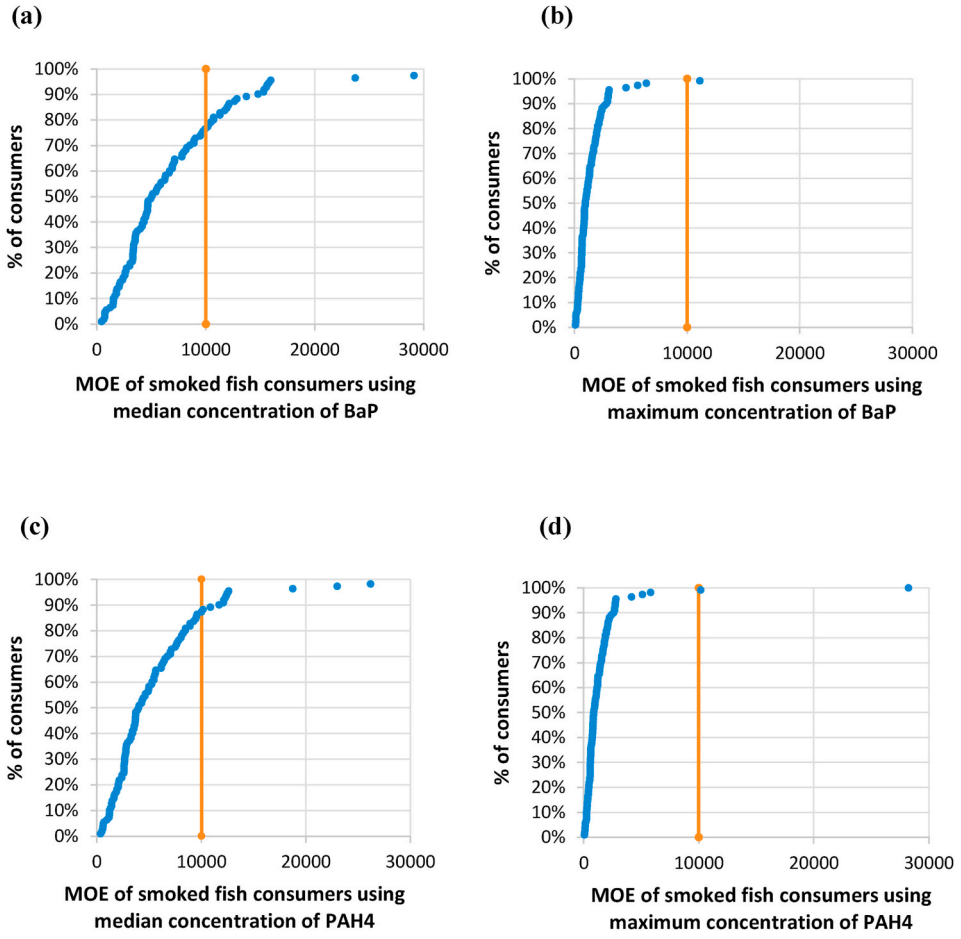

Fig. 1. MOE of smoked fish consumers calculated using median and maximum concentrations of BaP (a and b) or PAH4 (c and d) recorded in smoked fish samples.

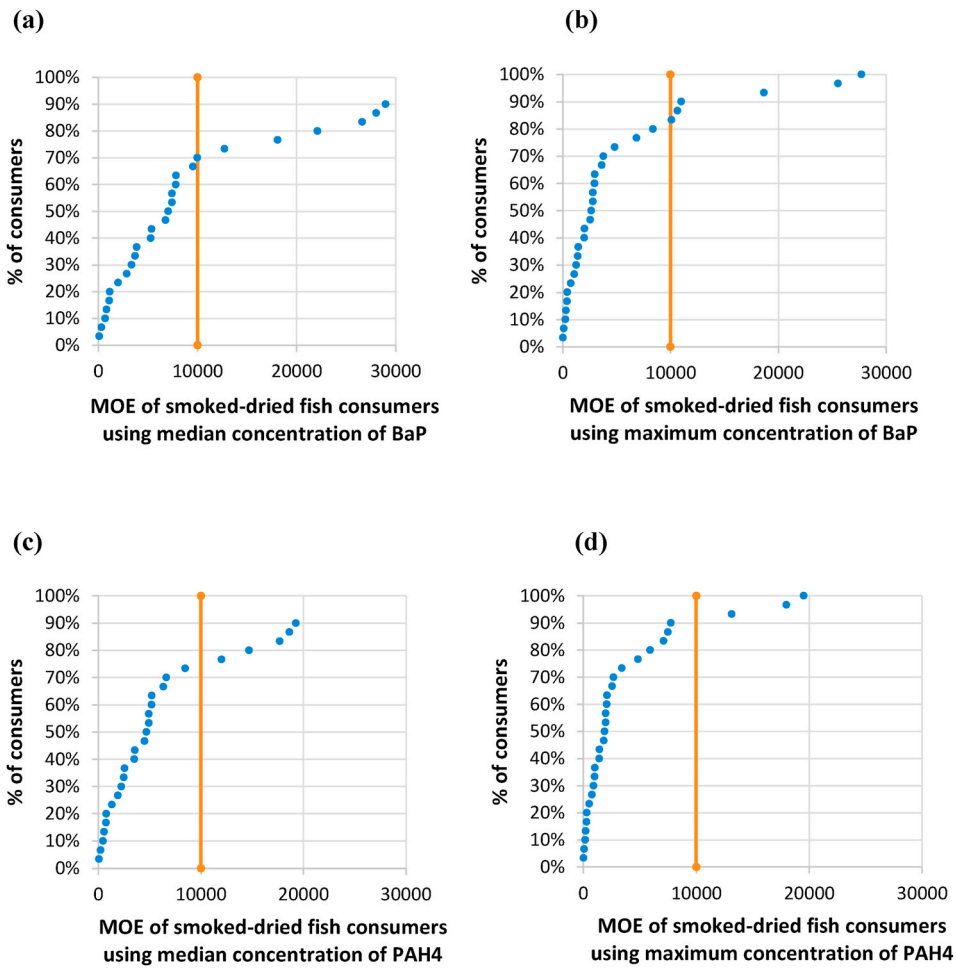

Fig. 2. MOE of smoked-dried fish consumers calculated using median and maximum concentrations of BaP (a and b) or PAH4 (c and d) recorded in smoked-dried fish samples.

Moreover, when including this extremely high PAH concentration in the calculation of the means and standard deviations of all samples, standard deviations exceed the means values. This very high PAH concentration could be due to the presence of some burned parts in the fish samples, which is not usual at all for marketed smoked-dried fish.

Significantly higher levels ($p < 0.05$) of BaP, PAH4 and PAH15 were found in smoked-dried fish than in smoked fish (Table 4A). However, no significant differences ($p < 0.05$) were found among PAH contamination levels when considering their collection places (processing sites or markets) (Table 4B). Table 5 shows minimum and maximum concentrations of PAHs recorded in the 35 samples included in the risk assessment.

3.4.2. PAH risk assessment

3.4.2.1. PAH exposure assessment. The PAHs EDI (expressed as ng/kg b.w/day) of smoked fish consumers considering the median contamination levels, ranged from 0.4 to 156.2 for BaP, and 2.7 to 959.7 for PAH4 (supplementary data 5). In the "worst-case" scenario using maximum PAHs contamination levels, the PAHs EDI ranged from 2.3 to 809.9 ng/kg b.w/day for BaP, and 12.0 to 4,314.9 ng/kg b.w/day for PAH4. For smoked-dried fish consumers, considering the median contamination levels, BaP intake ranged from 1.0 to 750.2 ng/kg b.w/day, and 7.0–5481.2 ng/kg b.w/day for PAH4, while in the "worst-case" scenario, the EDI ranged from 2.5 to 1974.8 ng/kg b.w/day for BaP, and 17.4 to 13,627.2 ng/kg b.w/day for PAH4 (supplementary data 6). When consuming both smoked and smoked-dried fish, the PAHs EDI of consumers considering the median contamination levels, ranged from 3.0 to 395.5 ng/kg b.w/day for BaP and 19.2–2858.3 ng/kg b.w/day for PAH4. In the "worst-case" scenario, the EDI ranged from 14.3 to 1110.4 ng/kg b.w/day for BaP and 78.6–7441.0 ng/kg b.w/day for PAH4 (supplementary data 7). The EDI calculated in this study were higher than EDI reported by Sahin et al. (2020) for grilled fish consumers in Turkey (BaP: 0.2 ng/kg bw/day and PAH4: 0.8 ng/kg bw/day). The high EDI values in this study are probably due to the high level of consumption of smoked and smoked-dried fish in Benin as reported by Iko Afé et al. (2020a). Wang et al. (2021) also reported lower PAHs EDI than in this study for grilled fish consumers in China (BaP: 1.1 ng/kg bw/day and PAH4: 3.4 ng/kg bw/day). In Nigeria however, Akpambang et al. (2009) reported BaP EDI, from consumption of commercial smoked fish, of 52, 4, 8.7 and 31.6 ng/kg bw/day for *Clarias gariepinus*, *Selar crumenophthalmus*, *Scomber scombrus* and *Pseudotolithus senegalensis*, respectively. These EDI are higher than those reported by Sahin et al. (2020) and Wang et al. (2021) but very close to the median EDI recorded in this study.

3.4.2.2. Risk characterization. When using the median PAHs contamination of smoked fish, the MOE to Bap and PAH4 was below 10,000 for 76% and 87% of consumers, respectively (Fig. 1a and c). In the worst-case scenario (using the maximum level of PAH contamination found in smoked fish), the MOE was below 10,000 for about 98% of smoked fish consumers for BaP and PAH4 (Fig. 1b and d).

The MOE to both BaP and PAH4 were below 10,000 in the "median" scenario (Fig. 2a and c), for 70% of smoked-dried fish consumers, while

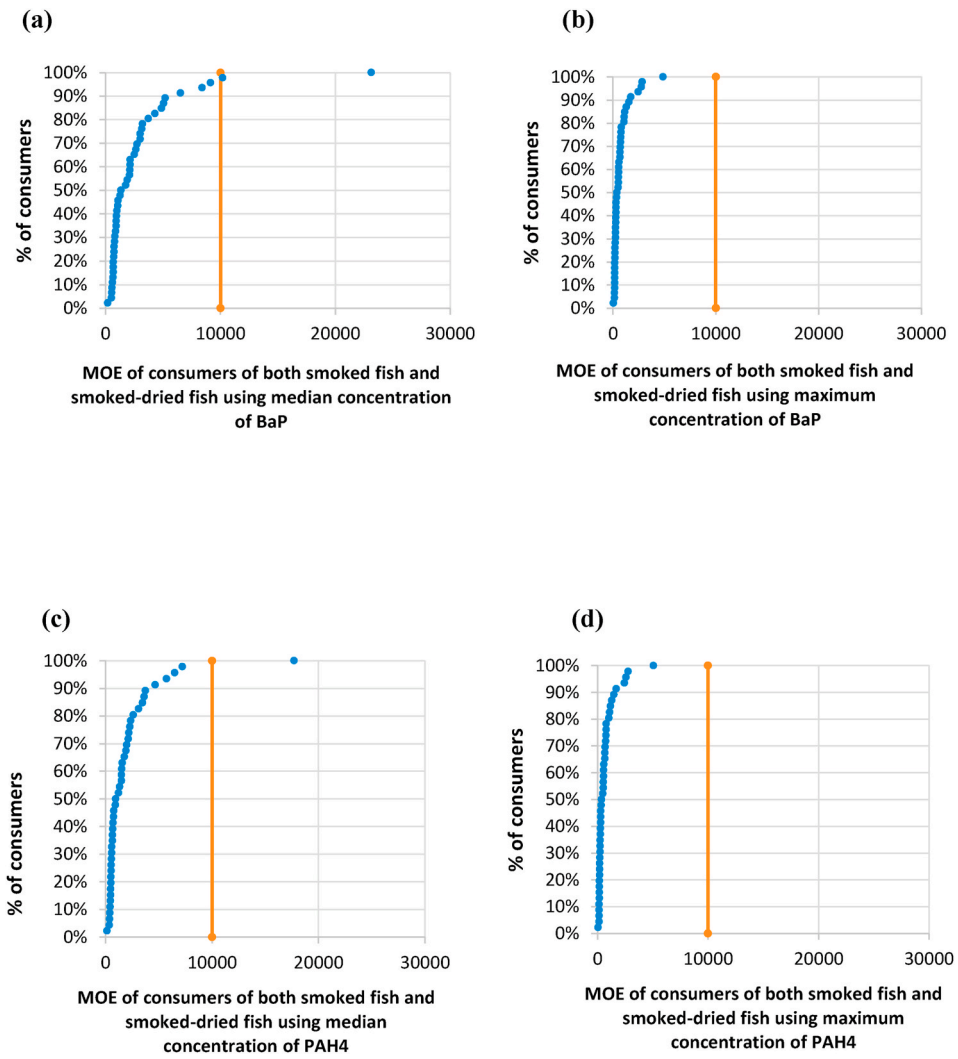

Fig. 3. MOE of consumers of both smoked fish and smoked-dried fish calculated using median and maximum concentrations of BaP (a and b) or PAH4 (c and d) recorded in smoked and smoked-dried fish samples.

in the worst-case scenario, these MOE were below 10,000 for about 80% and 90%, of smoked-dried fish consumers, respectively (Fig. 2b and d).

The MOE were below 10,000 for 96% (BaP) and 98% (PAH4) of consumers (Fig. 3a and c) for consumers of both smoked and smoked-dried fish, in the scenario using the median PAHs contamination levels. In the worst-case scenario, the MOE to both BaP and PAH4 was below 10,000 for 100% of these consumers BaP (Fig. 3b and d).

MOE below 10,000 indicate a concern (risk of cancer) for consumers for carcinogenic compounds such as PAHs. The risk of cancer was more important for people who consumed both smoked fish and smoked-dried fish than those who consumed only smoked fish or smoked-dried fish.

Akpamang et al. (2009) reported MOE for BaP ranging between 1346 and 58,464 for Nigerian consumers of traditionally smoked fish from different species: African sharptooth catfish (*Clarias gariepinus*), croaker (*Pseudotolithus senegalensis*), Atlantic mackerel (*S. scombrus*) and bigeye scad (*Selar crumenophthalmus*) and in particular below 10,000 (between 1346 and 8008) for smoked *P. senegalensis* and *S. scombrus* consumption.

Fish smoking can result in a concern for both consumers and processors. Agodokpessi et al. (2011) reported rhinitis, cough, and dyspnoea as main respiratory troubles which frequently affect female processors of smoked fish in Benin. Smoked fish processors might also be subjected to PAHs exposure through inhalation as reported in several papers (Boström et al., 2002; Zhang et al., 2009). The link between PAH exposure and cancer incidence in the Beninese population have never been assessed till now. About cancer incidence data in Benin, Bagnan, Padonou, Kodjoh, and Houansou (1994) reported breast, stomach, oesophagus to be the widespread cancers found among patients of the national hospital of Benin and noticed that men were the most affected. The liver cancer represented the most developed cancer among Beninese men while among Beninese women, it represented the third most recorded cancer (WHO, 2014). In 2019, the Global Cancer Observatory (GCO) and the International Agency for Research on Cancer (IARC) reported different cases of cancer in Beninese men including stomach (n = 274), liver (n = 233) and oesophagus (n = 190) (GCO/IARC, 2019).

The risk calculated in this study could be underestimated as the present study did not include other dietary sources of PAH exposure such as grilled or smoked products like grilled pork, which has been recently shown to be of concern (MOE <10,000) for the same population (Iko Afé et al., 2020b). These findings indicate that management strategies of PAHs presence in processed food should be developed, in particular to decrease PAHs levels in smoked fish and smoked-dried fish. Recently, Iko Afé et al. (2020c) reported different techniques to be applied before or after smoking in order to improve traditional methods of smoking and decrease the consumer's exposure to PAHs. Moreover, Akpambang et al. (2009) found that the application of smoking methods which enable to reduce PAHs formation in smoked fish resulted in MOE above 10,000 (between 19,698 and 58,464), which means less concern for consumers' health.

4. Conclusion

Biogenic amines and PAHs were monitored in 36 samples of smoked and smoked-dried fish (including 6 different species), collected in South Benin. The determination of the AA profile of these species showed that the maximum histidine content was found in the *C. cyanopterus* species. A sample of this species also displayed the highest histamine level, which exceeded by 44 times the Benin and EU maximal limit of 100 mg/kg, indicating a potential health concern for consumers. In addition, considering the individual fish consumption, the histamine intake calculated in a "worst-case" scenario (i.e. for the highest histamine contamination level) exceeded the ARfD of 50 mg/kg proposed by EFSA for more than 90% of the consumers. As biogenic amines, including histamine, might be formed at the stage of fish catching already, good hygiene practices for fishermen and good manufacturing practices for processors need to be reinforced to reduce contamination before and after processing. Concerning contamination with PAHs, none of the smoked and smoked-dried fishes were compliant with EU regulation (EC, 2006). MOE values were below 10,000 for more than 90% of consumers in a "worst-case" scenario, indicating a concern for food safety. Improvement of traditional smoking methods of fish is also required to manage this risk and to protect the consumers' health.

CRediT authorship contribution statement

Ogouyôm Herbert Iko Afé: Investigation, Writing – original draft. **Claude Saegerman:** Supervision. **Yénoukounmè Euloge Kpoclou:** Conceptualization, Methodology. **Caroline Douny:** Methodology, Supervision, Writing - reviewing and editing. **Ahmed Igout:** Supervision, Writing - reviewing and editing. **Jacques Mahillon:** Supervision, Writing - reviewing and editing. **Victor Bienvenu Anihouvi:** Conceptualization, Methodology. **Djidjoho Joseph Hounhouigan:** Conceptualization, Methodology, Supervision. **Marie-Louise Scippo:** Methodology, Supervision, Writing - reviewing and editing.

Declaration of competing interest

The authors declare that there is no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodcont.2021.108089.

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