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Polycyclic aromatic hydrocarbons (PAHs) in fish smoked using traditional and improved kilns: Levels and human health risk implications through dietary exposure in Ghana

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ABSTRACT

The concentrations of sixteen polycyclic aromatic hydrocarbons (PAHs) in fish smoked using the traditional Chorkor, the improved Cabin and Abuesi gas fish smoker (AGFS) kilns were investigated. Fresh mackerel and barracuda were smoked using LPG gas and two firewood species and the PAH levels were determined using gas chromatography-mass spectrometry. Based on consumption trends, the potential carcinogenic risks associated with consuming the products from these kilns were also assessed. The AGFS-smoked products had mean benzo(*a*)pyrene and PAH4 concentrations up to 0.66 and 2.52 μg/kg respectively, which were below the European Union maximum limits (2 and 12 μg/kg respectively). The benzo(*a*)pyrene and PAH4 concentrations were up to 3.59 and 67.99 μg/kg respectively for Cabin kiln and 15.51 and 121.60 μg/kg respectively for Chorkor kiln. Depending on the type of firewood used, the Cabin kiln produced BAP below the maximum limits when 'Esa' (*Celtis mildbraedii*) was used, while the Chorkor kiln had levels 3 to 8 times higher than the maximum limits. The PAH4 levels in the Cabin and Chorkor products were all above the maximum limits (4 and 8 times higher respectively). Based on the frequency and quantities of smoked mackerel and barracuda consumed by an average Ghanaian adult, the potential carcinogenic risks were lowest in the gas smoked mackerel and all barracuda samples (2.72×10^{-6} to 1.54×10^{-5}), moderate in the Cabin smoked mackerel (2.82×10^{-5} and 5.73×10^{-5}) and highest in the Chorkor smoked mackerel (7.06×10^{-5} and 1.71×10^{-4}).

1. Introduction

Smoking preserves fish, or other food products, by exposing it to smoke from smouldering wood or plant materials (FAO/WHO, 2012). In many developing countries, including Ghana, smoking is the most popular preserving method, using mostly firewood in traditional smoking kilns (Essumang, Dodoo, & Adjei, 2012, 2013). This process reduces the water activity, provides a physical barrier against microorganisms and can result in the deposition of beneficial antimicrobial and antioxidant compounds like phenols and carboxylic acid (Arason, Nguyen, Thorarinsdottir, & Thorkelsson, 2014). Unfortunately, contaminants, like polycyclic aromatic hydrocarbons (PAHs), dioxins, formaldehyde, nitrogen, sulphur oxides and heavy metals can also be deposited on smoked products (Codex Alimentarius Commission CAC, 2009).

PAHs are a class of persistent organic ecological toxicants (Codex Alimentarius Commission CAC, 2009). PAHs may be present in unprocessed fish, owing to environmental contamination of the water or resulting from lignin pyrolysis of biomass fuels, such as firewood, during fish smoking (Codex Alimentarius Commission CAC, 2009). Factors that determine the degree of PAH contamination in smoked fish include the type and composition of fuel used, smoking method (direct or indirect), distance between fish and heat source, fat content of fish, duration of smoking and the design of smoking chamber (Codex Alimentarius Commission CAC, 2009).

The United States Environmental Protection Agency (USEPA) has identified sixteen priority PAHs of health concerns, as they have been observed to be carcinogenic, teratogenic, and mutagenic to humans (Codex Alimentarius Commission CAC, 2009; International Agency for Research on Cancer IARC, 2018). Of these, benzo(*a*)pyrene has been

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classified as a definite carcinogenic (Group 1) (International Agency for Research on Cancer IARC, 2018). The European Union (EU) has set maximim limits (MLs) for benzo(*a*)pyrene and PAH4 (i.e. the sum of benzo(*a*)pyrene, chrysene, benzo(*a*)anthracene and benzo(*b*)fluoranthene) at 2 and 12 μg/kg respectively in smoked fish products (European Commission, 2011). The PAH4 concentration was proposed as the most suitable indicator of PAH contamination in foods.

In Ghana, the "Chorkor" kiln (also known as the "Chorkor" smoker (Fig. 1a), which was introduced in Ghana in 1969 (UNDP/TCDC, 2001) was an improvement over the existing kilns due to its higher product throughput and fuel efficiency, longer lifespan, shorter operation time and lower labour input. The main drawback of this kiln is in relation to the high concentrations of PAHs in the smoked products, resulting from using firewood (Essumang, Dodoo, & Adjei, 2014, 2013; IRI-CSIR & Kwarteng, 2016; Bomfeh et al., 2019). This reduces the quality of fish products and poses health risks to consumers, which often leads to the rejection of smoked products on international markets (Bomfeh et al., 2019). To address these concerns, the value chain development component of the West Africa Regional Fisheries Programme (WARFP), that ended in 2018, aimed at developing fish smoking technologies that reduce the levels of PAH in smoked fish to conform to international standards and thereby making the product safe for consumption. Such technologies will further increase the marketability of smoked fish products and contribute to the country's economic growth (The World Bank, 2011).

The WARFP led to the introduction of an improved kiln, the 'Ahotor', in Ghana. This kiln performed well in terms of fuel efficiency and throughput capacity, however, the PAH concentrations in the smoked products are still higher than the EU MLs (IRI-CSIR & Kwarteng, 2016). Two improved, but lesser known kilns, the Cabin (Fig. 1b) and Abuesi Gas Fish Smoker, AGFS (Fig. 1c) were therefore explored in this study and compared with the Chorkor kiln. The Cabin kiln, developed by the United Nations University-Fisheries Training Programme (UNU-FTP) and Matis Ltd, while the AGFS was also developed by GRATIS

(a)

(b)

(c)

Fig. 1. Fish smoking kilns: Chorkor smoker (a), Cabin (b) and the Abuesi gas fish smoker (AGFS).

Foundation in Ghana.

Both kilns are enclosed units and are expected to ensure minimal exposure of processors to smoke and heat, while offering fish of good nutritional value. It is therefore anticipated that the adoption of these technologies in Ghana will be of benefit to the fishing industry (for both small scale and industrial processors) and the country as a whole. Prior to their adoption in Ghana, however, it is imperative to assess the quality of smoked products from these technologies in order to protect human health.

This study therefore aimed to assess the PAH levels in fresh Atlantic chub mackerel (*Scomber colias*) and European barracuda (*Sphyraena*) in Ghana's coastal waters, compare the PAH levels in fish smoked using the AGFS and Cabin kilns to the existing Chorkor kiln using different fuel sources, and determine the possible carcinogenic health risks associated with smoked fish consumption from these kilns.

2. Materials and methods

2.1. Description of fish kilns used

The Chorkor, Cabin and AGFS (Fig. 1) are described in Table 1. The Chorkor kiln is normally built with cement or brick blocks, and has a front loading firepot and trays that are loaded atop the fireplace with no locking mechanism. The Cabin kiln Fig. 1 is constructed from fired bricks, and also, a front loading firepot that is sealed by a metal door. There is a curved metal plate that separates the smoking chamber from the firepot and there is a vent on top of the kiln that serves as a conduit for smoke release. The AGFS is an industrial, double-chamber fish smoking oven built from stainless steel, as described in Nunoo, Tornyeviadzi, Asamoah, and Addo (2019).

2.2. Fish sampling and preparation

A total of 10 kg each of fresh Atlantic chub mackerel (*S. colias*) and European barracuda (*S. sphyraena*) were purchased from fishers at the Sekondi fish landing site in the Western Region of Ghana in December 2017; and January and July 2018. These species were selected because they are commercially important pelagic fish species that are mainly smoked and consumed locally by a large section of the Ghanaian population, and also exported to the EU and the USA (Entee, 2015).

The fishes were weighed, degutted, washed, brined, drained and smoked using the Abuesi Gas Fish Smoker (AGFS), Cabin kiln and Chorkor kiln. Two readily available and common hardwood species used for smoking in coastal communities i.e. 'Afena' (*Strombosia glaucescens*) and 'Esa' (*Celtis mildbraedii*) were chosen as fuel sources in the Cabin and Chorkor smokers.

2.3. Fish smoking process

Fresh mackerel and barracuda measuring between 25.2 cm and 40.7 cm in total length and weighing between 225.46 and 474.67 g respectively were smoked for approximately 4 h using the Cabin (C), Chorkor (Ch) and Gas (G) smoking kilns. The smoking was in two phases: cooking and smoking of the fish for about 2 h and drying of the samples for another 2 h. For the AGFS, pieces of sugarcane were placed in the smoke chamber and lighted during the smoking phase. The AGFS is equipped with a fan that helps with the heat distribution and the drying of the fish. After smoking, the samples were cooled, wrapped in aluminium foil and transported to the laboratory for PAH analyses.

Table 1
Description of fish smoking kilns used in the study.

Kiln	Body dimensions (LxBxH) (cm)	Wall Width (cm)	Firepot dimensions (LxBxH) (cm)	Number of trays	Estimated capacity (kg)
Chorkor (one side)	120 × 150 × 59	13	94 × 78 × 80	8–12	120
Cabin	140 × 138 × 187	12.5	77 × 65 × 65	7	90
AGFS (one side)	120 × 180 × 240	7.5	–	18–25	700

2.4. Analytical methods

2.4.1. Moisture and fat content analyses

The moisture and fat contents analyses were carried out at the Food Research Institute laboratory, Accra using the air-oven method (AOAC, 32.1.03) and Soxhlet method (AOAC 4.5.01) respectively (AOAC, 2005).

2.4.2. PAH analysis

2.4.2.1. Sample preparation. Fish samples were stored at −80 °C prior to analyses at the Ghana Standards Authority laboratory, Accra. Prior to the analysis, the heads and bones of the fish samples were removed. About 200 g of each sample, with the skins on, were then minced thoroughly to achieve sample homogeneity, bagged, labelled, and stored at −20 °C ready for extraction.

2.4.2.2. Reagents, chemicals, solution and standards. The 18-component PAH standard was acquired from Dr. Ehrenstorfer GmbH, Germany. The PAH stock standard solution (10 μg/ml of 18 polyaromatic hydrocarbons) was diluted in acetonitrile (purchased from VWR International, West Chester, PA, USA) to produce a spiking solution of 1 ppm (μg/ml). The spiking solution was used to prepare the 6 points multi-level calibration curve containing concentrations of 5, 10, 20, 50, 100 and 200 ppb.

2.4.2.3. Sample extraction and purification. The PAH analysis was carried out by GC/MS using the Agilent Bond Elut QuEChERS sample preparation (Brondi, De MacEdo, Vicente, & Nogueira, 2011; Aheto et al., 2017) and a High Efficiency DB-5ms Ultra Inert GC column. 3.0 g (±0.05 g) of each minced sample was weighed into a 50 ml centrifuge tube. 12 ml of de-ionized water and 15 ml of acetonitrile (ACN) were added to the sample, which was then macerated for 2 min. The QuEChERS extraction salt containing 6 g $MgSO_4$ and 1.5 g NaCl was then added to the samples in centrifuge tubes. The tubes were capped and vortexed for 1 min at 1500 rpm for liquid-liquid partitioning and then centrifuged for 3 min at 3000 rpm 6 ml of the ACN layer was transferred into a 15 ml centrifuge tube with dSPE clean-up agents containing 150 mg primary secondary amines (PSA), 300 mg C18 and 900 mg $MgSO_4$. The mixture was vortexed for 1 min and then centrifuged for 3 min at 3000 rpm 4 ml of the upper ACN layer was transferred to a 50 ml pear-shaped flask and concentrated to near-dryness using rotary evaporator at below 40 °C. The near-dry extract was then re-dissolved in 1 ml ethyl acetate, and then transferred into a 2 ml autosampler vial, ready for PAH quantification.

2.4.2.4. Gas chromatography-mass spectrometry (GC-MS) analysis. The determination of PAHs in samples was performed by gas chromatography-mass spectrometry (GC-MS), with conditions summarized in Table 2. Sixteen (16) US-EPA priority PAHs were targeted. For quality control (QC), samples were spiked with an appropriate amount of the PAH spiking solution to yield a QC sample with concentration of 50 and 100 ng/ml (ppb). Identification of PAHs in the samples was based on comparison of the retention times with those in a standard solution, and quantification on the corresponding areas of the respective chromatograms (Fig. 2). The limit of detection (LOD) for the various PAHs was 0.1 μg/kg.

Table 2
Summary of chromatographic conditions for PAH.

Apparatus:	
Instrument	Description
Gas Chromatograph	Agilent 7890 B GC with a Agilent Technologies GC sampler 80
Analytical column	30 m + 10 m EZ Guard x 0.25 mm internal diameter fused silica capillary coated with VF-5ms (0.25 μm film) from Agilent or equivalent.
Liner	Agilent 5190–3167:900UL Single Tapered
Mass Spectrometer	Agilent 7000C Triple Quadrupole
Temperatures:	
Item	**Conditions**
Injector	Splitless mode, temperature 280 °C
Oven	Initially set at 70 °C (2 min), increased to 150 °C (at 25 °C/min), then to 200 °C (at 3 °C/min) and finally to 280 °C (at 8 °C/min for 12 min)
MSD Transfer Line	325 °C
Gases:	
Gas	**Flow rate**
Helium (carrier)	2.25 ml/min constant flow
Collision gas (Nitrogen)	1.5 ml/min constant flow
Septum purge	30 mL/min at 0.75min
Pressure	27.5 psi
Mass Spectrometry	
Segment start time	4.0 min
Segment end time	45.0 min
Source Temperature	300 °C
Scan Mode	MRM
Ionization mode	EI

2.5. Human health risk assessment

2.5.1. Toxicological risk associated with PAH concentrations

The observed concentrations of PAHs in the fish samples were compared to regulatory limits and guidelines to determine their toxicity. Individual PAH concentrations, the sum of all measured PAHs concentrations (Σ16 PAHs) and total carcinogenic PAHs i.e. PAH4 were assessed. Also, the ratio of the sum of all lower molecular weight (LMW) PAHs (2–3 rings) to that of higher molecular weight (HMW) PAHs (4–6 rings) were used to assess the source of the PAH in the fresh fish samples, whether petrogenic or pyrogenic (Nyarko, Botwe, & Klubi, 2011).

2.5.2. Carcinogenic risk (CR) assessment

The CR was calculated using concentrations of the seven carcinogenic PAHs (CPAHs) i.e. benzo(*a*)anthracene, chrysene, benzo(*a*)pyrene, benzo(*b*)fluoranthene, indeno (1,2,3-*cd*)pyrene, dibenzo (a,- h) anthracene and benzo(k)fluoranthene. The dietary daily PAH exposure level (E_D) was assessed for the adult population using Eq. (2) and (3) (Xia et al., 2010; Bandowe et al., 2014; Li et al., 2016):

$$E_D = BEC \; x \; IFR_i \tag{2}$$

$$BEC = \sum_{i}^{7} C_i \; x \; PEF_i \tag{3}$$

where C_i is the concentration of PAHs (μg/kg) in the fish tissue; *BEC* is the converted sum of seven carcinogenic PAHs based on the potency equivalency factors (PEFs) of BAPeq (μg/kg) (USEPA, 2013) and *IFR* is the fish ingestion rate in Ghana (g/day). The IFR was calculated, based on survey of one hundred consumers, as 28.6 g/day, 42.9 g/day and 8.7 g/day for fresh and smoked mackerel and barracuda, respectively.

The incremental lifetime cancer risk (ILCR) caused by dietary exposure to PAH from daily intake was then calculated using Eq. (4) (Xia et al., 2010; Li et al., 2016):

$$ILCR = E_D \; x \; EF \; x \; ED \; x \; SF \; x \; CF/BW \; x \; AT \tag{4}$$

where E_D is the daily PAH exposure level (μg/day); *EF* is the exposure frequency of 365 days/year (Bandowe et al., 2014); *ED* is the exposure duration/life expectancy of 63 years (UNDP, 2018); *SF* is the oral cancer slope factor for benz(*a*)pyrene [(with geographic mean of 7.3 mg/kg/day)$^{-1}$]; *CF* is the conversion factor (10^{-6} kg/mg) (Li et al., 2016); *BW* is the adult body weight of 64.5 kg (WorldData.info, 2019) and *AT* is the average lifespan for carcinogens (365 days/year × exposure duration).

2.5.3. Non-carcinogenic risk (non-CR) estimates

The non-CR estimates were based on the Hazard Index (HI) relating to eight non-carcinogenic PAHs (naphthalene, acenaphthalene, acenaphthene, fluorene, phenanthrene, anthracene, pyrene and fluoranthene). The Hazard Index (HI) was calculated using Eq. (5) (Li et al., 2016).

$$HI = \sum_{i}^{8} C_i \; x \; IFR \; x \; CF \; x \; ED \; x \; EF \Big/ BW \; x \; AT \; x \; RfDo \tag{5}$$

where *RfDo* is the oral ingestion reference dose (mg/kg/day)$^{-1}$.

2.6. Data analysis

Statistical analysis was performed using XLSTAT (Addinsoft, New York, USA). Comparisons of means of moisture and fat contents, across kilns and species were tested by two-way ANOVA followed by Tukey's HSD test at the $p < 0.05$ significance level. All PAH concentrations were expressed as μg/kg wet weight, respectively. For statistical purposes, individual PAH concentrations that were below the LOD of 0.1 μg/kg were assumed to be half of the respective LOD (Bandowe et al., 2014). Normality of the PAH data was assessed using the Shapiro-Wilk test. The data were not normally distributed, even after log transformation; therefore, only nonparametric tests were used. The Kruskal-Wallis with Dunn's multiple comparison tests were performed to detect significant differences between raw and smoked samples from the different kilns. Results are presented as mean ± standard deviation.

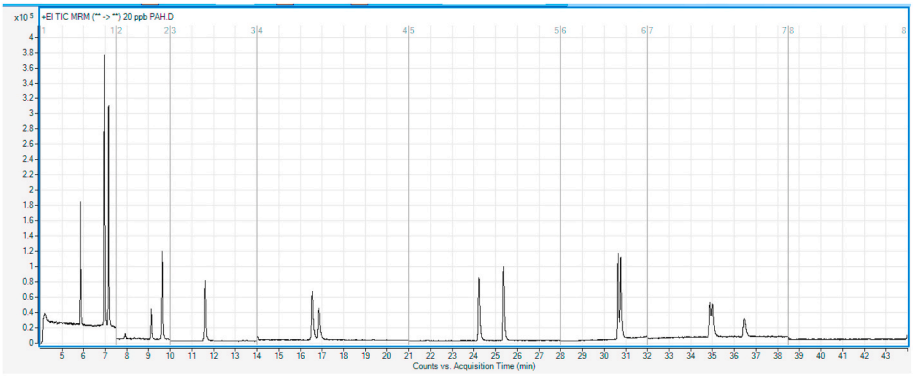
3. Results and discussion

3.1. Quality of fresh and smoked fish

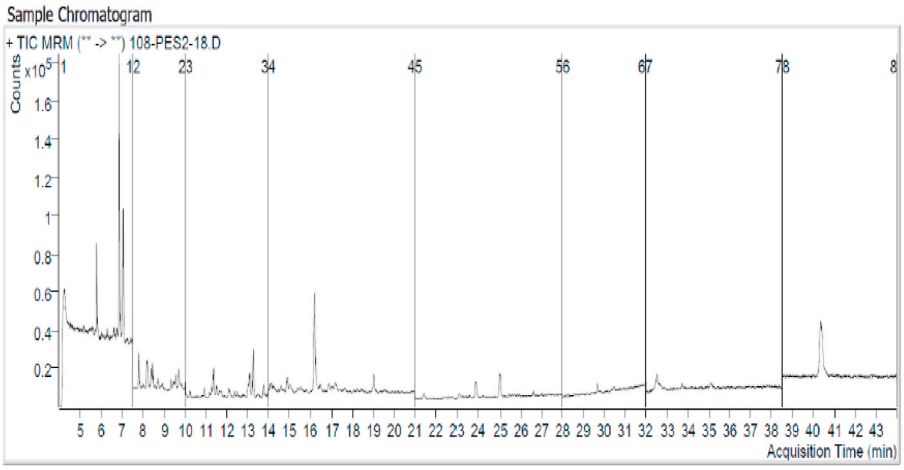
The fresh mackerel (FM) had moisture and fat contents of 71.31 ± 2.24 and 7.12 ± 3.93 g/100 g, respectively. The fresh barracuda (FB) on the other hand had moisture and fat contents of 75.52 ± 0.94 and 2.32 ± 1.79 g/100 g, respectively. With respect to the moisture contents, the smoked samples had significantly lower ($p < 0.05$) levels (between 27.19 and 35.70 g/100 for FM and 43.14–54.11 g/100 g for FB) than in the fresh samples. This could inhibit microbial growth, and thus improve the shelf life of the smoked products, while at the same time maintaining their sensory properties (Cardinal et al., 2004). The fat contents were higher in the smoked samples (6.16–21.53 g/100 g) but only the GSM and GSB (9.66 and 21.53 g/100 g respectively) were significantly higher ($p < 0.05$) compared to the fresh samples. The higher fat content, resulting from dehydration, increases the energy value of the fish, though it can easily cause lipid oxidation (Arason et al., 2014).

3.2. PAHs levels in fresh fish

The types and concentrations of PAHs in FM and FB are presented in Tables 3 and 4. The total PAH in FM ranged between 0.87 and 23.72 μg/kg, with a mean of 11.27 ± 10.98 μg/kg (Table 3). For individual PAH compounds, the concentrations ranged from below detection limits of 0.1 μg/kg (i.e. for chrysene and benzo(*b*)fluoranthene) to 2.5 μg/kg (i.e. for anthracene) in FM. The sum of four carcinogenic PAHs (PAH4) was 0.40 ± 0.45 μg/kg. Likewise, FB had total PAH concentrations ranging from 0.80 to 30.95 μg/kg, with a mean of 13.56 ± 12.58 μg/kg (Table 4). Chrysene, benzo(*a*)pyrene and benzo(*b*)fluoranthene were below detection limits, with phenanthrene having the highest concentration.



(a)



(b)

Fig. 2. Chromatograms showing PAH standard; 16 USEPA PAH mix (a) and smoked mackerel (b)
1 = Naphthalene; 2 = Acenaphthene, Acenaphthalene, Fluorene; 3 = Anthracene, Phenanthrene; 4 = Fluoranthene, Pyrene; 5 = Benzo(*a*)anthracene, Chrysene; 6 = Benzo(*a*)pyrene, Benzo(*b*)fluoranthene, Benzo(k)fluoranthene; Benzo (g,h,i)perylene, Indeno (1,2,3-*cd*)pyrene, Dibenzo (a,-h)anthracene.

Table 3
Concentrations (μg/kg wet weight) of individual PAHs in fresh (F), gas smoked (GS) and Afena (A) and Esa (E) Cabin smoked (CS) and Chorkor smoked (ChS) mackerel (M).

PAH	Fresh (n = 5)	Smoked (n = 6)				
		Chorkor		Cabin		AGFS
	FM	A-ChSM	E-ChSM	A-CSM	E-CSM	GSM
Naphthalene	1.69 ± 2.32	28.95 ± 15.60	26.91 ± 21.22	28.95 ± 22.13	23.26 ± 21.23	15.17 ± 12.59
Acenaphthalene	0.22 ± 0.21	23.27 ± 18.56	17.99 ± 26.53	15.68 ± 23.00	15.33 ± 21.75	0.95 ± 1.17
Acenaphthene	0.14 ± 0.20	1.32 ± 1.22	1.58 ± 1.92	1.37 ± 1.32	5.14 ± 7.09	0.17 ± 0.19
Fluorene	1.81 ± 2.17	34.77 ± 19.18	23.29 ± 25.09	20.99 ± 16.22	23.11 ± 20.98	2.71 ± 3.71
Phenanthrene	1.97 ± 3.14	38.26 ± 17.94	27.75 ± 26.06	45.15 ± 27.07	53.24 ± 54.73	1.22 ± 1.49
Anthracene	2.50 ± 5.34	131.08 ± 120.79	81.99 ± 97.77	61.58 ± 81.32	108.13 ± 98.85	1.09 ± 2.46
Fluoranthene	0.06 ± 0.02	73.76 ± 48.45	49.59 ± 52.10	39.76 ± 42.72	50.87 ± 46.99	3.83 ± 4.74
Pyrene	0.06 ± 0.02	73.8O±48.40	49.44 ± 52.20	39.81 ± 42.67	50.87 ± 46.99	3.83 ± 4.74
*Benzo(*a*)anthracene	0.10 ± 0.11	27.55 ± 20.17	22.68 ± 19.46	18.56 ± 18.83	39.15 ± 13.26	0.81 ± 1.15
*Chrysene	ND	40.30 ± 34.59	25.59 ± 25.39	16.46 ± 22.33	24.01 ± 26.54	1.01 ± 1.26
*Benzo(*b*)fluoranthene	ND	38.25 ± 48.13	11.33 ± 19.18	11.11 ± 20.61	1.42 ± 2.16	ND
Benzo(k)fluoranthene	0.25 ± 0.36	27.38 ± 19.85	17.17 ± 19.63	10.60 ± 9.51	9.09 ± 10.93	0.53 ± 0.42
*Benzo(*a*)pyrene	0.20 ± 0.34	15.51 ± 16.63	5.68 ± 7.36	3.59 ± 3.24	1.29 ± 1.67	0.66 ± 0.61
Indeno (1,2,3-*cd*)pyrene	0.66 ± 1.36	11.82 ± 10.24	4.90 ± 8.47	5.30 ± 6.49	0.32 ± 0.54	2.53 ± 3.89
Dibenzo (a,-h)anthracene	0.78 ± 1.63	11.61 ± 10.34	4.79 ± 8.32	4.61 ± 5.63	0.33 ± 0.42	2.17 ± 3.32
Benzo (g,h,i)perylene	0.72 ± 1.50	11.98 ± 9.90	4.78 ± 8.40	4.93 ± 5.97	ND	2.42 ± 3.68
∑PAHs	**11.27 ± 10.98**	**589.59 ± 378.34**	**375.42 ± 347.09**	**328.42 ± 231.71**	**405.58 ± 273.52**	**39.13 ± 27.74**
∑PAH4	**0.40 ± 0.45**	**121.60 ± 98.88**	**65.27 ± 65.78**	**49.71 ± 45.56**	**65.87 ± 35.09**	**2.52 ± 2.55**

Table 4
Concentrations (μg/kg wet weight) of individual PAHs in fresh (F), gas smoked (GS) and Afena (A) and Esa (E) Cabin smoked (CS) barracuda (B) (n = 5).

PAH	Fresh	Smoked		
	FB	A-CSB	E-CSB	GSB
Naphthalene	2.67 ± 2.53	12.36 ± 10.54	17.50 ± 10.41	6.61 ± 2.63
Acenaphthalene	0.72 ± 0.70	26.51 ± 32.84	17.51 ± 11.31	0.44 ± 0.41
Acenaphthene	0.92 ± 1.26	11.59 ± 24.37	0.08 ± 0.07	0.17 ± 0.17
Fluorene	1.65 ± 2.91	17.07 ± 29.47	20.16 ± 12.33	2.05 ± 2.08
Phenanthrene	3.04 ± 4.52	14.52 ± 19.17	30.87 ± 22.93	14.96 ± 19.40
Anthracene	2.19 ± 4.54	32.16 ± 53.51	75.30 ± 64.80	9.80 ± 13.69
Fluoranthene	0.11 ± 0.08	34.00 ± 51.14	58.25 ± 40.24	2.29 ± 1.63
Pyrene	0.11 ± 0.08	33.99 ± 51.14	58.25 ± 40.22	2.29 ± 1.63
[a]Benzo(*a*)anthracene	0.30 ± 0.56	13.48 ± 11.14	29.11 ± 16.47	0.44 ± 0.55
[a]Chrysene	ND	13.47 ± 12.25	35.73 ± 22.78	0.47 ± 0.66
[a]Benzo(*b*) fluoranthene	ND	4.78 ± 7.01	1.83 ± 3.23	0.35 ± 0.66
Benzo(k) fluoranthene	0.30 ± 0.48	3.68 ± 3.04	8.41 ± 10.63	0.21 ± 0.24
[a]Benzo(*a*)pyrene	ND	2.57 ± 3.54	1.32 ± 2.09	0.32 ± 0.28
Indeno (1,2,3-*cd*) pyrene	0.45 ± 0.76	3.10 ± 5.24	2.54 ± 5.57	3.56 ± 3.86
Dibenzo (a,- h) anthracene	0.53 ± 0.89	4.76 ± 6.46	2.18 ± 4.76	2.02 ± 2.04
Benzo (g,h,i) perylene	0.42 ± 0.83	5.14 ± 6.98	2.34 ± 5.12	2.56 ± 2.49
∑PAHs	**13.56 ± 12.58**	**233.19 ± 290.49**	**361.39 ± 155.58**	**48.53 ± 34.86**
∑PAH4	**0.45 ± 0.56**	**34.30 ± 30.51**	**67.99 ± 32.63**	**1.57 ± 1.44**

[a] Denotes PAHs used in ∑PAH4 calculation; ND denotes not detected (i.e. below the detection limit of 0.1 μg/kg); ∑ denotes total PAH concentration derived from the sum of individual mass concentrations of all 16 PAH congeners measured; ∑PAH4 denotes sum of benzo(*a*)anthracene, chrysene, benzo(*a*) pyrene and benzo(*b*)fluoranthene. 'Afena' (*Strombosia glaucescens*); 'Esa' (*Celtis mildbraedii*).

The PAH4 for FB averaged 0.45 ± 0.56 μg/kg.

The PAH concentrations in the fresh mackerel and barracuda were all low, indicating that these fishes accumulate low levels of PAHs in the marine environment, as suggested by Stołyhwo and Sikorski (2005). The maximum levels of benzo(*a*)pyrene and PAH4 concentrations in muscle of fish other than smoked fish are used as indicators of potential environmental pollution (European Commission, 2006). However, the current EU regulations has established that PAHs are rapidly oxidized and metabolized in fresh fish and hence do not accumulate in the muscles, thus no maximum level is prescribed (European Commission, 2011).

The lower molecular weight (LMW) PAHs accounted for 74% and 83% of the total PAHs in FM and FB, respectively. The ∑LMW-PAH/∑HMW-PAH ratios were 2.96 and 5.21 for FM and FB respectively, implying that the source of PAH was petrogenic (∑LMW/∑HMW > 1.0). This could be due to the higher solubility of LMW-PAHs in water, which makes them more bioavailable, and the ease of metabolism and removal of higher molecular weight PAHs (Bandowe et al., 2014; European Commission, 2011). Again, the site is within the main fishing harbour and hence its close proximity to vehicular activities, fuel discharge points, among other activities, could have caused petrogenic PAH contamination of the fish (Essumang et al., 2012).

3.3. PAH levels in smoked fish samples

Smoking caused an increase in the concentrations of the individual PAHs with the exception of acenaphthene which was significantly lower ($p < 0.05$) in gas smoked mackerel (GSM) compared to FM (Table 3). The mean concentrations of individual PAHs in smoked mackerel ranged from below detection limit (benzo (g,h,i)perylene and benzo(*b*)fluoranthene) to 131.08 μg/kg (i.e. anthracene). Naphtalaene, acenaphthalene, fluorene, fluoranthene, pyrene and chrysene were significantly higher ($p < 0.05$) in Chorkor-smoked mackerel using 'Afena' wood (A-ChSM) compared to FM. Again, the concentrations of fluorene, phenanthrene, anthracene, benzo(*a*)anthracene were also significantly lower ($p < 0.05$) in FM compared to 'Esa' Cabin smoked mackerel (E-CSM) and 'Afena'-Cabin smoked mackerel A-CSM. Fluoranthene and benzo (g,h,i) perylene concentrations were significantly different ($p < 0.05$) between 'Esa' Chorkor-smoked mackerel (E-ChSM) and E-CSM and A-ChSM and E-CSM, respectively. GSM samples significantly differed from A-ChSM, E-CSM, A-CSM with respect to fluorene, phenanthrene, anthracene and benzo(*b*)fluoranthene concentrations. There were no significant differences ($p > 0.05$) in indeno (1,2,3-*cd*)pyrene, dibenzo (a,- h)anthracene and benzo(k)fluoranthene between all the smoked samples and FM. The concentration of benzo(*a*)pyrene ranged from 0.66 μg/kg in GSM to 15.51 μg/kg in A-ChSM. The Chorkor smoked samples generally had higher benzo(*a*)pyrene concentrations than the Cabin smoked samples, irrespective of the firewood type used. There were however no significant differences ($p > 0.05$) in PAH concentrations between the samples smoked with the different kilns and also FM.

The mean total PAH concentrations in smoked mackerel ranged from 39.13 μg/kg in GSM to 589.59 μg/kg in A-ChSM (Table 3). The total concentrations in FM and GSM were significantly lower ($p < 0.05$) than in E-CSM and A-ChSM. The PAH4 concentrations also ranged from 2.52 to 121.60 μg/kg in GSM and A-ChSM, respectively. PAH4 was significantly lower ($p < 0.05$) in FM and GSM with respect to E-CSM and A-ChSM. The A-CSM had about 80% less total PAHs compared to the A-ChSM, whereas the E-ChSM had 8% less than the E-CSM (the main contributor being anthracene in the E-CSM). The GSM had on average, 91% and 89% less PAH than the Chorkor and Cabin smoked mackerel, respectively. Also, the GSB had 87% lower PAH than the Cabin smoked barracuda. The LMW PAHs accounted for between 53% and 56% of the total PAHs of A-CSM, GSM and E-CSM and 44%–48% in A-ChSM and E-ChSM.

The individual PAH concentrations in smoked barracuda ranged from a mean of 0.08–75.30 μg/kg for acenaphthene and anthracene, respectively (Table 4). There were no significant differences ($p < 0.05$) in individual PAH concentrations between the smoked barracuda samples from the different kilns and also the FB for naphtalaene, acenaphthalene, acenaphthene, benzo(*b*)fluoranthene, benzo(k)fluoranthene, indeno (1,2,3-*cd*)pyrene, dibenzo (a,- h)anthracene and benzo (g,h,i) perylene. The concentrations of fluoranthene, phenanthrene, anthracene, fluorene, pyrene, and chrysene were significantly higher ($p < 0.05$) in E-CSB compared to FB. Benzo(*a*)anthracene had a significantly higher ($p < 0.05$) concentration in E-CSB compared to FB and GSB. Benzo(*a*)pyrene concentrations were 0.32, 1.32 and 2.57 μg/kg in GSB, E-CSB and A-CSB, respectively. The mean total PAH concentrations ranged from 48.53 to 361.39 μg/kg in GSB and E-CSB respectively. The Cabin smoked samples (A-CSB and E-CSB) recorded total PAH concentrations that were significantly higher ($p < 0.05$) than in the FB. Only E-CSB had significantly higher ($p < 0.05$) PAH4 concentration than FB. The HMW PAHs contributed 51% and 55% to the total PAHs in CSB and 30% to the total PAHs in GSB.

The total PAH content in the smoked mackerel and barracuda, based on fuel type was of the order Gas < 'Afena (*C. mildbraedii*) < 'Esa' (*S. glaucescens*) for Gas-Chorkor and Gas < *S. glaucescens* < *C. mildbraedii* in Gas-Cabin combinations. The gas-smoked fish samples had less PAHs than the Chorkor and Cabin smoked fish samples. This implies that in terms of PAH content, the gas kiln had a better product than the other

two kilns. The high PAH content in wood-smoked fish could be a result of high lignin content of both *C. mildbraedii* and *S. glaucescens* (25% and 34.5% respectively) (Brauns & Brauns, 2013), which caused them to burn hot (averagely between 345.9 and 465.8 °C) (Essumang, Dodoo, & Adjei, 2013). Again, the high PAH content in smoked mackerel compared to barracuda might be related to the high fat content in the mackerel samples, which has also been observed by several authors (Codex Alimentarius Commission CAC, 2009; Bandowe et al., 2014; Essumang et al., 2013, 2014; Aheto et al., 2017).

With respect to kiln type, the levels were of the order AGFS < Cabin < Chorkor. The Cabin kiln produced mackerel having lower total PAH than the Chorkor kiln when *S. glaucescens* was used. When *C. mildbraedii* was, however, used for the smoking, the Chorkor kiln had a lower total PAH than the Cabin kiln and this was as a result of the higher percentage of lower molecular weight PAHs in the E-CSM compared to the E-ChSM. The high levels of PAH obtained in the Chorkor and Cabin kilns could be due to the stacking of several trays of fish and covering the topmost tray in the former, and the closed nature of the latter. These could lead to a buildup of smoke around the fish (Bomfeh et al., 2019). Again, the high levels could be as a result of testing for PAHs from a mince of both the skin and muscles in the fish samples tested. This was because both species are usually consumed in Ghana with skin on. Stołyhwo and Sikorski (2005) in their study reported that the traditional kilns mostly produce fish that are heavily smoked, with benzo(*a*)pyrene concentrations up to about 50 μg/kg, especially on their surfaces. Essumang et al. (2013) found higher levels of total PAHs in Chorkor kiln-smoked sardine, cigar minnows, tuna and mackerel using three different fuel sources (acacia, sugarcane bagasse and mangrove). Aheto et al. (2017) also found higher levels (ranging between 1156.60 and 4443.40 μg/kg) of total PAHs in market samples of sardines, mackerel and anchoives. Their study however did not differentiate between the types of fuel wood and kiln used. Another study by Essumang et al. (2014) using modified traditional kilns with or without activated charcoal filters found an average of more than 40% reduction in the PAH content of fish smoked with filters, which was less than concentrations obtained in the AGFS in the present study.

The maximum limits (MLs) of benzo(*a*)pyrene and PAH4 in the muscle meat of smoked fish have been set by the EU at 2 and 12 μg/kg (European Commission, 2011). The lowest concentration of benzo(*a*)pyrene in this study was measured in GSB, with the highest in A-ChSM. From the results, benzo(*a*)pyrene levels in the gas-smoked and 'Esa' Cabin-smoked samples were all below the EU recommended MLs. The A-CSB, A-CSM, E-ChSM and A-ChSM were 1.3, 1.8, 2.8, times 7.8 times higher than the recommended limits. With respect to the estimated PAH4, all the gas-smoked samples met the EU ML criterion. The Cabin smoked mackerel and barracuda were 4.1, 5.5, 2.9 and 5.7 times higher (for A-CSM, E-CSM, A-CSB and E-CSB respectively). The chrysene and benzo(*a*)anthracene concentrations contributed the most to these results. The Chorkor smoked mackerel 5.4 and 10.1 times higher (for E-ChSM and A-ChSM respectively).

Several studies have compared the PAHs in smoked fish using different technologies in Ghana and beyond. IRI-CSIR and Kwarteng (2016) compared the PAH concentrations of smoked fish from two traditional smoking kilns (i.e. the Chorkor, and Morrison) to the Ahotor (an improved kiln) in Ghana. The Chorkor, Morrison and Ahotor recorded benzo(*a*)pyrene and PAH4 concentrations of 22 and 84 μg/kg; 30 and 110 μg/kg and 5.9 and 53.1 μg/kg, respectively. Aidoo (2017) compared the PAH in mackerel (whole and fillet) from two fish smoking kilns (Cabin and Bradley) in Iceland. The PAH and PAH4 concentrations were 2.8 and 25.85 μg/kg in the smoked fillets and 1.05 and 8.3 μg/kg in the whole smoked mackerel from the Cabin kiln. The Bradley kiln gave concentrations below the EU MLs. These estimates were lower than in the present study and this could be attributed to the type of wood used and the shorter length of time used for smoking.

Essumang et al. (2013) reported benzo(*a*)pyrene and PAH4 concentrations of 41.27 and 452.33; 1.26 and 169.4 and 15.22 and 225.75 μg/kg respectively in acacia, sugarcane bagasse and mangrove smoked mackerel from the Chorkor smoker in Ghana. This supported the fact that different firewood sources influenced the level of PAH in smoked fish. The authors also concluded that the elevated levels of benzo(*a*)pyrene and PAH4 in smoked mackerel may have been due, in part to its high lipid contents.

Bomfeh et al. (2019) also compared barracuda smoked with the FAO FTT, Chorkor and metal drum kilns. Soft and dry-smoked barracuda recorded benzo(*a*)pyrene concentrations of 0.6, 50.3 and 37.4 μg/kg and 1.8, 61.1 and 69.8 μg/kg respectively in FTT, Chorkor and metal drum smoked fish. These results were however from the use of different sources of fuel. The FTT used charcoal for cooking and sugarcane bagasse for smoke flavouring, while the Chorkor and metal drum used firewood. Also, the FTT represented indirect smoking while the other two were direct smoking methods and this could account for the different levels of PAH in fish products. This was comparable to results from the present study where the gas-smoked barracuda had lower PAH concentrations compared to the Cabin smoked ones that used firewood. Another comparision was made between the FTT and the Chorkor smoker using the same types of fuel for smoking sardines. The FTT recorded benzo(*a*)pyrene and PAH4 concentrations of 0.2 and 1.5; 1.9 and 37.0 and 7.7 and 28.9 μg/kg for charcoal, *Pterocarpus erinaceus* and *Azadirachta indica* respectively. The Chorkor smoked fish recorded benzo(*a*)pyrene and PAH4 concentrations that were 51 and 26; 32 and 10 and 8 and 7 times higher than the FTT using charcoal, *Pterocarpus erinaceus* and *Azadirachta indica* respectively as fuels. This also indicates that the type of kiln and fuel used can have an impact on the PAH outcomes, which agreed with the findings from the Cabin and Chorkor kilns smoked mackerel using the *S. glaucescens* and *C. mildbraedii* wood for smoking in this study.

Another study by Diei-Ouadi (2013) to test different smoking kilns and fuel sources found high levels of benzo(*a*)pyrene and PAH4 (4.6 and 7.2 μg/kg) in smoked fish when the FTT was used with LPG gas as the fuel source. This was higher than findings from the recent study, implying that the gas smoker used here performed better than the FTT, when LPG gas was used.

An advantage of the AGFS over the Chorkor, Cabin, FTT and Ahotor kilns, among other traditional fish smoking kilns in Ghana is that it does not rely on firewood or charcoal, derived from burning firewood, as source of fuel. This implies deforestation and its attendant problems could be largely eliminated in the fish smoking industry.

3.4. Human health risk assessment and dietary exposure of PAHs

3.4.1. Carcinogenic health risk assessment

The carcinogenic risks associated with the consumption of fresh and smoked mackerel and barracuda are presented in Table 5. It was estimated that an adult Ghanaian (with a life expectancy of 63 years) would consume 28.6, 42.9 and 8.7 g/day of fresh mackerel, smoked mackerel and barracuda respectively. The carcinogenic risk associated with the consumption of fresh mackerel and barracuda were therefore estimated to be between 3.44×10^{-6} and 6.50×10^{-6}. This implied that an estimated 3 in 1,000,000 and 7 in 10,000,000 adults respectively were potentially at risk of suffering from cancer in their lifetime. The gas smoked mackerel and barracuda estimated 2 in 100,000 and 3 in 1,000,000 adults were likely to suffer from cancer. Likewise, Cabin smoked fish had estimates between 3 and 6 in 100,000 whereas Chorkor smoked mackerel fell between 7 per 100,000 and 2 per 10,000 adults (for *C. mildbraedii* and *S. glaucescens* respectively in each kiln). The US-EPA has estimated a 1 in 1,000,000 (ILCR of 1×10^{-6}) chance of additional human cancer given a 70-year exposure time (USEPA, 2004). This level is considered acceptable or inconsequential and compares with risks resulting from 'normal' human activities like diagnostic x-rays, and fishing, among others (Xia et al., 2010). A level of risk of 1 in 100,000 (ILCR of 1×10^{-5}) is considered the carcinogenesis threshold, with 1 in 10,000 or greater (ILCR $\geq 1 \times 10^{-4}$) deemed serious and

Table 5
Estimated carcinogenic risks associated with the consumption of fresh (F), gas smoked (GS) and Afena (A) and Esa (E) Cabin smoked (CS) and Chorkor smoked (ChS) mackerel (M) and barracuda (B).

Carcinogenic equivalency	Mackerel						Barracuda (n = 5)			
	FM (n = 5)	Chorkor (n = 6)		Cabin (n = 6)		GSM (n = 6)	FB	Cabin		GSB
		A-ChSM	E-ChSM	A-CSM	E-CSM			A-CSB	E-CSB	
Benzo(*a*)anthracene	0.01	2.75	0.08	1.86	3.92	0.08	0.03	1.35	2.91	0.04
Chrysene	0.00	0.04	0.00	0.02	0.02	0.00	0.00	0.01	0.04	0.00
Benzo(*b*)fluoranthene	0.01	3.82	0.01	1.11	0.14	0.01	0.01	0.48	0.18	0.03
Benzo(k)fluoranthene	0.00	0.27	0.01	0.11	0.09	0.01	0.00	0.04	0.08	0.00
Benzo(*a*)pyrene	0.20	15.51	0.66	3.59	1.29	0.66	0.05	2.57	1.32	0.32
Indeno (1,2,3-*cd*)pyrene	0.07	1.18	0.25	0.53	0.03	0.25	0.05	0.31	0.25	0.36
Dibenzo (a,- h)anthracene	0.78	11.61	2.17	4.61	0.33	2.17	0.53	4.76	2.18	2.02
BEC (μg/kg)	1.06	35.19	3.17	11.81	5.82	3.17	0.66	9.52	6.97	2.78
E_D (μg/day)	30.39	1508.21	623.63	506.32	249.29	135.86	5.75	82.53	60.37	24.07
Carcinogenic risk	3.44E-06	1.71E-04	7.06E-05	5.73E-05	2.82E-05	1.54E-05	6.50E-07	9.34E-06	6.83E-06	2.72E-06

BEC denotes the converted sum of seven carcinogenic PAHs based on the potency equivalency factors (PEFs) of BAPeq (μg/kg); E_D denotes the daily PAH exposure level (μg/day).

therefore requiring attention (USEPA, 2004; Xia et al., 2010; Essumang et al., 2013). From the results, fresh mackerel and all barracuda samples were within the acceptable limits and may therefore pose a very low risk. All the smoked mackerel samples were above the limits, with the gas smoked being lowest, followed by the Cabin smoked and the Chorkor smoked the highest potential risks. Based on the risks with respect to the fuel type, it could be inferred that LPG was most suitable, followed by *C. mildbraedii* with *S. glaucescens* being very unfavorable, especially when used in the Chorkor kiln.

Bandowe et al. (2014) estimated risks of magnitude between 7×10^{-7} to 4×10^{-4} in fresh *Cynoglossus senegalensis*, *Pomadasys peroteti* and *Drepane africana* from different coastal marine areas in Ghana. A report by Essumang et al. (2013) found 5, 15 and 29 out of 100,000 adults were at risk of developing cancer in their lifetime from consuming Chorkor smoked mackerel using sugarcane bagasse, mangrove, and acacia wood in Ghana. Other smoked fish samples (tuna, sardines, and cigar minnow) assessed posed low to moderate risks. The authors concluded that the use of sugarcane bagasse was more suitable for fish smoking compared to the use of hardwood.

3.4.2. Non-carcinogenic risk assessment

The hazard indices estimated for fresh mackerel and barracuda were 1.12×10^{-4} and 4.38×10^{-5} respectively. That for smoked mackerel were 5.86×10^{-4}, 6.12×10^{-3}, 4.29×10^{-3}, 5.38×10^{-3} and 6.08×10^{-3} for GSM, E-CSM, A-CSM, E-ChSM and A-ChSM, respectively. Smoked barracuda recorded estimates of 1.64×10^{-4}, 1.13×10^{-3} and 6.08×10^{-3} for GSB, E-CSB and A-CSB, respectively.

The non-carcinogenic risks (hazard index, HI) from the results were all less than one. It could therefore be inferred that consumers that eat the smoked products were unlikely to experience non-carcinogenic effects, as was also reported in a study by Li et al. (2016).

4. Conclusions

The smoking process, while aiding in the preservation of fish, also produces some carcinogenic substances such as PAHs in the processed fish. The fresh mackerel and barracuda were of good quality for smoking and posed minimal carcinogenic risks to consumers. With respect to the kilns, AGFS performed best, by producing smoked products with benzo(*a*)pyrene and PAH4 concentrations below the EU MLs (2 and 12 μg/kg respectively). The Cabin kiln also produced smoked mackerel with 77% and 59% lower benzo(*a*)pyrene and PAH4 (only in *C. mildbraedii* smoked fish) than the Chorkor. The levels of benzo(*a*)pyrene and PAH4 were however greater than the EU's MLs in all Chorkor and Cabin smoked samples (except for benzo(*a*)pyrene in E-CSM and E-CSB). Based on the frequency and quantities of smoked mackerel and barracuda consumed by an average Ghanaian adult (with a life expectancy of 63 years), the potential carcinogenic risks were of least concern in the gas smoked and all barracuda samples, moderate in the Cabin kiln smoked mackerel and high in the Chorkor kiln smoked mackerel. It could therefore be inferred that the presence of PAHs in the smoked fish was due to the type of kiln, smoking method (direct and indirect) and fuel used (LPG and firewood) for the treatment. Again, the magnitude of the carcinogenic risks depended largely on the fish ingestion rate, with higher benzo(*a*)pyrene and PAH4 levels not always corresponding to increased risks (as shown in the barracuda).

CRediT authorship contribution statement

Eunice Konadu Asamoah: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing - original draft, Project administration, Visualization, Funding acquisition. **Francis Kofi Ewusie Nunoo:** Conceptualization, Methodology, Writing - review & editing, Supervision, Project administration, Funding acquisition. **Samuel Addo:** Conceptualization, Writing - review & editing, Supervision, Funding acquisition. **Josephine O. Nyarko:** Methodology, Investigation. **Grethe Hyldig:** Resources, Writing - review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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