academicJournals

Vol. 9(3) pp. 126-135, March, 2015
DOI: 10.5897/AJFS2014.1227
Article Number: FBD534550813
ISSN 1996-0794
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African Journal of Food Science

Full Length Research Paper

Influence of fish smoking methods on polycyclic aromatic hydrocarbons content and possible risks to human health

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Received 3 November, 2014; Accepted 16 February, 2015

Polycyclic aromatic hydrocarbons (PAHs) are widespread environmental contaminants representing an important group of carcinogens that have been detected in smoked fish. This work investigated the effect of fish smoking methods on dietary exposure to PAHs and potential risks to human health. The smoking methods considered accounted for differences in smoked catfish/solefish content of 16 PAHs. The results revealed traditional method of smoking had 7 genotoxic PAHs. Traditionally smoked catfish/solefish were 18 - 24 times higher than those measured by modern method. Risk assessment conducted using benzo[a]pyrene carcinogenic and mutagenic toxicity equivalency factors (TEF and MEF, respectively) showed low risk (2.01×10^{-8} - 2.86×10^{-8} and 1.09×10^{-8} - 1.83×10^{-8}, respectively for carcinogenicity and mutagenicity) associated with consuming smoked catfish/solefish and below the USEPA guideline (1.0×10^{-5}) for potential cancer risk. Mean hazard indexes were below 1 (below an acceptable cumulative threshold) ranging from 1.43×10^{-6} - 9.96×10^{-8}. A significantly high accumulation of PAHs was found in the smoked fish as compared to the non-smoked fish control samples. This study indicates that there is no adverse health effect of PAHs content on consumers of smoked fish species but levels of PAHs present in smoked catfish/solefish prepared using traditional methods may pose elevated cancer risks if consumed at high consumption rates over many years.

Key words: Smoked fish, polycyclic aromatic hydrocarbons, mutagenic, carcinogenic, human health, hazard index.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) compounds are groups of potent carcinogens that are present in the environment; traces of these substances have been found in various food products (Guillen and Sopelana,

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2003). PAHs are formed by incomplete combustion processes which occur whenever wood, coal or oil are burnt. The possible sources of PAHs in food are environmental contamination, as well as thermal treatment of varying severity which is used in the preparation and manufacturing of foods (Guillen, 1994), (Guillen, 1994), the absorption and deposition of particulates during food processing such as smoking, grilling, boiling and toasting, the pyrolysis of fats and the incomplete combustion of charcoal (Larsson et al., 1983; Guillen, 1994; Moret et al., 1997). Regarding food of animal origin, one hypothesis suggests that the lipophilic character of PAHs is responsible for the accumulation in the fat of animals which eat contaminated plants (Guillen et al., 1997). PAHs occur as contaminants in different food categories and beverages including water (Belykh et al., 1999), fruit, cereals, oils (Dennis et al., 1983, 1991; Moret and Conte, 2002), smoked meat (Potthast, 1977; Simko, 2002) and smoked fish (Simko, 1991; Akpan et al., 1994; Lodovici et al., 1995; Moret et al., 1999). Non-processed fish contains low PAHs concentration even when it comes from contaminated water because fishes rapidly metabolize PAHs, resulting in low steady-state level in the tissue (Moret et al., 2000; Chen and Chen, 2005; Wretling et al., 2010; Essumang et al., 2013). The health effects resulting from PAH exposure have recently been discussed extensively in the literature (Shen et al., 2008). These include growth retardation, low birth weight, small head circumference, low IQ, damaged DNA in unborn children and the disruption of endocrine systems, such as estrogens, thyroid and steroids (Essumang et al., 2012).

Skin changes (thickening, darkening and pimples) and reproductive-related effects such as early menopause due to destruction of ova have also been identified with PAHs (Essumang et al., 2011, 2012). It is known that in mammalian cells, PAHs undergo metabolic activation to diol, and epoxides that bind covalently to cellular macromolecules, including DNA, thereby causing errors in DNA replication and mutations that initiate the carcinogenic process (Rodriguez et al., 1997; Schoket, 1999; Lightfoot et al., 2000; Essumang et al., 2012, 2013). Polymorphisms causing glutathione transferase deficiencies (GSTM1) may result in elevated breast cancer, lung cancer and other forms of human cancer risk from PAHs (IARC, 1999; Van der Hel et al., 2003). Because of their mutagenic and carcinogenic effects, PAHs have been included in several priority pollutant lists of the Agency of Toxic Substances and Disease Register (ATSDR), the International Agency for Research on Cancer (IARC), the European Community (EC) and the Environmental Protection Agency (USEPA). Several studies have been carried out to determine the levels of exposure of humans to PAHs (De Vos, 1990).

Smoking is one of the oldest food preservation technologies and can be used to achieve the characteristic taste, colour and aroma for food (especially meat and meat products, fish and fish products) (Djinovic et al., 2008). In Europe, about 15% of the total quantity of fish for human consumption is smoked prior to release to the market (Stolyhwo and Sikorski, 2005). However, foods are nowadays smoked for sensory quality rather than for the preservative effect. Yanar et al. (2006) reported that the acceptance of smoked fish in developed countries is based primarily on the sensory characteristics it imparts to the product while Akintola et al. (2013) confirmed the nutritional qualities and adequacies. In addition to this, smoking enhances preservation due to the dehydrating bactericidal and antioxidant properties of smoke such as phenol derivates, carbonyls, furan derivates, organic acids and their esters (Simko, 2002).

The actual levels of PAHs in smoked foods depend on several variables in the smoking process, including type of smoke generator, combustion temperature, and degree of smoking (Moret et al., 1997). Smoke is generated by thermal pyrolysis of a certain kind of wood when there is limited access of oxygen. Temperature of smoke generally plays a very important role, because the amount of PAHs in smoke formed during pyrolysis increases linearly with the smoking temperature within the interval 400-1000°C (Toth and Blaas, 1972).

In modern industrial ovens, the smoke is usually generated in a separate chamber cleaned by using various techniques, such as electrostatic filters or smoke washing, and then led into the smoking chamber. This, together with the control of some important parameters such as temperature, humidity, smoke concentration, and circulation rate, can contribute to the minimization of PAHs contamination (Moret et al., 1999).

Incomplete wood combustion during smoking can produce considerable amounts of PAHs which can penetrate through the surface of products (Jira et al., 2006). In a study performed by Gomma et al. (1993), the total PAH concentrations were detected between 2.6-29.8 and 9.3-86.6 µg/kg in smoked meat and fish, respectively.

In another study conducted by Panalaks (1976) in Canada, smoked fish and meat samples were analyzed and PAH compounds were detected in 18 out of 25 smoked fish samples (maximum of 141 µg/kg) and in 19 out of 43 smoked meat samples (maximum of 13 µg/kg). Petrun and Rubenchik (1966) found the levels of BaP ranged from 4.2 to 60 µg/kg in hot and cold-smoked fish samples. In their study, Storelli et al. (2001) reported that the concentration of total PAHs in seafood varied from 46.5 to 124 µg/kg.

Reinik et al. (2007) found the highest total PAH concentrations in smoked meat, sausage and chicken samples as 16, 19 and 6.5 µg/kg, respectively. In another study, Djinovic et al. (2008) stated that there are differences in PAH contents between final smoked beef ham samples from traditional smokehouse (3.9 µg/kg) and industrial smokehouse (1.9 µg/kg).

This study seeks to determine the effect of smoking process on PAHs content in smoked fish samples (catfish and solefish) in Nigeria. The data from the study will also

be used to assess dietary intake of PAHs and the carcinogenic health hazards via smoked fish consumption.

MATERIALS AND METHODS

Sample collection and preparation

Fresh fish and commercially smoked fish of two different species commonly consumed in Nigeria, namely catfish and solefish were purchased from 3 different local fish vendors in Lagos. The fresh fish was gutted, cleaned and a part was placed over a wire gauze that was on burning hardwood charcoal (15 cm away from the hot hardwood charcoal ember). The catfish and solefish were allowed to cook for 90 min on both sides to obtain a greater level of drying. Fresh, laboratory smoked and commercially smoked fish samples from different vendors were pooled together to obtain represent-tative samples for each of 2 types of fish species. The fish samples were separately composited, homogenized, packed in amber bottles and kept in the freezer prior to analysis.

Reagents

Methanolic 2 M-KOH (methanol/water 9 + 1) and hexane analytical grade were redistilled in glass before use. Methanol (analytical grade), Silica gel (mesh: 70 – 230), glass wool and potassium hydroxide pellets (Purity: 86.1%) were obtained from Sigma Aldrich. PAH standard mixture containing 16 PAHs compounds (purity: 95.9-99.9%) including naphthalene (Naph), acenaphthylene (Acy), acenaphthene (Ace), fluorene (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (Fla), pyrene (Py), benzo[a]anthracene (BaA), chrysene (Chr), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), indeno[1,2,3-cd]pyrene (IcdP), dibenzo[a,h]anthracene (DBahA) and benzo[g,h,i]perylene (BghiP) in a 80 mg/L mixture solution were obtained from AccuStandard Chem. Co. (New Haven, CT, USA). Deuterated PAH internal standard solutions (naphthalene-d8, acenaphthene-d10, phenanthrene-d10, and chrysene-d12) at 4,000 mg/L and surrogate standard solutions (2-fluorobiphenyl and 4-terphenyl-d14) at 2,000 mg/L were obtained from AccuStandard Chem. Co. PAHs working standards, internal standard mixture solutions and surrogate standard mixture solutions were properly diluted in *n*-hexane and prepared daily before the analysis. Glassware were washed with detergent, soaked 24 h in dichromic acid rinsed severally with tap water, deionized distilled water, acetone and dried in an oven at 105°C. Helium and nitrogen gases were obtained from Air Liquid Nigeria Plc.

Extraction and sample clean-up

PAH extraction was carried out by applying the method described by Wretling et al. (2010). All the samples were analyzed in duplicate. Aliquot of 10 g of homogenized smoked fish were weighed into a 250 mL Erlenmeyer flask and spiked with 1 mL of a perdeuterated PAH internal standard mixture. Saponification was achieved by adding 60 mL of methanolic 2 M–KOH, the sample was extracted under reflux for 2 h. 50 mL of n-hexane was added and the refluxing was continued for another 5 min. The extract was cooled to ambient temperature and transferred to a 250 mL separating funnel using 30 mL of methanol/water (4+1). The funnel was shaken and the layers were allowed to separate. The aqueous layer was drained into a second 250 mL separating funnel and shaken with another 30 mL of n-hexane. The aqueous layer was discarded and the hexane phases were combined and washed successively with 30 mL of methanol/water (4+1), 30 mL of methanol/water (1+1) and 2 x 30 mL of water. The washed hexane solution was transferred to a 250 mL round-bottomed flask and concentrated to about 1 mL in a rotary evaporator under reduced pressure at 40°C and cleaned by silica gel column chromatography. The eluent was re-concentrated to 0.5 ml in a rotary evaporator (at 40°C) and concentrated further under a nitrogen flow to 200 μL before transferring to a GC sample vial with a conical glass insert.

GC–FID analysis

The polycyclic aromatic hydrocarbon analysis was carried out by an Agilent 7890A gas chromatograph system coupled with a flame ionisation detector. 2 μL of sample solution was injected in the pulsed splitless mode onto a 30 m x 0.32 mm i.d. fused capillary column with a film thickness of 0.25 μm (HP-5). Helium gas was used as the carrier gas. Other operating conditions were: pulse pressure 10.74 psi, purge time 0.75 min, purge flow 15.0 mL. An injection temperature was set at 300°C. The column temperature was initially held at 80°C for 1 min, and ramped to 320°C at a rate of 20°C/min and then 320°C was held for 20 min. 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. Procedural blanks were analyzed and quantified.

Analytical quality control

A spiking procedure was used to calculate recoveries. The recoveries (mean of 2 replicate analyses) were calculated by comparing the difference between spiked (4.6 – 8.1 μg/kg) and unspiked sample with the known amount of PAHs added. Recoveries obtained for different PAH standards ranged from 72 and 108% and their relative standard deviation ranged from 15.9 to 21.3%.

Benzo[a]pyrene equivalent estimation

Toxic equivalency factors (TEFs) have been developed for a number of individual PAHs to expresses its potency relative to benzo(a)pyrene, which has a TEF of unity. The concentration of each of the individual PAH compounds is multiplied by its TEF proposed by (Nisbet and LaGoy, 1992) (Table 1), and these values are summed to yield benzo(a) pyrene equivalent concentrations, TEQ_{BaP} (AFSSA, 2003). This technique has been applied successfully to smoked and fresh seafood monitoring studies, and other wider monitoring programmes (Law et al., 2002). The mutagenicity of individual PAHs relative to B(a)P had also been computed using the mutagenic equivalency factor (MEF) proposed by Durant et al. (1996, 1999) as shown in Table 1. The sum of the concentration of each individual PAH multiplied by the corresponding MEF gives the mutagenic equivalents (MEQ).

$$TEQ_{BaP} = \sum(TEF_i \times C_i) \quad (1)$$

$$MEQ_{BaP} = \sum(MEF_i \times C_i) \quad (2)$$

where Ci is the measured individual PAHs concentrations for the 'ith' compound with the assigned TEFi or MEFi.

Dietary exposure to PAHs

Estimates of human dietary PAH exposure doses (mg kg^{-1} BW d^{-1}) occurring over a lifetime were determined. The daily BaP equivalent dose of mixtures of carcinogenic (mutagenic) PAH compounds was calculated for carcinogenicity and mutagenicity using the following

Table 1. Proposed benzo(a)pyrene equivalent factors for carcinogenic (TEF) and mutagenic toxicity (MEF).

PAH compound	TEF (Nisbet and LaGoy, 1992)	MEF Durant et al. (1996, 1999)
Naphthalene	0.001	
Acenaphthylene	0.001	
Acenaphthene	0.001	
Fluorene	0.001	
Phenanthrene	0.001	
Anthracene	0.01	
Fluoranthene	0.001	
Pyrene	0.001	
Benzo(a)anthracene	0.1	0.082
Chrysene	0.001	0.017
Benzo(b)Fluoranthene	0.1	0.25
Benzo(k)fluoranthene	0.01	0.11
Benzo(a)pyrene	1.0	1.0
Dibenzo(a,h)anthracene	1.0	0.29
Indeno(1,2,3-cd)pyrene	0.1	0.31
Benzo(g,h,i)perylene	0.01	

Table 2. Toxicity values for PAHs contaminants

PAHs	RfD (mg / kg-d)		CSF (USEPA, 2004) (1/mg / kg-d)
Naphthalene	2.00×10^{-02}	Chrysene	7.30×10^{-3}
Acenaphthylene	2.00×10^{-02}	Benzo(a)anthracene	7.30×10^{-1}
Acenaphthene	6.00×10^{-02}	Benzo(b)Fluoranthene	7.30×10^{-1}
Fluorene	4.00×10^{-02}	Benzo(k)fluoranthene	7.30×10^{-2}
Phenanthrene		Benzo(a)pyrene	7.30
Anthracene	3.00×10^{-01}	Dibenzo(a,h)anthracene	7.30
Fluoranthene	4.00×10^{-02}	Indeno(1,2,3-cd)pyrene	7.30×10^{-1}
Pyrene	3.00×10^{-02}		
Benzo(g,h,i)perylene	4.00×10^{-02}		

equation.
Average daily dose of carcinogenic (mutagenic) PAHs is:

$$\frac{\text{TEQ (or MEQ)} \times \text{IR} \times \text{CF}}{\text{BW}} \quad (3)$$

These exposure assumptions were made to be consistent with EPA guidance on default assumption on ‘‘reasonable maximum exposure’’ (USEPA, 1991). Where IR is the ingestion or intake rate of carcinogenic (mutagenic) PAHs based on average fish consumption rate set to 68.5 g day^{-1} per person from the annual per capita fish consumption of 25 kg for Nigeria (FAO, 2008). CF is the conversion factor (0.001 mg μg^{-1}) and BW represents body weight which was set at 70 kg.

Non-cancer hazard, carcinogenic and mutagenic risk calculations

Risk associated with dietary exposure to non-carcinogenic PAHs was evaluated using a hazard quotient approach. Hazard quotients represent a ratio of the exposure dose for each PAH divided by an oral chronic reference dose (RfD).

$$\text{Hazard quotient (HQ)} = \text{Average daily dose (ADD)} / \text{RfD} \quad (4)$$

Pertinent RfD values (mg / kg day) are listed in Table 2. Summation of individual hazard quotients results in hazard index.

$$\text{Hazard index (HI)} = \Sigma(HQ_1 + HQ_2 + HQ_3 + \ldots\ldots HQ_n) \quad (5)$$

The calculated TEQ_{BaP} and MEQ_{BaP} for the seven USEPA classified carcinogens (mutagens) were used to estimate carcinogenic and mutagenic risk involved in ingestion of smoked fish used herein for life time of 70 years (USEPA, 2000). The total risk due to exposure to mixtures of carcinogenic (or mutagenic) PAHs is the product of the dietary carcinogen exposure dose (mg kg^{-1} BW d^{-1}) and benzo[*a*]pyrene’s slope factor value in Table 2.

Risk (carcinogenic or mutagenic) = average daily dose x slope factor (6)

Statistical analysis

Analysis of variance (ANOVA)) for $\alpha = 0.05$ were performed to estimate the significance of the differences between the means of total and individual PAHs content in traditionally and modern

Table 3. Concentration (µg/kg) (mean± SD) of PAHs for catfish / solefish smoked with different smoking methods.

PAHs	Traditional smoked fish		Modern smoked fish		Fresh fish	
	A1	B1	A2	B2	A3	B3
Naphthalene	12.89±0.22	5.48±0.68	0.03	0.02	0.01	0.01
Acenaphthylene	0.41±0.15	0.19±0.05	0.93±0.04	0.31±0.24	0.02	0.01
Acenaphthene	1.05±0.32	2.02±0.32	0.29±0.01	0.41±0.15	0.01	nd
Fluorene	4.04±1.19	2.62±0.82	0.02	nd	nd	nd
Phenanthrene	15.94±1.30	9.85±2.22	0.99±0.06	0.38±0.18	nd	nd
Anthracene	2.09±0.39	0.27±0.13	0.01	0.02	nd	nd
Fluoranthene	2.89±2.33	5.07±0.84	nd	nd	nd	nd
Pyrene	1.30±0.01	1.10±0.39	nd	nd	nd	nd
Chrysene	nd	nd	nd	nd	nd	nd
Benzo(a)anthracene	0.29±0.06	0.16±0.13	nd	nd	nd	nd
Benzo(b)Fluoranthene	0.11±0.09	0.12±0.15	nd	nd	nd	nd
Benzo(k)Fluoranthene	nd	nd	nd	nd	nd	nd
Benzo(a)pyrene	nd	nd	nd	nd	nd	nd
Dibenzo(a,h)anthracene	nd	nd	nd	nd	nd	nd
Benzo(g,h,i)perylene	0.26	0.35	nd	nd	nd	nd
Indeno(1,2,3-cd)pyrene	nd	nd	nd	nd	nd	nd
Total PAHs	41.27	27.23	2.27	1.14	0.04	0.02
Carcinogenic PAHs	0.40	0.28	0.00	0.00	0.00	0.00
Non-Carcinogenic	PAHs 40.87	26.95	2.27	1.14	0.04	0.02

A = catfish; B = solefish; Non- carcinogenic PAHs = Naphthalene, Acenaphthylene, Acenaphthene, Fluorene, Phenanthrene; Anthracene, Pyrene, Fluoranthene, Benzo(ghi)perylene; Carcinogenic PAHs = Chrysene, Benzo(a)anthracene, Benzo(b)Fluoranthene, Benzo(k)fluoranthene, Benzo(a)pyrene, Dibenzo(a,h)anthracene, Indeno(1,2,3-cd)pyrene, nd = no data.

smoked fish using both SPSS and Microsoft Excel.

RESULTS AND DISCUSSION

PAHs levels in fresh fish samples

The PAHs concentrations in non-smoked fresh catfish-/solefish samples used as control were below detection limits as presented in Table 3 (0.002 – 0.005 µg kg^{-1}). This is in conformity with the statement made by Stołyhwo and Sikorski (2005) that fish and marine invertebrates may naturally contain small or undetectable amounts of different PAH absorbed from the environment. The lack of PAHs in fresh catfish/solefish samples indicates that the PAHs measured in smoked catfish/solefish samples were wholly attributable to smoking processes as affirmed by Forsberg et al. (2013) The results in non-smoked fresh fish samples were considerably lower than those reported by other authors (Silva et al., 2011; Wretling et al., 2010).

PAHs levels in smoke fish samples

The mean values of PAHs content measured in traditional and modern smoked catfish/solefish as presented in Table 3 were predominantly those with ≤4 rings. Similar studies were reported in traditional Nigerian smoked fish (Akpambang et al., 2009) and fish prepared using traditional German smoking kilns (Karl and Leinemann, 1996). The two smoked fish had different PAH (levels) contribution from the smoking process. This could be attributed to the differences in fat and moisture contents and the nature of skin cover (Nakamura et al., 2008). Smoked catfish on the average recorded the highest mean levels of PAHs for both traditional and modern techniques. Individual PAH levels ranged from 0.01– 15.94 µg kg^{-1}. Phe was the most abundant PAH followed by Naph, Flu, Fla, Ant, Py, Ace, Acy and BghiP. The summation of these 9 analytes accounted for 98% of the total mass of PAHs measured across all smoked catfish and solefish. Together, 2-ring, 2+3-ring, and 2+3+4-ring PAHs accounted for roughly 36, > 75 and > 98% of the total PAHs mass measured across all smoked catfish and solefish samples, respectively. The individual PAHs of lower molecular weight found in high level could be attributed to the lower average wood temperature used in the smoking process (Nakamura et al., 2008). This shows that smoking process contributed to the increase percentage composition of these PAHs. Irrespec-tive of the smoking method applied, benzo[a]pyrene used as biomarker in monitoring carcinogenic PAHs recorded mean concentrations below detection (Table 3) limit which was much lower than maximum tolerable limit of 5.0 and 2.0 µg/kg in smoked fish established by the European

Table 4. Source characterization and assessment of PAHs.

PAH ratios	Petroleum	Wood	This study	Reference
[Ant/(Ant + Phe)]	<0.10	>0.10	0.01-0.12	Yunker et al. (2002), Zhang et al. (2004), Li et al. (2006) Pies et al. (2008) and Placha´ et al. (2009)
(Fla/(Fla + Py)]	0.40	>0.5	0.69-0.82	Yunker et al. (2002), Zhu and Wang (2003) and Placha´ et al. (2009)
BaA/(BaA + Chr)]	<0.20	1.2–5.0	1.00	Maher and Aislabie (1992), Gilbert et al. (2006), Zhang et al. (2006), Pies et al. (2008), Essumag et al. (2012)
[BaP/(BghiP)]	>0.6	1.2–5.0	-	Park et al. (2002), Yin et al. (2008), Maliszewska-Kordybach et al. (2008), and Essumag et al. (2012)
[IcdP/(IcdP + BghiP)]	<0.5	>0.5	-	Maliszewska-Kordybach et al. (2008) and Yin et al. (2008)

Ant = anthracene, Phe = phenanthrene, Fla = fluoranthene, BaA = benz[a]anthracene, Chr= chrysene, BaP = benzo[a]pyrene, BghiP = benzo[g,h,i]perylene, and IcdP = indeno[1,2,3-cd]pyrene.

Commission (Regulation (2005) and Turkish Codex Regulation (2008), respectively. The results obtained in this work therefore indicated that the smoked fish may contribute no levels of cancer and cancer-related cases in the study area, because BaP is widely known for its carcinogenicity and mutagenicity; further epidemiological studies may be required to prove this conclusion. Fish species smoked by traditional method was 18 times greater than corresponding modern smoked fish samples. The observed differences probably reflect the highly controlled and standardized smoking systems used in modern smoking (Vaz-Velho, 2003). Furthermore, in order to increase the shelf-life of the product, fish vendors may re-smoke the product many times until they are sold, thus contributing to increase PAH formation (Akpambang et al., 2009).

One-way ANOVA conducted at 95% confidence level on the numbers of aromatic rings showed no statistical significant differences ($p > 0.05$) between the numbers of aromatic rings with respect to each of the fish type smoked. Thus the number of aromatic rings was species independent. Further analysis of variance (one-way ANOVA) conducted on the data at 95% CL showed significant difference ($p < 0.05$) in PAH levels between fish type with respect to the smoke type used. Thus PAHs levels in smoked fish were species dependent.

Sources of PAHs in smoked fish

PAH ratios of selected compounds are generally considered to be a good indicator of the pollution and the mechanism of PAH distribution in foods. Yunker et al. (2002) have summarized the literature on PAH ratios (Table 4). The ratio of [An/(An + Phen)] in this study ranged from 0.01 to 0.12 with a mean of 0.05 (Table 5). This indicates a predominance of petroleum as a source for PAHs (ratio < 0.1) in the smoked fish. The [Fl/(Fl + Py)] ratio in this work also ranged from 0.69 to 0.82 which is an indication of wood or coal combustion as a source of the PAHs in the smoked fish samples. The results from the [BaA/(BaA + Chry)] ratio again confirm wood combustion as the primary and major source of PAH contamination in smoked fish. These PAH ratios reveal that the major source of PAHs in the smoked fish is the wood combustion with vehicular traffic source contributing a comparatively insignificant amount.

Cancer and non-cancer risk assessment of PAHs in smoked fish

The carcinogenic toxicity (TEQ_{BaP}) and mutagenic toxicity (MEQ_{BaP}) relative to B(a)P were calculated for the carcinogenic and mutagenic risk associated with ingestion of the smoked fish (Tables 1 and 3). While TEQ_{BaP} is directly associated with carcinogenicity, MEQ_{BaP} (mutagenic activity) may not be directly associated with cancer (Zeiger, 1998, 2001; Essumang et al., 2013) and may have implications for other non-cancerous adverse health effects like pulmonary diseases, birth defects, impotency, low intelligent quotient, etc. (DeMarini et al., 2004; Essumang et al, 2013). From the result in Table 6, the TEQ for the seven USEPA priority carcinogens were 0.040 and 0.028 for catfish and solefish smoked traditionally. Known carcinogenic PAHs were not found in smoked fish prepared by modern method (Tables 6 and 7).The corresponding EQ_{BaP} daily dose and carcinogenic risk for an adult involved in life time of 70 years ingestion of the smoked fish products were also calculated to be 3.92×10^{-8} and 2.75×10^{-8} mg kg^{-1} day^{-1} for a risk of 2.86×10^{-8} and 2.01×10^{-8}, respectively (Tables 2 and 6).

Table 5. Fish species and PAH isomer ratios for source assessment in smoked fish sampled.

Fish species/ Isomer ratio	Ant/ Ant + Phe	Fla/ Fla + Py	BaA/ BaA + Chr	BaP/ BghiP	Ind/ IcdP + BghiP
S1	0.12	0.69	1.00	-	-
S2	0.03	0.82	1.00	-	-
S3	0.01	-	-	-	-
S4	0.05	-	-	-	-

S1 = traditional smoked catfish; S2 = traditional smoked solefish; S3 = modern smoked catfish; S4 = modern smoked solefish

Table 6. Risk assessment based on carcinogenic equivalency, average daily dose and risks (Mean±SD) for traditional and modern smoked fish species.

Carcinogenic equivalency	T. smoked Catfish	T. smoked Solefish	M. smoked Catfish	M. smoked Solefish
Benzo(a)anthracene	0.029	0.016	nd	nd
Benzo(b)Fluoranthene	0.011	0.012	nd	nd
Benzo(k)fluoranthene	nd	nd	nd	nd
Benzo(a)pyrene	nd	nd	nd	nd
Dibenzo(a,h)anthracene	nd	nd	nd	nd
Chrysene	nd	nd	nd	nd
Indeno(1,2,3-cd)pyrene	nd	nd	nd	nd
∑BaP-TEQ	0.040	0.028		
BaPEQ daily dose ($mgkg^{-1}day^{-1}$)	3.92×10^{-08}	2.75×10^{-08}		
LECR	2.86×10^{-08}	2.01×10^{-08}		

T. = traditional; M. = modern; LECR = life-time excess carcinogenic risk, nd = no data.

Table 7. Risk assessment based on mutagenic equivalency, average daily doses and risks (Mean±SD) for traditional and modern smoked fish species.

Mutagenic equivalency	T. smoked Catfish	T. smoked Solefish	M. smoked Catfish	M. smoked Solefish
Benzo(a)anthracene	0.024	0.013	nd	nd
Benzo(b)Fluoranthene	0.019	0.002	nd	nd
Benzo(k)fluoranthene	nd	nd	nd	nd
Benzo(a)pyrene	nd	nd	nd	nd
Dibenzo(a,h)anthracene	nd	nd	nd	nd
Chrysene	nd	nd	nd	nd
Indeno(1,2,3-cd)pyrene	nd	nd	nd	nd
∑BaP-TEQ	0.0259	0.015	0	0
BaPEQ daily dose ($mgkg^{-1}day^{-1}$)	2.51×10^{-08}	1.49×10^{-08}		
LECR	1.83×10^{-08}	1.09×10^{-08}		

T. = traditional; M. = modern; LECR = life-time excess carcinogenic risk, nd = no data.

These risk values mean that for ingestion of catfish prepared by traditional smoking, 3 out of 10,000,000 adults are likely to suffer from cancer in their life time and for ingestion of solefish prepared by traditional smoking, 2 out of 10,000,000 people are likely to suffer from cancer in their life time. This means that the consumption of catfish and solefish prepared by traditional smoking pose no risk, because it is lower than the USEPA (1993, 2009) carcinogenic unit risk of 1×10^{-5} (carcinogenesis threshold). Generally, relatively lower $\sum TEQ_{BaP}$ and cancer risk values below the acceptable USEPA (1993, 2009) carcinogenic unit risk of 1×10^{-5} (carcinogenesis threshold) were recorded for the fish samples prepared by traditional smoking. Also, the mutagenic equivalent for

Table 8. Risk assessment based on non-carcinogenic equivalency, average daily doses and hazard index (Mean±SD) for traditional and modern smoked fish species.

Non-Carcinogenic equivalency	T. smoked Catfish	T. smoked Solefish	M. smoked Catfish	M. smoked Solefish
Naphthalene	0.013	0.0055	0.00001	0.00001
Acenaphthylene	0.0004	0.00019	0.00093	0.00031
Acenaphthene	0.00105	0.00202	0.00029	0.00041
Fluorene	0.00404	0.00262	0.00002	nd
Phenanthrene	0.016	0.0099	0.00099	0.00038
Anthracene	0.0209	0.0027	0.00001	0.00002
Fluoranthene	0.0029	0.0051	nd	nd
Pyrene	0.0013	0.0011	nd	nd
Benzo(g,h,i)perylene	0.00026	0.00035	nd	nd
∑BaP-TEQ	0.060	0.029	0.002	0.001
BaPEQ daily dose $mgkg^{-1}day^{-1}$	5.86×10^{-08}	2.88×10^{-08}	2.20×10^{-09}	1.11×10^{-09}
Hazard index	1.43×10^{-06}	9.02×10^{-07}	9.96×10^{-08}	4.08×10^{-08}

nd = no data.

these PAHs calculated were 0.026 and 0.015 for catfish and solefish prepared by traditional smoking (Table 7). The corresponding EQ_{BaP} daily doses were also calculated to be 2.52×10^{-8} and 1.49×10^{-8} mg kg^{-1} day^{-1} for catfish and solefish prepared by traditional smoking respectively (Tables 2 and 7). Hence, the mutagenic risk involved in ingestion of these smoked fish products of 70 years was calculated to be 1.83×10^{-8} and 1.09×10^{-8}, respectively. This imply that for adult's life time ingestion of catfish prepared by traditional smoking; 2 out of 10,000,000 and 1out of 10,000,000 people are like to suffer from non-cancer and other cancer related disease in their life time, respectively. Generally, relatively lower $\sum MEQ_{BaP}$ and mutagenic risk values below the acceptable USEPA (1993, 2009) unit risk of 10^{-5} were recorded for catfish and solefish samples prepared by traditional smoking. Catfish prepared by traditional smoking produced the largest observed values for carcinogenic and mutagenic PAHs. From these results, it may be said that catfish and solefish prepared by traditional smoking had low cancer and mutagenic risk and may be considered safe for consumption. Exposure to non-carcinogenic PAHs resulted in hazard indexes ($\sum PAH_{16}$ HQs) ranging from 9.02×10^{-7} to 9.96×10^{-8} across the two smoking methods (Tables 2 and 8). The non-carcinogenic PAHs produced hazard indexes less than 1; a level described by the EPA as generally having no appreciable risk for the development of non-cancer health effects through the ingestion of these hazardous PAHs from smoked fish in their diets. Taken together, risks associated with carcinogenesis pose the largest threat to human health.

Conclusion

From the results discussed above, it may be concluded that smoked catfish/solefish could be deemed fit for human consumption. Smoked catfish/solefish from commercial fish mongers (traditional method of smoking) showed elevated levels of polycyclic aromatic hydrocarbons (PAHs) as compared to modern method of smoking, and this may result in cases of cancer and cancer-related ailments in Nigeria. The high levels of PAHs in smoked catfish/solefish prepared by traditional method is a result of uncontrolled fish-smoking practices that burn wood at higher temperatures, coupled with thermal pyrolysis of fat in fatty fish at higher temperatures to give the fish a longer shelf-life, but which also promotes PAH production. This study found that fish smoking practices employed by fishmongers in Nigeria are similar throughout the nation. There is therefore a need to educate fishmongers about safe smoking practices, and also most importantly to adopt a fish smoking procedure that would reduce considerably the levels of toxins in fishes smoked with traditional kilns in order to ensure not only the health safety of consumers but also that of fishmongers exposed to smoke during fish-smoking processes.

Conflict of interests

The authors did not declare any conflict of interest.

ACKNOWLEDGEMENTS

The authors acknowledge members of STERG, most especially Prof. P. O. Okebukola for their positive input during proposal overview presentation.

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