Levels of Polycyclic Aromatic Hydrocarbons in Smoked Elephant-snout Fish [*Mormyrus caschive* (L.)] and Tilapia [*reochromis niloticus* (L.)] from Terekeka, South Sudan

Borodi C. Mondo[1*], Peter Akoll[2] and Margaret Masette[2]

[1] Department of Fisheries Science, School of Natural Resources and Environmental Studies
University of Juba, P. O. Box, 82, Juba-South Sudan

[2] Department of Zoology, Entomology and Fisheries Sciences, SBS, College of Natural Sciences
Makerere University, P. O. Box 7062, Kampala-Uganda.

*Corresponding Author: mondocharles1982@gmail.com

ABSTRACT

The level of polycyclic aromatic hydrocarbons (PAHs) in pit and *chorkor* smoked fish was determined to adopt appropriate technology that maintains the quality and safety of smoked fish to enhance nutrition and food security in South Sudan. A total of 60 fresh Elephant-snout Fish (*Mormyrus caschive*) and Tilapia (*Oreochromis niloticus*) were purchased from Sur-num landing site in Terekeka; 12 fresh samples were iced and the remaining 48 samples were divided into two batches for pit and *chorkor* smoking. Fish samples were extracted and purified using organic solvents, and dried under nitrogen flow. Fish samples were analyzed for polycyclic aromatic hydrocarbons using gas chromatography-mass spectrometry. Results revealed that, seven types of PAHs consisting of low molecular weight, 2-ringed-Naphthalene, 3-ringed; Anthracene, Fluorene, acenaphthene and Phenanthrene, and medium molecular weight; Benzo [a] anthracene and Chrysene, 4-ringed were recorded from both smoked fish species. Fluorene and Naphthalene dominated the PAHs with pit smoked fish containing significantly higher levels of Fluorene (3.83±0.10 µg/kg) and Naphthalene (5.86±4.16 µg/kg) than *chorkor* smoked samples. Higher PAHs in pit smoked samples is attributed to open combustion of firewood resulting in higher temperatures which consequently lead to increase in the amount of oils on fish surface with subsequent deposition of PAHs. Low level of PAHs in *chorko*r smoked samples is attributed to the structural design and standardization of *chorkor* kiln that significantly influences the level of PAHs accumulation in smoked fish. Improve *chorkor* kilns characterized with easily controlled smoking parameters; oxygen, temperature and time reduces the level of PAHs in smoked fish samples. The study therefore, recommends *chorkor* adoption for artisan fisheries in South Sudan.

Keywords: Polycyclic Aromatic Hydrocarbons, Levels, Species, Technologies, Pyrolysis

INTRODUCTION

Fish smoking in artisan fisheries of the under-developing countries has received greater attention due to technological simplicity and acceptability of smoked fish products (Magawata and Musa, 2015). It has been estimated that over 70 percent of fish produced in tropical countries is consumed in smoked form (Adeyeye and Oyewole, 2016). Fisher-folks have used smoking to preserve fish due to lack of cool preservation facilities to deliver fresh fish to distant markets (Odoli *et al.,* 2015). In addition, fish smoking is used to reduce fish post-harvest losses estimated at 30% and 40% in dry and wet seasons respectively. South Sudan's water bodies and wetlands are inhabited by diverse fish species of commercial importance, of which Tilapia (*Oreochromis niloticus*) and Elephant-snout Fish (*Mormyrus caschive (L.)*) are ranked the second and third after Nile Perch (*Lates niloticus (L.)*) (Miller and Benansio, 2010). These commercial fish species are processed and preserved by smoking in an attempt to cope up with fish post-harvest loss challenges (FAO and WFP, 2013), and to ensure sustainable supply of fish throughout the year (Ikenweiwe *et al.,* 2010; FAO, 2015; Adeolu *et al.,* 2017).

Efficient and effective smoking have multiple advantages: maintains good smoky flavour, taste, aroma and texture of fish (Kumolu-Johnson *et al.,* 2010; FAO, 2016; Adeolu *et al.,* 2017). As such, smoked fish products attract more income to fisher-folks (Akande and Diei-ouadi, 2010; Emere and Dibal, 2013) and reduce post-harvest losses (Getu and Misgamaw, 2015). Smoking ultimately reduces

moisture content which eventually retards the autolytic activity of bacteria, prevents mold growth, fish discoloration and ultimately increase the shelf-life of fish and guarantees a sustainable supply of fish during lean seasons (Magawata and Musa, 2015).

In South Sudan, fish processors have various traditional smoking technologies ranging from pits, round mud kilns, rectangular mud kilns and drum kilns (UNIDO, 2015). In Terekeka, where more than 70% of South Sudan's domestic fish are harvested (CAMP, 2013), traditional pit smoking is the dominant processing and preservation technique (Miller, 2011). Nevertheless, traditional smoking technology is associated with excessive fire wood consumption, unregulated temperature, uneven smoking of fish (Tawari and Abowei, 2011), and exposure of fish products to dust and smoke contaminants during the smoking process (Yusuf *et al.,* 2015).

Although smoking of fish plays a significant role in fish preservation, smoke chemicals produced by different technologies have different quantities and may influence the quality of smoked fish products (Silva *et al.,* 2011). Polycyclic aromatic hydrocarbons (PAHs) which are obtained from incomplete pyrolysis of fuel wood, coal and oils can penetrate into the muscles of smoked fish products (Ongwech *et al.,* 2013). Majority of PAHs are mutagenic and carcinogenic to human health (Anderson *et al.,* 2010; Forsberg *et al.,* 2013). Some of the documented effects of PAHs include; low birth weight, growth retardation, small head circumferences (Deliang *et al.,* 2014), low intelligence quotient (Edwards *et al.,* 2010), damage of de-oxy-ribose-nucleic acid (DNA) in unborn children (Yusuf *et al.,* 2015), and endocrine systems' disorders including estrogens, thyroid and steroids (Essumang *et al.,* 2013; Tang *et al.,* 2014). In addition, related reproductive effects such as early menopause due to ova destruction have been identified with PAHs (Forsberg *et al.,* 2013). PAHs are also known to have cancer causing effects in humans (Alomirah *et al.,* 2011).

Due to the difference in structural design and smoking parameters, smoking technologies may release different quantities of smoke chemicals, which may influence the generation and deposition capacities of PAHs on fish muscles (Duedahl-Olesen *et al.,* 2010). As such, some technologies may exceed the maximum acceptable limit of carcinogenic PAHs (5µg kg^{-1}) permitted in smoked fish by World Health Organization (WHO, 1998; 2000) and European Commission (Regulation EC No 835/2011) which is adopted by many countries (European Commission, 2011) including the developing economies.

Indeed, elevated concentrations of PAHs in smoked fish products have potential risks on consumers' health (Forsberg *et al.,* 2012; Singh *et al.,* 2016). For instance, heavy polycyclic aromatic hydrocarbons are reported to have implication on breast, lung and colon cancers (Ongwech *et al.,* 2013; Tongo *et al.,* 2017). Higher levels of polycyclic aromatic hydrocarbons are known to cause hematological, cardio, renal, neurological, immunological, reproductive and developmental toxicities in humans (Anderson *et al.,* 2010; Edwards *et al.,* 2010; Forsberg *et al.,* 2013; Tang *et al.,* 2014). Higher concentrations of polycyclic aromatic hydrocarbons can bind with proteins and DNA causing biochemical disruption and cell damage in animals and cancer in humans (Yusuf *et al.,* 2015).

Although, PAHs are known to be present in preserved food and their levels are critical to human health, concentration levels of PAHs produced by pit and *chorkor* smoking technologies have not been determined for smoked fish products of South Sudan. This study was therefore, carried-out to assess the effect of smoking technologies on the level of PAHs in smoked fish in order to adopt appropriate technology that maintains the quality and safety of smoked fish for enhanced nutrition and food security in South Sudan.

MATERIALS AND METHODS

Study area

The study was conducted in June, 2018 in Terekeka County of Central Equatoria State, South Sudan. Terekeka County is located approximately, 136 km north of Juba on the western bank of the Nile (Miller and Benansio, 2010). Terekeka lies within Latitudes of 5˚26'38"N and Longitudes of 31˚45'02"E, respectively (FAO and WFP, 2019). The County covers an area of about 10,358.232 km^2

and is occupied by an estimated population of 246,483 (South Sudan Centre for Census, Statistics and Evaluation, 2018). Terekeka has tropical climate with comparatively small seasonal variation of temperature, humidity and wind throughout the year. Normally, the County receives rainfall between the months of April to November with an average annual rainfall of 907 mm (Climate-data.org. 2018). The area experiences dry periods between the months of December and March with an average annual temperature of 27.7°C (Climate-data.org. 2018). It is within the dry season that most people are actively involved in fisheries activities. However, plentiful fish catch occurs during the months of June to August particularly when the flood recedes (Personal communication).

Study design

The current study was conducted in two phases, field smoking and laboratory analysis. Experimental field smoking of two fish species using pit and *chorkor* technologies was conducted three times in a completely randomized design. In the laboratory, the PAHs levels were determined to compare the two smoking technologies.

Sampling and processing procedures

A total of 60 fresh *M. caschive* and *O. niloticus* were purchased at Sur-num landing site situated about one kilometer North of Terekeka Town. Cleaning, scaling, gutting, and washing of fresh fish samples were done at the landing site. From the procured fish samples, twelve fresh fish were kept in cooler boxes packed with ice blocks immediately after being harvested at the fishing grounds. A purposeful smoking of the remaining 48 fish samples was done using improved *chorkor* and traditional pit kilns, respectively.

Lokeyi women fish group *chorkor* oven was used to represent improved smoking kiln. The *chorkor* was measuring 2 m long, 1 m wide and 1 m high with 3 trays attached after every 30 cm from base to top. The *chorkor* was constructed using unburnt bricks and the interior part plastered with clay soil. The top part of the *chorkor* was roofed with perforated flat iron sheets. It had two inlets at the base for aeration and smoke production by burning firewood. Its smoking chamber had a movable door that remained closed except during monitoring periods. A traditional pit was constructed alongside improved *chorkor* kiln. The pit kiln measured 1 m long, 0.5 m wide and 0.5 m high as practised by fisher-folks in the area. Four wooden planks were placed at the edges where a wire mesh was sitting. During fish smoking, flat iron sheet was used to cover fish samples.

From the procured samples, 12 specimens were ice stored at 4^{o}C and transported by vehicle to the laboratory where 6 specimens from each specie were used for PAHs analysis. The remaining 48 samples were divided equally into two batches for pit and *chorkor* experimental smoking using *Acacia seyal,* the dominant tree species for smoking fish in the area. All the procured fresh fish samples were washed to remove slime, descaled, eviscerated and rewashed thoroughly with clean water to remove blood. Fresh fish samples were then immersed in a freshly prepared salt solution (a mixture of 100 g salt in 10 L of clean water) for 15 minutes followed by draining for 15 minutes. Fire was set in both pit and *chorkor* kilns to generate smoke heat by burning *Acacia seyal* wood. The pre-treated fish samples were randomly loaded on the wooden trays and wire mesh on top of *chorkor* and pit kilns, respectively. The desired temperature of 60-80^{o} C for *chorkor* kiln was maintained manually by a thermometer until fish were smoke dried.

During smoking, the position of fish samples on the wooden trays were changed in *chorkor* to attain uniformity of the products and turned upside down in pit kiln in the mid periods in order to make samples smooth and steady in texture and appearance. The smoked samples were then, cooled for 12 hours at ambient temperature and later wrapped in aluminum foil, labeled for easy identification and packed in carton boxes before delivery. Smoked samples were transported to the Laboratory in Makerere University for analyses. While in the Lab, samples were stored in deep freezers.

Sample preparation for PAHs analyses

From each sample, 100g of smoked fish muscle was removed from the edible parts, grinded to powder form using blender, labeled and stored in deep freezers at -18°C for PAHs analysis. One

hundred grams of fresh fish muscles from each sample were taken as control and prepared in the same way as the smoked samples.

Extraction and purification of polycyclic aromatic hydrocarbons

The Wretling *et al.* (2010) method was used with minor modifications. Briefly, to the 10 g homogenized fish muscles per sample in a 250 mL round bottomed Erlenmeyer flask, 40 mL of dichloromethane (99.8% pure) extraction solvent was added and the flask was thoroughly sealed with aluminum foil for 30 minutes to prevent evaporation. To the mixture in the flask, 5 g of anhydrous sodium sulphate was added. The content was shaken vigorously in a reciprocating shakers for 15 minutes. The content was further vortexed in an auto vertex mixer for 5 minutes to homogenize the mixture and allowed to stand for 10 minutes so that the layers separate.

The aqueous layer was filtered to a second 250 mL round bottomed Erlenmeyer flask by passing it through a separating funnel packed with a glass wool to a height of 2 cm. The flask was rinsed with methanol/water (20 ml; 4:1 v/v) and the content added to the funnel. The extract was then dehydrated by passing it through anhydrous sodium sulphate in a separating funnel packed with florisil to a height of 5 cm and conditioned with 20 mL dichloromethane. The flask was rinsed with 10 mL dichloromethane and rinsing added to the separating funnel. The clean and clear aqueous content was decanted into a 50 mL spherical flask and concentrated to 1 mL using a rotary evaporator at 35°C. The extracts were further purified as described by Mottier *et al.* (2000).

A chromatographic column (1 cm internal diameter, id) was plugged with glass wool at the base. Activated silica gel was loaded in the column to a height of 5 cm. Additional 2 g sodium sulphate was added to the column. The packed column was then conditioned with 10 mL dichloromethane, the 1 mL concentrate was loaded into the column and eluted with 20 mL dichloromethane. The eluate was transferred to a conical flask and concentrated to 1mL in a vacuum rotary evaporator at 35°C. The eluate was further concentrated under nitrogen flow at 37°C to near dryness. To the concentrate, 1 mL of cyclohexane (containing 99.5% purity) was added and the content transferred into 2 mL amber glass vials with Teflon lined screw cap using pipettes for gas chromatography-mass spectrometry (GC-MS) analysis. To minimize PAHs volatilization, vials were kept in deep freezer at -20°C prior to analysis.

Chromatographic analysis

Fish samples analyzed with an Agilent 6890N gas chromatography instrument coupled with a 5975-mass spectrometry (Agilent Technologies, Santa Clara, CA, USA) following standard procedures (Lee *et al.,* 2015). To separate the compounds, 1 µL sample solution was injected in the pulsed split-less mode onto a HP-5MS column with 30 m × 0.25 mm id. The column was programmed as follows: injector temperature; 300°C, oven temperature at 80°C and held for 1 minute, 245°C (6°C/minute), 270°C (30°C/minute) and held for 10 minutes, and 310°C held for 10 minutes. Other operating conditions were pulse pressure 45 psi, pulse time 0.9 minutes, purge flow 50 mL, purse time 1 minute. Helium gas at constant flow rate of 1.1 mL/minute was used as the carrier gas. The mass spectrometry was operated in electron impact ionization mode at 70 eV with a solvent delay of 3.75 minutes. Identification and quantification of individual PAHs was confirmed by comparing the mass spectra and the peak retention time with those of PAHs standards in the equivalent conditions. A retention time match of ±1% was considered for confirmation (Samuel *et al.,* 2010).

Identification and quantitation of polycyclic aromatic hydrocarbons

External standard was used for the identification and quantitation of PAHs. A stock solution of 20 µL/mL was prepared by diluting 0.2 mL of a 1000 µg/mL PAHs standard to 10 mL with cyclohexane. 1 µg/mL standard working solution was prepared by diluting 0.5 mL of a 20 µg/mL PAHs standard stock solution to 10 mL with cyclohexane. PAHs in the samples were identified and quantified by a combination of a retention time and mass spectral match against the calibration standards. Sample peak areas were compared to peak areas of the standard. A minimum of three concentration levels of the standards ranging from 0.1 to 5 ppm were injected into the GC-MS and calibration curve for each standard was obtained by plotting peak area against concentration of the standards. Each PAH in the sample was then quantified using the formula (Ongwech *et al.,* 2013):

$$Cs = \frac{(Ac \times 100)}{Ws \times R} \times 100$$

Where, *Cs* is the concentration of PAH in the sample in µg/kg, *Ac* is the concentration (ng/ml) relative to the peak area in the injection volume (µl), *Ws* is the mass (in grams) of the sample extracted and *R* is the recovery. For control purpose, solvent blanks were included in every run during the analysis. Arithmetic means and standard deviations were calculated from quantifiable samples only. A computer program XLSTAT (version 7.5.2) was used for the computation.

Statistical analysis

Data collected from the study was analyzed using R statistical package (R Core Team, 2018). A two-way analysis of variance (ANOVA) was used to test the difference between means of PAHs values detected and quantified in fish samples smoked using pit and *chorkor* with fresh fish samples to determine the effect of smoking technologies on the concentration level of PAHs in smoked fish. Tukey's Honest Significant Difference Test was performed where the means of the two groups under comparison were significantly different. Level of significance was computed at $P \leq 0.05$.

RESULTS

This study recorded seven types of PAHs from smoked fish samples: Chrysene, Benzo [a] anthracene, Anthracene, Phenanthrene, Fluorene, Acenaphthene and Naphthalene (Table 1). The PAHs types recorded in pit and *chorkor* smoked fish products were mostly low and medium molecular weight (LMW, for example, 2-ringed; naphthalene, 3-ringed; anthracene, fluorene, acenaphthene and phenanthrene, and MMW, 4- ringed; benzo [a] anthracene and chrysene) compounds. In terms of abundance, naphthalene was the most occurring PAH followed by fluorene, benzo [a] anthracene, chrysene, phenanthrene, anthracene and acenaphthene. Although naphthalene was the only PAH detected in fresh *O. niloticus*, fluorene and naphthalene were found in fresh samples of *M. caschive* (Table 1). The higher molecular weight (HMW, Benzo [b] fluoranthene, Benzo [k] fluoranthene, Benzo [a] pyrene and Dibenzo [a, h] anthracene, 5-ringed; Benzo [g, h, i] perylene and Indenol [1, 2, 3-c, d] pyrene, 6-ringed) compounds were not detected in all the samples analyzed. As such, PAH4 (Benzo [a] anthracene, Chrysene, Benzo [b] fluoranthene, Benzo [a] pyrene) was employed to determine the carcinogenic potency of PAHs measured in smoked fish samples (Table 1).

Table 1. Types of PAHs detected and quantified in smoked fish (µg/kg wet weight basis).

PAHs types	*M. caschive* (χ±SD)			*O. niloticus* (χ±SD)		
	Fresh	Pit	*Chorkor*	Fresh	Pit	*Chorkor*
Chrysene	n.d	4.65±0.00	n.d	n.d	n.d	n.d
Benzo [a] anthracene	n.d	7.83±0.00	n.d	n.d	n.d	n.d
Anthracene	n.d	2.31±0.00	n.d	n.d	n.d	n.d
Phenanthrene	n.d	2.69±0.00	n.d	n.d	n.d	n.d
Fluorene	3.75±0.1	3.83±0.1	3.64±0.1	n.d	3.81±0.09	n.d
Acenaphthene	n.d	0.82±0.00	n.d	n.d	n.d	n.d
Naphthalene	2.86±1.2	5.86±4.16	5.53±1.9	2.37±0.6	3.64±1.59	2.82±2.10
ΣPAHs	6.61	27.98	9.17	2.37	7.45	2.82
ΣPAH4	0.00	12.47	0.00	0.00	0.00	0.00

Note: n=6 samples per treatment, n.d=not detected; PAH4 is the sum of Benzo [a] anthracene, Chrysene, Benzo [b] fluoranthene and Benzo [a] pyrene and ΣPAHs is the sum of PAHs detected and quantified in the samples.

The means of total PAHs levels showed that, fresh samples have significantly lower means PAHs (3.01±2.95µg/kg for *M. caschive* and 1.58±1.32 µg/kg for *O. niloticus*) than the smoked samples (Table 2). With regards to technologies, the mean of PAHs in pit smoked fish (9.55±9.81µg/kg in *M.*

caschive and 6.18±3.21 µg/kg in *O. niloticus*) was significantly higher than *chorkor* (6.72±2.72 and 2.82±2.10 µg/kg), P<0.05 for *M. caschive* and *O. niloticus*, respectively.

The concentrations of each types in smoked *M. caschive* and *O. niloticus* are presented in table 1. The concentration levels of fluorene (3.75±0.10 µg/kg) and naphthalene (2.86 ±1.22 µg/kg) recorded in fresh samples of *M. caschive* were significantly lower than those recorded in smoked samples. Pit smoked *M. caschive* had higher levels of fluorene (3.83±0.10 µg/kg) and naphthalene (5.86±4.16µg/kg) than *chorkor* smoked samples (n.d and 5.53±1.90 µg/kg, P<0.05).

With regards to *O. niloticus*, naphthalene was the only PAHs detected in the fresh samples. The fresh *O. niloticus* sampled had significantly lower naphthalene concentration (2.37±0.62 µg/kg) than smoked products (P<0.05). Furthermore, *chorkor* smoked *O. niloticus* had significantly lower naphthalene concentration levels (2.82±2.10 µg/kg) than pit smoked products (3.64±1.59 µg/kg, P<0.05). Fluorene (3.81±0.09 µg/kg) was detected in pit smoked *O. niloticus* but not in fresh and *chorkor* smoked samples.

Table 2. Total PAHs levels (µg/kg) quantified in smoked *M. caschive and O. niloticus*

Parameters	*M. caschive* (χ±SD)			*O. niloticus* (χ±SD)		
Samples	Fresh	Pit	*Chorkor*	Fresh	Pit	*Chorkor*
1	8.66	26.88	3.06	0.00	5.37	1.23
2	0.00	3.77	10.98	1.66	10.12	1.70
3	2.52	9.66	5.33	0.00	2.79	6.37
4	1.64	1.00	7.96	2.68	2.90	1.38
5	2.55	13.87	7.53	2.09	8.25	1.80
6	2.68	2.12	5.49	3.06	7.66	4.42
ΣPAHs	18.05	57.31	40.34	9.50	37.08	16.91
((χ±SD)	3.01±2.95	9.55±9.81	6.72±2.72	1.58±1.32	6.18±3.21	2.82±2.10

DISCUSSION

The levels of PAHs in fresh samples of *M. caschive* and *O. niloticus* were lower than recorded in the smoked fish samples. Fluorene and naphthalene levels in the fresh fish samples were significantly lower than recorded in smoked products. This is in conformity with observation that naturally, fish and aquatic invertebrates contain small and or undetected quantity of PAHs absorbed from the environment (Stołyhwo and Sikorski, 2005; Ongwech *et al.,* 2013; Yusuf *et al.*, 2015). Low PAHs recorded in fresh fish samples could be due to degradation of the compounds into other components during metabolic processes in fish. More than 85% of PAHs in fresh fish samples were below the limit of detection. This indicates that the PAHs recorded in smoked fish samples were exclusively attributed to smoking process as observed by earlier studies (Stołyhwo and Sikorski, 2005; Linda, *et al.,* 2011: Olabemiwo *et al.,* 2013; Forsberg *et al.,* 2013).

With regards to the samples, low molecular weight (LMW; for example, naphthalene, 2-ringed; ace-naphthalene, fluorene, anthracene and phenanthrene; 3-ringed) and medium molecular weight (MMW; pyrene and chrysene; 4-ringed PAHs) compounds were detected. This could be related to the type of firewood used during the smoking process. Studies have shown that smoke produced by hard woods usually generates higher concentrations of low molecular weight PAHs (Ezike & Ohen, 2018). Although individual PAHs of low molecular weights were detected in quantifiable amount, their levels were much lower than those of PAH4 (Chrysene and Benzo *[a]* anthracene). This could be attributed to the fact that the medium (Benzo *[a]* anthracene, Pyrene, Chrysene and Fluoranthene; 4-ringed) and high molecular weight PAHs (Benzo *[a]* pyrene, Benzo *[b]* fluoranthene, Benzo *[k]* fluoranthene and Dibenzo *[a,h]* anthracene; 5-ringed; Benzo *[g,h,i]* perylene and Indeno *[1,2,3-c,d]* pyrene; 6-ringed) compounds are more resistant to breaking up by combustion particularly at temperatures below 100 ^{o}C (Essumang *et al.,* 2013).

Additionally, higher molecular weight PAHs are resistant to degradation both in aquatic organisms including fish and the environment (Ongwech *et al.,* 2013). A similar observation was made by Anyakora and Coker (2007), when they assessed PAHs content in four fish species obtained from the Niger Delta. They attributed such a difference to the fact that fish samples used in their study were procured in an environment heavily polluted by petroleum products. Linda *et al.* (2011) noted higher levels of high molecular weight compared to low molecular weight PAHs when they characterized PAHs in smoked fish products from Ghana. Their study attributed the difference to residues of previous combustion processes that might have occurred in the smoking chamber. Ongwech *et al.* (2013) also reported higher concentration levels of high molecular weight compared to low molecular weight PAHs when they analyzed PAHs in smoked *Lates niloticus* collected from three markets in Gulu District, Uganda. They attributed that difference to exposure of fish markets to heavy air pollution from automobiles as the markets are situated along Gulu-Kampala high way. Ongwech *et al.* (2013) further explained the higher levels of high molecular weight compounds in relations to the mechanism of PAHs formation in that, during prolonged smoking, chances are that low molecular weight PAHs formed are subsequently converted to high molecular weight compounds through addition of combustion products from the continued wood pyrolysis. Likewise, further burning of aromatic hydrocarbons' residues may lead to formation of additional high molecular weight PAHs and subsequently increases their concentrations in the smoked fish samples.

In this study, fish species smoked by pit technology have higher PAHs concentration levels than the corresponding *chorkor* smoked fish samples. Additionally, pit smoked samples recorded higher mean values of quantifiable PAHs than *chorkor* smoked fish samples. The difference could be explained in relations to the structural design and standardization of the smoking kilns. On one hand, *chorkor* kiln has standard structural design and materials; perforated iron sheet, wood trays, enclosed system which helps in moderate control and standardization of smoking process (oxygen and temperature). On the other hand, excessive combustion of firewood due to free air supply in pit kiln resulted in increased temperatures which consequently led to variation in the rate of fat exudation. High rate of fat exudation may lead to modification of fish surface due to the presence of oils (Linda *et al.,* 2011). The oily surface allows deposition of PAHs and other smoke chemicals on fish surface with subsequent penetration into the smoked fish muscles (Lee *et al.,* 2019).

Furthermore, differences in fat exudation as a result of elevated temperature may have increased the rate of PAHs deposition and penetration into the muscles of pit smoked fish (Alcicek, 2011; Chung *et al.*, 2011). Consistent with other studies, the rate of PAHs deposition and penetration is modified by the level of fat content on the surface and in the muscles of fish (Alcicek, 2011; Alomirah *et al.,* 2011). The presence of individual PAHs in higher levels in pit smoked fish samples could be related to excessive heat treatment and proximity of fish products to heat that may have increased the penetration of smoke chemicals into fish flesh (Forsberg *et al.,* 2013). However, the presence of low molecular weight individual PAHs in high level could be as a result of average wood combustion temperature (60-80 ^{0}C) used in the smoking process that could not indeed degrade PAHs of higher molecular weight (Yusuf et al., 2015; Slámová *et al.,* 2017). It also suggests that the wood type; *Acacia seyal* used in this experiment may probably contain smoke chemicals of low and medium molecular weight (LMW, 3 and MMW, 4-ringed PAHs).

In regards to the use of PAH4 as indicator for carcinogenicity, *chorkor* smoking can be adopted as a safer processing technology than pit smoking method. Although the results of the current study revealed that smoked fish samples from the two technologies could be deemed safe for human consumption, chances are that consumption of pit smoked fish products may pose health risk due to cumulative effect. This current study therefore, calls for regular monitoring of PAHs concentration levels especially the higher molecular weight (HMW, PAHs) compounds in smoked fish products of South Sudan.

The study concluded that the best fish quality in terms of concentration levels of polycyclic aromatic hydrocarbons were the samples smoked using improved *chorkor* kiln. This is due to the structural design and standardization of *chorkor* kiln that significantly influenced the level of polycyclic aromatic hydrocarbons' deposition, penetration and accumulation in the smoked fish

products. Adoption of improved *chorkor* oven characterized with easily controlled smoking parameters; oxygen and temperature will therefore, reduce the level of PAHs in smoked fish. Additionally, uptake of improved *chorkor* will consolidate smoked fish value chain by maintaining the quality and safety, and the quantity of smoked fish. The study recommends regular monitoring of PAHs' concentration levels especially the heavy molecular weight compounds in smoked fish products of South Sudan. Besides, other improved smoking facilities where smoke chemicals are maximally controlled to reduce the levels of PAHs in the final products to acceptable international limits should be developed and tested for adoption. Finally, tree species survey and smoke profile to minimize the use of species with higher risk of PAHs for smoking fish should be undertaken.

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