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Polycyclic aromatic hydrocarbons in smoked Lates niloticus from selected markets, Gulu District, Uganda

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Hot smoking is among the oldest methods of preservation which mankind has used in food processing. Potential health hazards associated with smoked foods may be caused by carcinogenic components of wood smoke – mainly polycyclic aromatic hydrocarbons (PAHs) and their derivatives. This paper presents results based on the determination of PAHs in smoked Lates niloticus from three markets in Gulu district, northern Uganda. The samples were analysed using gas chromatography-mass spectrometry (GC-MS). The PAHs detected in the fish samples were acenaphthylene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, chrysene, benzo[b]fluoranthene and indeno [1,2,3-cd]pyrene. The analysed samples showed PAH levels ranging from non-detectable (n.d) levels to 53.23 µg/kg of smoked fish. High molecular weight (HMW) PAHs constituted 71.1% by mass of the total PAHs detected and quantified in the samples, with indeno [1,2,3-cd]pyrene and benzo[b]fluoranthene featuring substantially in 95.8% of all the samples analysed. However, seven of the nine (77.8%) compounds detected in the samples were low molecular weight (LMW) PAHs. The higher concentrations of HMW PAHs suggest that the fish could have been smoked using soft wood or smoked for longer time and, also, may be due to the resistance of these PAHs to environmental degradation. Generally most of the samples analysed had $\sum$BaPeq and $\sum$PAH4 levels within the maximum acceptable risk limits of 5 and 30 µg/kg respectively, as recommended by European Commission Regulations for muscle meat of smoked fish. Hence the fish could therefore be deemed fit for human consumption.

Key words: Lates niloticus, polycyclic aromatic hydrocarbons; smoked fish, gas chromatography-mass spectrometry (GC-MS), toxic equivalency.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are a large class of organic compounds containing two or more fused aromatic rings without heteroatoms (Anyakora et al., 2005; Doris and Ken, 2009). The compounds are lipophilic, chemically stable and poorly degraded by hydrolysis. The majority of PAHs are readily metabolised and broken down in mammals though, some are bioaccumulated especially in organisms higher up the

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food chain (Atlas, 1991; WHO, 2000). They occur in every type of environment as complex mixtures that originate from environmental sources, industrial food processing (e.g. drying and smoking processes), packaging materials and certain alimentary practices (Agerstad and Skog, 2005; Duedahl-Olesen et al., 2006; EFSA, 2008). Pyrosynthesis and pyrolysis are two processes that may explain formation of PAHs during combustion. Lower hydrocarbons form PAHs by pyrosynthesis (Chen and Chen, 2001). Higher alkanes present in fuels and plant materials form PAHs by pyrolysis (Ravindraa et al., 2008).

Many of the heavy PAHs reported to be highly mutagenic and carcinogenic in laboratory animals have also been implicated in breast, lung and colon cancers in humans (Alonge, 1988; Simko, 2002). Some PAHs that do not exhibit carcinogenicity have been reported to act as synergists (Simko, 2002). PAHs have also been reported to cause hemato, cardio, renal, neuro, immuno, reproductive and developmental toxicities in humans and laboratory animals (Ramesh et al., 2004). Prenatal exposures of humans to PAHs have been reported to affect IQ at the age of five (Edwards et al., 2010). In 2001, PAHs were ranked the ninth most life threatening compounds to human health (King et al., 2002). More than 100 PAHs have been characterized in nature, 16 of which were classified by United States Environmental Protection Agency (USEPA), as priority pollutants. They include: naphthalene, acenaphthylene, benzo[b]fluoranthene, phenanthrene, dibenzo[a,h]anthracene, chrysene, benzo[a]pyrene, acenaphthene, benzo[k]fluoranthene, fluorene, pyrene, benzo[a]anthracene, anthracene, fluoranthene, indeno[1,2,3-cd]pyrene, and benzo[g,h,i]perylene (Chen and Chen, 2001; EPA, 1993). Benzo[a]pyrene (BaP), the most carcinogenic PAH in soot, has for long been used as a marker for the occurrence and carcinogenicity of PAHs (Simko, 2002). However, the total carcinogenic potency of all PAHs contained in a food product is 10 times higher than the content of BaP alone (SCF, 2002). European Food Safety Authority (EFSA, 2008) suggested the use of PAH8 (benzo[a]anthracene, chrysene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[k]fluoranthene, dibenzo[a,h]anthracene, indeno[1,2,3-cp]pyrene, and benzo[g,h,i]perylene) or a subgroup of these, PAH4 (the sum of the first four in PAH8) as more suitable indicators for the occurrence and toxicity of PAHs. European Commission Regulation (EC) No 1881/2006 was amended in August 2011 and the use of PAH4 as marker was proposed to take effect on the 1st September 2012 (European Commission, 2011).

Food products can be contaminated through various ways including hot smoking, contaminated soil and packaging materials, polluted air and water sources (Chung et al., 2011). Although high levels of PAHs are not usually observed in raw foods (WHO, 1998), with uncontrolled smoking procedures, PAH levels up to 200

µg/kg of smoked fish have been reported (Bonny, 1983). During smoking, heating and drying processes, combustion products come into direct contact with foods and PAH contamination can occur. The compounds have been detected in various food and non-food substances and beverages including duck meat (Chen and Lin, 1997), smoked cheese (Pagliuca et al., 2003), fish (Anyakora and Coker, 2007; Linda et al., 2011; Moret et al., 1999; Serden et al., 2010; Wretling et al., 2010) and pork (Chung et al., 2011) among others.

Gulu district, where the study was conducted, experienced over two decades of insurgency since 1986. This decimated domestic animals and smoked fish become the dominant source of animal proteins. *Lates niloticus*, the dominant fish species on the markets, is transported from the regions around Lake Victoria, over 300 km away. The catch is normally smoke cured, since consignments must be bulked and stored to await transportation to the markets. The hot smoking procedure to which the fish are subjected has been reported to generate a lot of PAHs (Larsson et al., 1983) that get diffused to the fish muscles. Very little, if any, information exists on the occurrence and levels of PAHs in the smoked fish consumed in Gulu district in particular and Uganda in general. This study aimed at establishing the presence and amounts of PAHs in smoked *L. niloticus* sold in the selected markets in Gulu district, Uganda.

MATERIALS AND METHODS

Reagents and standards

Analytical grade reagents and chemicals used, including dichloromethane, anhydrous sodium sulphate, silica gel, potassium hydroxide, cyclohexane and methanol were purchased from British Drug House (BDH, England) store in Kampala. The 16 USEPA's priority PAH standards were obtained from Supelco Inc. (Bellefonte, PA), Sigma Aldrich Co., Germany.

10 ml of each stock of PAH standard solution was prepared at a concentration of 100 mg/L in cyclohexane. The stock solutions were transferred into amber glass tubes with Teflon lined closures. The tubes, appropriately labeled with the name of the standard, and date of preparation were kept at 4°C. These stock solutions were later diluted to the appropriate concentrations to get the working standards for analysis after extraction and purification of the samples.

Sampling

Smoked fish samples were collected from three markets, G (Bobi, from Gulu municipality), R (Omoro, from Omoro county) and S (Unyama, from Aswa county) which lie 32°18'03.31"- 32°21'25.94"E and 2°33'23.84"- 2°48'59.90" N. From each of these markets, four fish samples each weighing about 500 g were purchased once a week for four weeks. The samples were collected, wrapped in aluminium foils and transported to Makerere University, Department of Chemistry, for laboratory analysis.

Extraction and purification

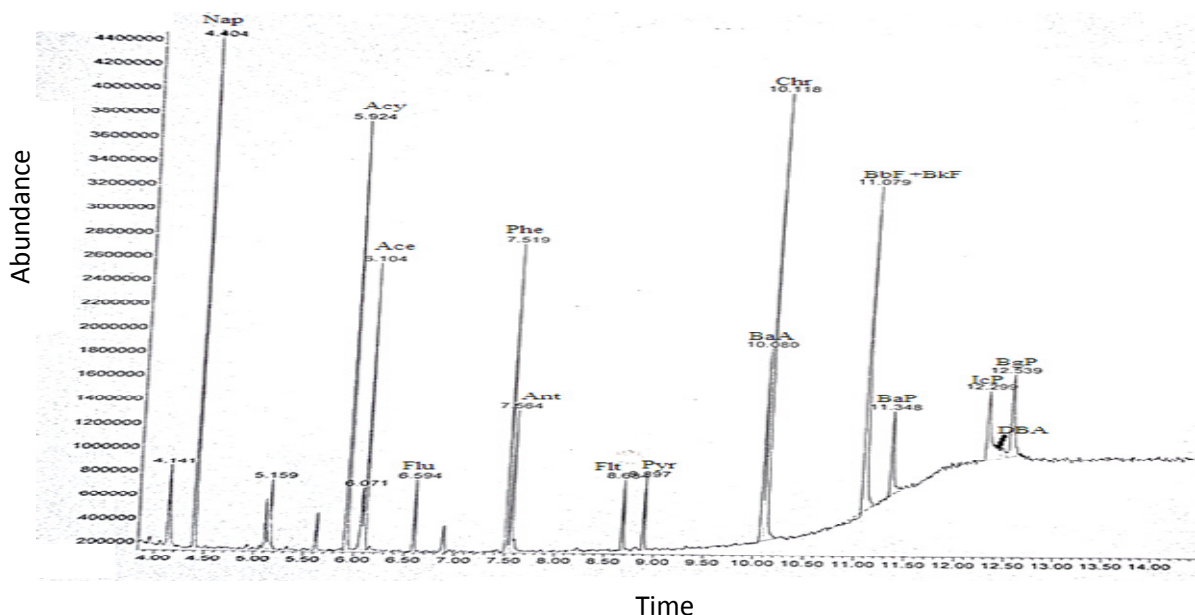The method employed (with minor modifications), were described

Figure 1. GC-MS chromatogram of PAHs standard mixture.

and used by Wretling et al. (2010) and Anyakora and Coker (2007). Homogenized fish muscles (30 g) were weighed in a 250 ml Erlenmeyer flask. The weighed amount was saponified by adding methanolic potassium hydroxide solution (methanol/water, 9:1v/v; 90 ml, 3.5 M), thoroughly sealing the flask and keeping it in a drying cabinet at 70°C for two hours. The flask was shaken after 1 h. Cyclohexane (50 ml) was added to the flask; the mixture was shaken and allowed to cool at ambient temperature. The contents in the flask were transferred to a 250 ml separation funnel, the flask rinsed with methanol/water (30 ml; 4:1 v/v) and the washing added to the funnel. The mixture was then shaken vigorously for 3 min and allowed to stand so that the layers separate. After separation, the aqueous layer was transferred to a second 250 ml separation funnel and washed with cyclohexane (30 ml). This second cyclohexane phase was combined with the first and the mixture washed with methanol/water (30 ml; 4:1 v/v), then with methanol/water (30 ml; 1:1 v/v) and three times with water (30 ml). The cleaned cyclohexane phase was then dried by passing it through anhydrous sodium sulphate, collected in a round bottomed flask and concentrated to 1 ml using a rotary evaporator. This was kept for purification.

The extracts were purified as described by Mottier et al. (2000); Wang et al. (1999). Chromatographic column (1 cm i.d. × 15 cm) was plugged with glass wool at the base. Activated silica gel was loaded in the column to a height of 5 cm. An additional 1 cm of anhydrous sodium sulphate was added to the column. The packed column was then conditioned with dichloromethane (15 ml), the 1 ml concentrate (extract) loaded onto it and eluted with dichloromethane (20 ml). The eluate was dried by passing it through anhydrous sodium sulphate, concentrated to 1 ml using a rotary evaporator and transferred to an amber glass sample vial for gas chromatography-mass spectrometry (GC-MS) analysis. The vials were kept at 4°C prior to the analysis.

Gas chromatographic analysis

An Agilent 6890N network GC system equipped with a 5975 MSD, 7683B auto sampler and a Zebron, ZB-5MSi column was used to

separate the compounds. The system was operated in a constant flow mode with an initial rate of 4.9 ml/min, nominal initial pressure of 35.53 psi and average velocity of 81 cm/s. The oven was initially set at 55°C for 1 min, ramped to 320°C at 25°C/min and held for 3 min.

The mode of injection used was pulsed (1.0 μl) splitless at an initial temperature of 300°C and pressure 35.53 psi, until 0.2 min. The purge flow rate was 30 ml/min with a purge time 0.75 min. The carrier gas was helium (99.999%) at a constant flow rate of 45 cm/s. The detector source was set at 300°C, quadrupole at 180°C, transfer line at 280°C; scan range at 45 to 450 amu and electronic impact at 70eV with a solvent delay of 3.75 min. PAHs were identified based on the match in the retention times of the compounds in the samples against those of the PAH standards (Figure 1). A retention time match of ± 1% was considered for confirmation (Samuel et al., 2010). Mass spectral data base library search was also performed for retention time match outside this window. A total of nine PAHs were identified in the samples analysed (Figure 2).

Quantification of the PAHs

This was based on absolute calibration methods. A minimum of four concentration levels of the standards ranging from 0.1 to 15 ppm were injected into the GC-MS and calibration curve for each standard was obtained by plotting peak area against concentration of the standards. The regression equation and coefficient of determination (r^2) were determined. Each PAH in the sample was then quantified using the formula;

$$As(ppb) = \frac{Xs \times 100}{Ws \times R}$$

where, As is the concentration of PAH in the sample in μg/kg, Xs 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.
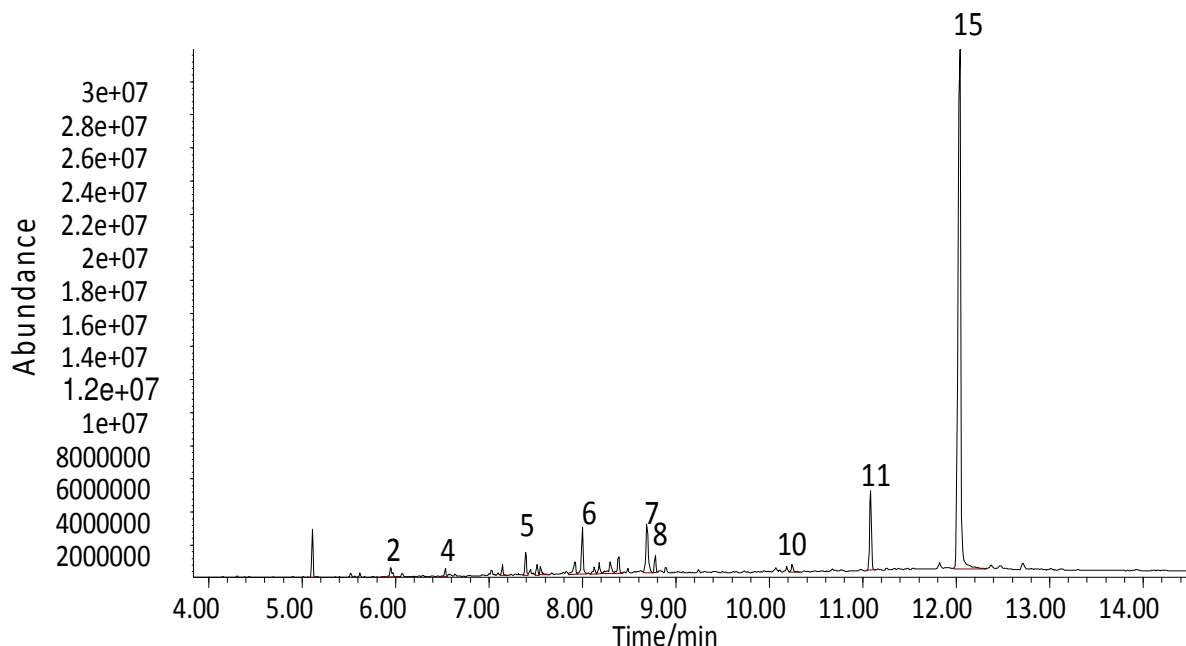
Figure 2. The 9 PAHs identified in the samples analysed: 2- acenaphthylene, 4- fluorene, 5- phenanthrene, 6- anthracene, 7- fluoranthene, 8- pyrene, 10- chrysene, 11- benzo[b]fluoranthene, 15- indeno[1,2,3-cd]pyrene.

Quality control

Both method's (prepared at a frequency of 12.5%), and solvent blanks were included in every run during the analysis. Individual reference standards were used to identify and quantify the PAHs. Arithmetic means and standard deviations were calculated from positive quantifiable samples only, and in all cases, the differences were considered significant, if the exact p value was $\alpha \leq 0.05$. A computer programme XLSTAT (version 7.5.2) was used for computation. Linearity was established by computing the relative standard deviations (RSDs) and observing the coefficient of determination (r^2) values. The RSDs obtained were all below 10% while the r^2 values ranged from 0.9930 to 0.9999.

To establish the mean percent recoveries, a set of three aliquots (30 g) of the homogenized fish muscles were spiked with 50 µl mixture of the 16 PAH standards of concentration ranging from 2 to 10 µg/L. Another set of three aliquots (30 g) were set as controls. Both the spiked and the control aliquots were allowed to stay overnight to allow for absorption of the added PAHs into the matrix. The two sets of the aliquots were then taken through the same analytical procedures. The percent recoveries were then computed from the formula

$$\mathrm{Re\,cov}\,ery = \%R = \frac{Cs - Cu}{Cn} \times 100$$

Where, Cs is the concentration of PAHs in the spiked sample, Cu is the concentration of PAHs in the unspiked (control) sample, Cn is the (nominal) theoretical concentration increase that results from spiking the samples.

The mean percent recoveries obtained were within the range (70 to 130%) with a mean of 80.23%. These values were relatively quantitative and were therefore used without any correction. Limits of detection (LODs) and limits of quantification (LOQs) ranged from

0.01 to 0.08 µg/kg and 0.04 to 0.24 µg/kg respectively.

RESULTS

PAHs in smoked *Lates niloticus*

In the samples from three sampled markets, 9 PAHs were detected in the smoked fish (*L. niloticus*) Figure 2, and the total concentrations were 58.10, 23.40 and 43.20 µg/kg at sites G, R, S, respectively, as shown in Table 1. Five, nine and eight PAHs were identified and quantified in samples from sites R, G and S, respectively. Acenaphthylene was present in samples from both sites G and S while indeno[1,2,3-cd]pyrene featured prominently in all except in samples from site S. Fluorene was in all samples collected from all the sites. Most of the PAHs (77.8%) identified in the samples were low molecular weight (LMW, 3-4 ringed) compounds though there were high concentrations of the HMW PAHs (indeno [1,2,3-cd]pyrene and benzo[b]fluoranthene).

Carcinogenity of PAHs

Toxic equivalency as a measure of carcinogenicity of PAHs

The carcinogenic potency of each PAH identified in the samples collected was also determined in terms of BaP

Table 1. Levels of PAHs (µg/kg) in smoked *L. niloticus* from three markets in Gulu district.

PAH	Sample collection site n = 16		
	G (Bobi)	R (Omoro)	S (Unyama)
Acenaphthylene	2.5 ± 0.5	n.d	2.50
Fluorene	0.9 ± 0.2	0.7 ± 0.1	0.85
Phenanthrene	5.5 ± 3.3	1.5 ± 0.1	5.54
Anthracene	3.7 ± 2.6	n.d	3.67
Fluoranthene	4.9 ± 1.3	n.d	4.85
Pyrene	3.4 ± 1.0	n.d	3.40
Chrysene	1.5 ± 1.0	4.4 ± 0.1	1.54
Benzo[b]fluoranthene (HMW)	9.5 ± 3.8	3.8 ± 2.7	9.46
Indeno[1,2,3-cd]pyrene (HMW)	26.2 ± 8.6	13.0 ± 5.6	26.18

Results are presented as mean ± standard deviation for samples from the three sites, n.d, not detected (p value 0.001).

Table 2. Toxic equivalency factors (TEF) for the 16 priority PAHs (Nisbet and LaGoy, 1992).

PAH	TEF	PAH	TEF
Naphthalene	0.001	Benzo[a]anthracene	0.1
Acenaphthylene	0.001	Chrysene	0.01
Acenaphthene	0.001	Benzo[b]fluoranthene	0.1
Fluorene	0.001	Benzo[k]fluoranthene	0.1
Phenanthrene	0.001	Benzo[a]pyrene	1.0
Anthracene	0.01	Indeno[1,2,3-cd]pyrene	0.1
Fluoranthene	0.001	Dibenzo[a,h]pyrene	1.0
Pyrene	0.001	Benzo[g,h,i]perylene	0.01

equivalent concentration, commonly referred to as the toxic equivalency (TEQ). A list of toxic equivalency factors (TEFs), completed by Nisbet and LaGoy (1992) (Table 2), was used to estimate the carcinogenic potency of total PAHs (that is, total BaP equivalent concentration) using the formula:

$$TEQ = (PAH_i \times TEF_i)$$

Where TEQ is the toxic equivalency of the reference compound, PAH_i and TEF_i the concentration and toxic equivalent factor of congener *i*.

The computed BaPeq (TEQ) values for PAHs detected and identified in the smoked fish sampled are shown in Table 3. Indeno[1,2,3-cd]pyrene had the highest mean TEQ, while Fluorene had the lowest. The sum TEQ of the PAHs in sampled area is G> S >R.

PAH4 as a measure of carcinogenicity of PAHs

Based on the conclusions of the Scientific Panel on Contaminants in the ood Chain (CONTAM Panel), new maximum levels for PAH4 were introduced whilst maintaining a separate one for BaP. The use of PAH4 ensure that PAH levels in foods are kept at low levels and that the amount of PAH can also be controlled in those samples in which BaP is not detectable, but where other PAHs are present. European Food Safety Authority (2008) concluded that the use of PAH4 should be preferred to PAH8 since the latter would not provide much added value.

Furthermore, both Scientific Committee on Foods (SCF, 2002) and Joint Expert Committee on Food Additives (JECFA, 2005), concluded that a TEQ approach to assessment of carcinogenic potency of PAHs was not very appropriate due to limitations in the available data and because of the different modes of action amongst different PAHs (EFSA, 2008). The use of PAH4 was also employed to estimate the carcinogenic potency of the PAHs detected and quantified in the samples (Table 4). The PAH4 values were 11.00, 8.14, and 10.98 µg/kg for sites G, R and S, respectively. The PAH4 values for each sample and for each of the sites were below the maximum level (30 µg/kg) set by Regulation (EU) 835/2011, for muscle meat of smoked fish and smoked fishery products.

Table 3. BaPeq PAH concentrations detected in the *L. niloticus* samples.

PAH	Site of sample collection				
	G	R	S	∑TEQ	Mean TEQ
Acenaphthylene	0.003	n.d	0.002	0.005	0.002
Fluorene	0.001	0.001	0.001	0.003	0.001
Phenanthrene	0.003	0.002	0.002	0.007	0.002
Anthracene	0.037	n.d	n.d	0.037	0.012
Fluoranthene	0.005	n.d	0.007	0.012	0.004
Pyrene	0.003	n.d	0.003	0.006	0.002
Chrysene	0.015	0.043	0.04	0.098	0.033
Benzo[b]fluoranthene	1.175	0.382	0.701	2.258	0.753
Indeno[1,2,3-cd]pyrene	2.618	1.303	1.767	5.688	1.896
∑TEQ	3.860	1.731	2.523	8.114	2.705

n.d = not detected, BaPeq = BaP equivalent concentration. TEQs were calculated on the basis of the content and TEFs for the 16 PAHs as given by Nisbet and LaGoy (1992).

Table 4. Levels of PAHs, PAH4 and PAH8 detected and quantified in the samples.

PAH	Levels of PAHs, PAH4 and PAH8 detected and quantified in the samples site of sample collection		
	G	R	S
Acenaphthylene	2.50	n.d	1.59
Fluorene	0.85	0.74	0.92
Phenanthrene	5.54	1.51	1.52
Anthracene	3.67	n.d	n.d
Fluoranthene	4.85	n.d	7.13
Pyrene	3.40	n.d	2.92
Chrysene	1.54	4.32	3.97
Benzo[b]fluoranthene	9.46	3.82	7.01
Indeno[1,2,3-cd]pyrene	26.18	13.03	17.67
∑PAH	56.14	23.41	42.73
∑PAH8	37.18	21.17	28.65
∑PAH4	11.00	8.14	10.98

n.d = not detected, PAH4 is the sum of benzo[a]anthracene, chrysene, benzo[b]fluoranthene and benzo[a]pyrene.

DISCUSSION

The relatively high levels of PAHs could be attributed to smoking of the fish. In a similar study by Olabemiwo et al. (2011), acenaphthylene, fluorene and indeno [1,2,3-cd] pyrene were not detected in non-smoked fish (used as control) but were present in all the smoked samples. Smoking as a method of preserving fish could have contributed to PAH content and amount in the smoked fish since they were not detected in the control samples by Olabemiwo et al. (2011).

In most of the samples (77.8%), low molecular weight (LMW, 3 to 4 ringed) compounds were detected. This could possibly be traced to the type of wood used during the smoking process. Studies by Pagliuca et al. (2003) showed that smoke produced by woods of deciduous trees (hard woods) show high concentrations of low molecular weight PAHs. However, although more of the LMW PAHs were detected, their concentrations were much lower than those of the high molecular weight (HMW, 5-6 ringed) PAHs that constituted up to 71.1% of the total mass. This can be attributed to the fact that the HMW PAHs are more resistant to degradation both in the fish and the environment. A similar profile was noted by Anyakora and Coker (2007), when they assessed PAH content in four species of fish from the Niger Delta. Linda et al. (2011) also noted higher concentrations of HMW compared to LMW PAHs when they characterized PAHs in smoked fish from Ghana. They attributed that difference to residues of previous pyrolytic processes that

occurred in the smoking chamber.

The high concentrations of indeno [1,2,3-cd]pyrene and benzo[b]fluoranthene, Table 1, could also be explained from the mechanism of formation of the PAHs. Pagliuca et al. (2003) noted that vendors sometimes re-smoke the fish in order to increase their shelf life. Linda et al. (2011) noted that during re-smoking, it is possible that the pyrolytic products from the wood combustion add to the intact PAH molecules forming HMW PAHs. Similarly, during prolonged smoking, chances are that the LMW PAHs formed are subsequently converted to the HMW compounds through addition of the pyrolytic products from the continued wood combustion. Furthermore, pyrolysis of aromatic hydrocarbon residues leads to the formation of additional HMW PAHs and consequently increases their concentrations in the samples (Guillen and Sopelana, 2004).

The difference in the number of PAHs from the sites could be due to the difference in the setup of the markets. Sites G and S are enclosed markets (with lockup shops) while site R is an open space close to the busy Gulu-Kampala highway. It is therefore possible that the intense heat and free wind movement at site R could have facilitated the volatilization and loss of especially the LMW PAHs from the fish. Furthermore, such open space could have allowed atmospheric fallouts of such compounds as sulphurdioxide, ozone and oxides of nitrogen with which PAHs are known to react readily forming sulphonic acid, diones, nitro and dinitro PAHs respectively (ATSDR, 1995). It is also important to note that the commercial samples used in the study were smoked and handled differently and that could result in varying PAH load. That could explain the slight variation in the PAH profile noted from the three sites particularly if these fish were not obtained from the same smoking kiln.

BaP, the marker for the occurrence and carcinogenicity of PAHs was not detected in any of the samples analysed. Similarly, a study by Olabemiwo et al. (2011), reported that BaP was conspicuously absent in both the control and smoked fish samples. In another study, Anyakora and Coker (2007), reported BaP levels as high as 2.32 µg/kg in *Clarias garieppinus* which they attributed to the fact that the fish were caught in highly polluted rivers of the Niger Delta region.

The reason for the absence of BaP in the samples could therefore be that the fish were not caught from aquatic environments polluted by petroleum. However, according to Lawrence and Weber (1984), the ability of fish to metabolise PAHs may also explain why BaP is not often detected or found only at very low levels in fish from environments heavily contaminated with PAHs. On the other hand Mottier et al. (2000), noted that a principal loss normally occurs due to partitioning of BaP into the alkaline methanolic saponification solvent. Takatsuki et al. (1985) added that such partitioning leads to formation of emulsions that are difficult to breakdown hence lowering the concentration of free BaP. A study by Larsen

and Poulsen (1987) as cited in Moret et al. (1999), concluded that smoked fish normally contains 0.1-0.5ppb of BaP but added that the level could be exceeded in heavily smoked products.

The total toxic equivalency (TEQ), (Table 3), for all samples considered at the three sites was 8.114 µg/kg, with a mean value of 2.705 µg/kg. This total was above the maximum risk limit for BaP level set by European Commission Regulation (EU) 835/2011 but the mean was below the limit for muscle meat of smoked fish. The sum of TEQ for each of the sites considered was 3.860, 1.731 and 2.523 µg/kg for sites G, R, S, respectively. All these values were within the maximum tolerable risk limit for BaP in muscle meat of smoked fish. Values for site G and S were however above that for muscle meat of fish other than smoked fish, set at 2 µg/kg (European Commission, 2011). The total TEQ for indeno[1,2,3-cd]pyrene(IcP), (5.688 µg/kg) was just above the limit while the mean (1.896 µg/kg) was well within the limit for BaP in muscle meat of smoked fish. According to earlier studies (Mottier et al., 2000), BaP could have simply been degraded during saponification and could have actually added to these TEQ values. There is therefore need to monitor the levels of PAHs especially the HMW components in the smoked fish sold in these markets. However this value is the total for all the samples from the three sites and would only raise serious health concern if an individual consumes all the samples and accumulate these PAHs without any breakdown or metabolism. However, studies have shown that PAHs are readily metabolised or broken down even in humans (WHO, 2000). This could therefore allay the fears of eating smoked *L. niloticus* from markets in Gulu.

Based on the use of PAH4 as indicator for the carcinogenicity of PAHs, (Table 4), smoked *L. niloticus* from each of these three markets could be deemed safe for human consumption. However, there is need for regular monitoring of the concentrations of especially the HMW PAHs in the smoked fish from these markets.

Conclusion

Traditionally smoked *L. niloticus* from selected three markets from Gulu district were found to contain BaP at undetectable levels as in none of the analysed samples was detected. Highest single concentration of PAH was noted for indeno[1,2,3-cd]pyrene from a smoked fish sampled from site R (53.23 µg/kg), while the lowest single quantifiable amount was for Fluorene (0.69 µg/kg) sourced from site G. Five PAHs were detected in samples from R, eight from S and nine from G. Generally the samples from the different sampling sites exhibited fairly uniform profiles, suggesting similarity in source of contamination. Benzo[b]fluoranthene and indeno[1,2,3-cd]pyrene were most representative, each being found in 95.8% of all samples analysed. The samples contained

varying amounts of both the LMW and HMW PAHs that could be attributed to the smoking process. BaPeq concentrations commonly referred to as TEQ values were within tolerable risk levels (5 µg/kg), set by European Commission regulations. The use of PAH4 as marker for the occurrence and carcinogenicity of PAHs was also employed and mean values obtained for individual samples (n = 3), and individual sites were all within the maximum tolerable risk limits (30 µg/kg), set by European Commission Regulation (EU) 835/2011, for muscle meat of smoked fish.

Generally, the fish could be deemed fit for human consumption. However, the higher levels obtained for the HMW PAHs and the inclusion of exposures from other dietary sources can augment the risk levels hence there is need for regular monitoring of the levels of PAHs in the smoked fish.

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