

Effects of Traditional Smoking Methods on the Concentrations of Polynuclear Aromatic Hydrocarbons (PAHs) in Some Species of Smoked Fish Traded in Benue State, Nigeria

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Abstract

The effects of three traditional smoking methods on the concentrations of polynuclear aromatic hydrocarbons (PAHs) in smoked fishes were studied. Samples of five different species of fish highly traded for immediate consumption were purchased from fishermen and processed using saw dust smoke, firewood smoke and charcoal smoke respectively. Some of the fresh fishes were also sun-dried and analyzed as control. The PAHs content were extracted with standard dichloromethane using solid-liquid extraction, and analyzed using Gas chromatography – Mass spectrophotometer (GC-MS) method. The results showed that fish samples processed with saw dust smoke recorded the highest concentrations of total PAHs, ranging from 815.75 µg/kg to 1550.28 µg/kg, followed by firewood smoked samples with total PAHs content varying between 738.14 µg/kg to 994.09 µg/kg while charcoal smoked samples recorded the least total PAHs levels of 135.02 µg/kg to 614.42 µg/kg. Benzo(a)pyrene concentrations of 5.68 µg/kg and 5.44 µg/kg respectively were detected in the samples of *Arius heude loti* and Mud minnow processed using saw dust smoke. The Benzo(a)pyrene levels exceeded the EC regulatory limit of 5 µg/kg. Because benzo(a)pyrene has been associated with intense carcinogenicity in humans, its levels recorded in the smoked *Arius heude loti* and Mud minnow may have implication for the quality and safety of these fish products. Therefore, it is imperative that regulatory bodies conduct awareness campaigns to educate both the smoked fish processors, traders and consumers on the need to discourage the use of saw dust in smoking fish and adopt safer and improved methods of smoking fishes.

Keywords: polynuclear aromatic hydrocarbons, traditional smoking methods, smoked fish, consumers

1. Introduction

Polynuclear aromatic hydrocarbons (PAHs) constitute the largest class of chemical compounds containing two or more fused aromatic rings made up of carbon and hydrogen atoms, known to be genotoxic agents (EC, 2002). They are generally classified as relatively persistent organic environmental contaminants (Deshpande, 2002; Harvey, 1997; Martson et al., 2001), formed during incomplete combustion processes which occur whenever wood, coal or oil are burnt (Ciecierska & Obiedzinski, 2007); with combustion sources being the most predominant (Simko, 2002). PAHs contamination can significantly affect smoked fish quality and safety. The United States Environmental Protection Agency (USEPA) and European Union (EU) identified PAHs as potential carcinogens and listed them among prioritized pollutants (EC, 2006). These contaminants are widespread in foodstuffs not only as a result of environmental pollution but also as a consequence of some thermal treatments, which are used in the preparation and manufacture of foods (Guillen et al., 1997; Philips, 1999). Human exposure to PAHs occurs in three ways: inhalation, dermal contact and consumption of contaminated foods (Silva et al., 2011), with diet being the major source as it accounts for 88 to 98% of such contamination (Farhadian et al., 2011).

Fish is one of the most common sources of animal protein in most parts of the World being consumed in various forms as either fresh, fried, canned (Silva et al., 2011) and as smoked products. Smoked fish marketing continues to expand as a vast economic activity and livelihood component of the communities living along river Benue,

particularly among women since it can be carried out with minimal investment. The production of fish forms a reasonable source of revenue base for a nation which underscores its importance (Al-Jufaili & Opara, 2006). Although, fish is a very important diet, its high moisture content posed a major challenge of spoilage by microbial activities on prolong storage under high ambient temperatures (Deudahl-Olesen et al., 2006). Several attempts have been made to reduce fish spoilage to the minimum through improved preservation technologies since at harvest time, the products availability is usually in excess of the demand. It has been reported that out of the total of 194,000 metric tons of dry fish produced in Nigeria, about 61% of it was smoked (Silva et al., 2011). The smoking preservation has become a means of offering diversified, high value added products as an additional marketing option for certain fish species where fresh consumption becomes limited (Gomez-Guillen et al., 2009).

Food processing methods such as smoking, drying, roasting, baking, and frying have been recognized as major sources of food contaminations by PAHs (EC, 2002; CCFAC, 2005; Moret et al., 2005; Yurchenko & Molder, 2005). In the smoked foods however, the actual levels of PAHs contamination depend on several variables including the type of smoke generator, combustion temperature, and degree of smoking (Garcia-Falcon & Sermal-Gandara, 2005).

The traditional smoking techniques of fishes involve drying pre-salted, whole, or filleted fishes with smoke produced during combustion of firewood, saw dust, and charcoal. This process may lead to contamination of smoked fishes with PAHs if the process is not adequately controlled or if very intense smoking procedures are employed (Gomez-Estaca et al., 2011). Considering the quality and safety of dried fishes for human consumption, smoking may not be an appropriate method of fish preservation. At present however, it is widely being used due to the cheap materials and low technology required in the smoking process. Therefore, it becomes imperative to monitor the effect of smoking on PAHs concentration of the processed fishes. This study investigated the effects of firewood smoke, saw dust smoke and charcoal smoke on the concentration of PAHs in five species of fishes (*Arius heudeloti*, *Cynoglossus senegalensis*, *Clarias gariepinus*, *Blunt hwake* and *Mud minnow*) sold as high delicacies in two major fish markets in Benue State Nigeria.

2. Materials and Methods

2.1 Study Area

The two major fish markets selected for this study are Abinsi (Guma Local Government Area) and Wadata (Makurdi Local Government Area) of Benue State Nigeria. At present, these areas are the biggest hub for smoked fish market in the Guma/Makurdi constituency in North-Eastern part of Benue State. Makurdi lies on latitude 7°30'N and longitude 8°35'E (Amuta et al., 2008) while Guma lies on latitude 13.08°N and longitude 7.88°E (Urbonu, 2011). In these areas, harvested fishes are mostly processed by hot smoking using traditional kiln (oven) lined either with firewood, saw dust or charcoal. The smoke produced by burning these materials helps to dry the fish laid on wire mesh placed over the kiln. Fresh leaves are sometimes, used to cover the fish on wire mesh to ensure proper circulation of smoke. Smoke-drying of fish with the traditional kiln usually takes between 2 to 8 hrs depending on the weight of the fish and intensity of smoke produced and these may constitute significant factors in the degree of PAHs contamination of the product.

2.2 Sample Collection

Five fresh samples each of *Clarias gariepinus*, *Arius heude loti*, *Cynoglossus senegalensis*, *Mud minnow* and *Blunt hwake* were bought from the fishermen at Abinsi in Guma Local Government Area and Wadata in Makurdi Local Government Area in March, 2013 during the dry season. The fish samples were identified and authenticated by a zoologist in the Department of Biological Sciences, Faculty of Science, Benue State University, Makurdi Nigeria.

2.3 Sample Preparation

The fishes were washed to remove adhered sand particles and then placed on wire mesh laid above the traditional kilns. Three kilns with different heat sources: firewood (mahogany), saw dust and charcoal respectively were used to smoke the fishes. The fishes were smoked under the same temperature conditions for 8 hours until they are fully dried and become brittle. Each species of the dried fishes were then homogenized using porcelain mortar and pestle, sieved through a 250 μm^2 sieve and packaged in labeled polythene bags according to the smoke methods prior to extraction.

2.4 Extraction of PAHs

The method used for extraction of PAHs in the samples was based on the solid-liquid extraction procedure described by Ajai et al. (2012). A 5 g anhydrous sodium sulphate (Na_2SO_4) and 5 pre-cleaned glass beads were

added into a pre-cleaned extraction flask. Then, 5 g of the sample was placed in a separatory funnel into which 20 ml of dichloromethane was added and the funnel tightly closed. The flask was shaken vigorously until slurry was formed after which anhydrous Na_2SO_4 was added and shaken vigorously to produce free flowing finely divided slurry. Then, the mixture was centrifuged and the solvent layer decanted into a collecting flask through a small glass funnel containing a layer of anhydrous Na_2SO_4 over a plug of glass wool. The extract was then filtered into a 25 cm^3 concentrator flasks using a glass funnel packed with plug of glass wool. The extraction procedure was repeated twice with 5 cm^3 of the extracting solvent in each case after which the extracts were combined and transferred into a flask. Then, boiling chips were added to the extract and concentrated on a water bath to reduce the volume to approximately 1 cm^3 ; cooled to room temperature and further concentrated using a Ribby R.E. 200B Vacuum rotary evaporator B890/528. The extract was then transferred into amber-coloured bottles and stored in a refrigerator prior to clean up.

The sample clean up was done using Gel Permeation Chromatography (GPC). The extracted samples were purified and conditioned using dichloromethane as described by Ajai et al. (2012).

2.5 Analyses of the Extracts

Gas chromatography-Mass spectrometry (GC-MS) method was used to analyze the PAHs levels of the extracts at the Laboratory Light House, Petroleum Company Limited Effurum Warri, Delta State-Nigeria.

3. Results

3.1 Performance Characteristics of the Smoking Methods

Table 1 shows the performance characteristics of the saw dust, firewood and charcoal smokes used in drying the fishes. It can be inferred from the table that the intensity of smoke produced differed with the smoke medium. Saw dust emitted the highest smoke intensity but it generated the lowest heat energy followed by firewood which produced moderate smoke and heat intensities. Charcoal produced the lowest smoke intensity but the highest amount of heat during the smoking process. Fish samples processed using charcoal smoke dried fastest, with all the different species taking less than 4 hrs to dry fully (become very brittle). Samples dried with firewood smoke took between 4 to 6 hrs to dry fully while samples dried using the saw dust smoke took longer time (> 6 hrs) to dry fully.

Table 1. Performance characteristics of the smoking methods

Smoking method	Observation	Drying process	Drying Duration
Charcoal	Low smoke intensity and glowing red hot	Fishes dry very fast	Less than 4 hrs
Firewood	Moderate smoke and red hot	Fishes dry slowly	4 to 6 hrs
Saw dust	High smoke intensity and less hot to touch	Fishes dry very slow	Greater than 6 hrs

3.2 Concentrations of Individual PAHs in *Arius heude loti*

Figure 1 shows the concentrations of the individual PAHs in *Arius heude loti* processed by the different smoking methods. The results revealed that most of the individual PAHs were recorded in the samples processed using saw dust smoke. However, the samples processed with charcoal smoke recorded the highest level of anthracene and phenanthrene while samples processed using firewood smoke recorded the highest level of pyrene and Benzo(a)anthracene. Most of the individual PAHs were not detected in the *Arius heude loti* samples dried using sun heat as control except acenaphthylene, acenaphthene and naphthalene. This suggests that smoke is the major contributor of the PAHs contamination in the processed fishes.

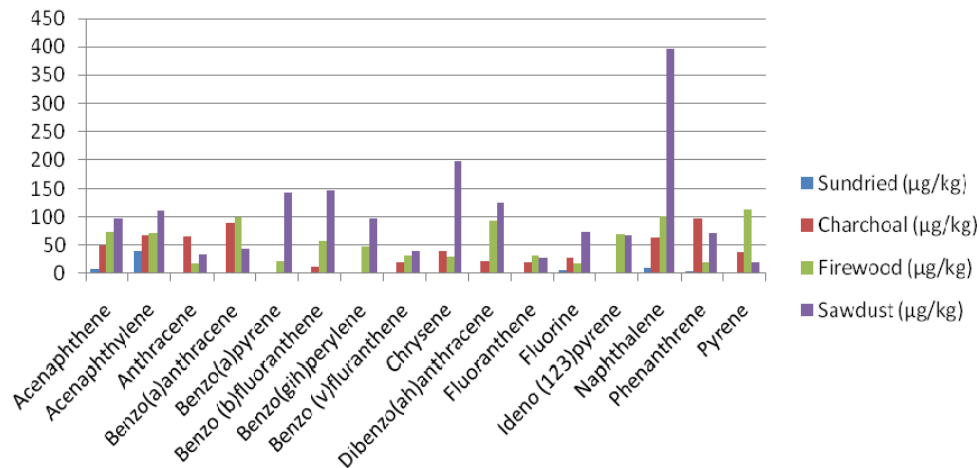


Figure 1. Concentration of individual PAHs in *Arius heude loti* according to the smoking methods

3.3 Concentrations of Individual PAHs in *Cynoglossus senegalensis*

Figure 2 shows the concentration of the individual PAHs recorded in *C. senegalensis*. The trend of the PAHs contamination also revealed that most of the PAHs were recorded in the samples processed using saw dust and firewood smoke respectively. *C. senegalensis* samples dried using charcoal smoke only recorded naphthalene and acenaphthylene. The results also showed that benzo(a)pyrene was not detected in any of the *C. senegalensis* samples processed using all the smoking methods.

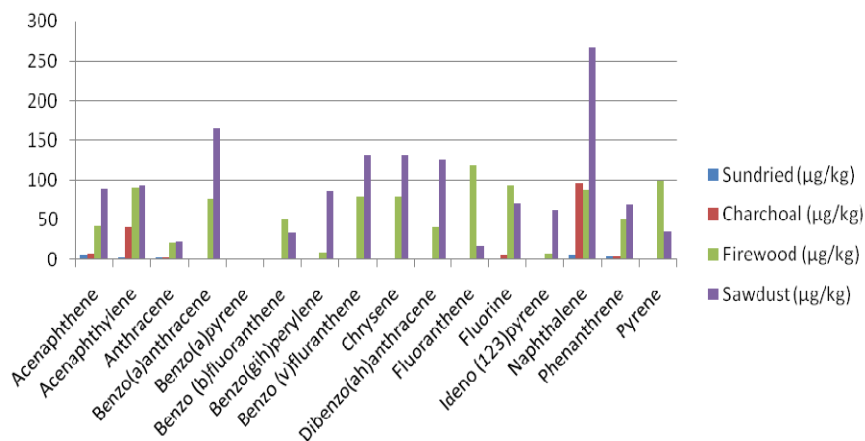


Figure 2. Concentrations of individual PAHs in *C. Senegalensis* according to the smoking methods

3.4 Concentration of Individual PAHs in *Mud minnow*

The results in Figure 3 shows the individual PAHs recorded in the *Mud minnow*. The samples dried using saw dust and firewood smoke respectively recorded higher PAHs contamination. Samples processed using charcoal smoke recorded low levels of naphthalene, acenaphthylene, acenaphthene while benzo(a)pyrene was not detected in all the mud minnow samples processed using the different methods.

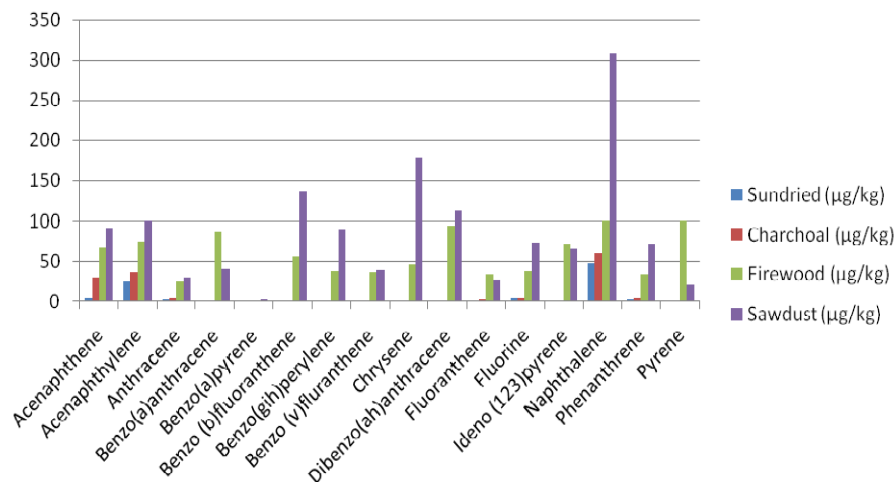


Figure 3. Concentrations of individual PAHs in *Mud minnow* according to the smoking methods

3.5 Concentrations of Individual PAHs in *Clarias gariepinus*

The results of the individual PAHs recorded in *Clarias gariepinus* samples processed by the various smoking methods (Figure 4), also recorded higher levels of the individual PAHs in the samples processed using saw dust and firewood smoke. No PAH was detected in the sun-dried samples while only the samples processed using saw dust smoke recorded benzo(a)pyrene.

3.6 Concentrations of Individual PAHs in *Blunt hwake*

The results presented in Figure 5 show the individual PAHs concentration of the smoked *Blunt hwake*. Higher levels were also recorded in the samples processed using saw dust and firewood smoke. No PAH was recorded in the sun-dried samples. Also, benzo(a)pyrene was not detected in all the processed samples while benzo(g,h,i)perylene was only detected in the samples dried with saw dust smoke.

3.7 Concentrations of Total PAHs in the Fishes

The results in Figure 6 show that the concentrations of total PAHs in *Arius heude loti* samples were 1,550.28 µg/kg, 884.17 µg/kg, 614.42 µg/kg and 70.51 µg/Kg respectively for the saw dust, firewood, charcoal smoked and sundried samples. In the *Mud minnow* samples, the concentrations of total PAHs were: 1372.90 µg/kg saw dust smoked, 887.68 µg/kg firewood smoked, 135.02 µg/kg charcoal smoked and 79.96 µg/kg sundried (control). The *Blunt hawke* recorded total PAHs levels of 815.75 µg/kg in the saw dust smoked sample, 738.14 µg/kg in the firewood smoked, and 136.15 µg/kg in the charcoal smoked samples. *C. senegalensis* recorded 1,301.26 µg/kg in the saw dust smoked samples, 891.35 µg/kg in the firewood smoked and 158.62 µg/kg in the charcoal smoked samples. The sundried *C. senegalensis* recorded 28.94 µg/kg total PAHs. *Clarias gariepinus* recorded 1,118.05 µg/kg, 994.09 µg/kg, and 373.62 µg/kg total PAHs in the saw dust, firewood and charcoal smoked samples respectively. No record was obtained for total PAHs concentration in the sundried samples of *Clarias gariepinus* and *Blunt hawke*.

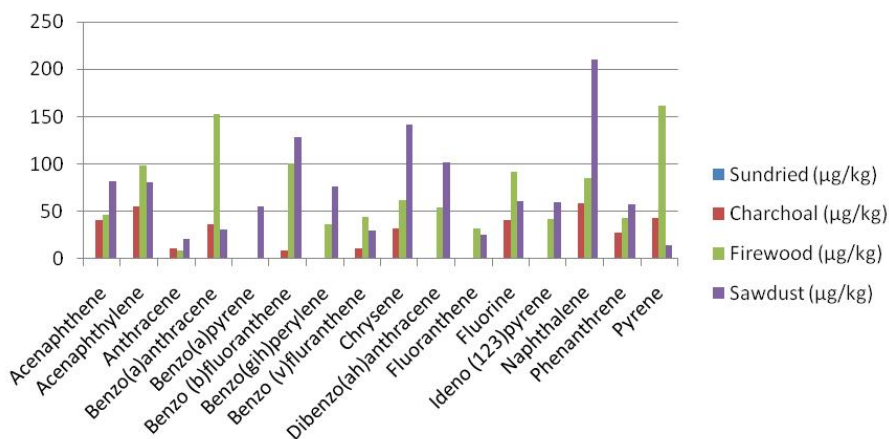


Figure 4. Concentrations of individual PAHs in *Clarias gariepinus* according to the smoking methods

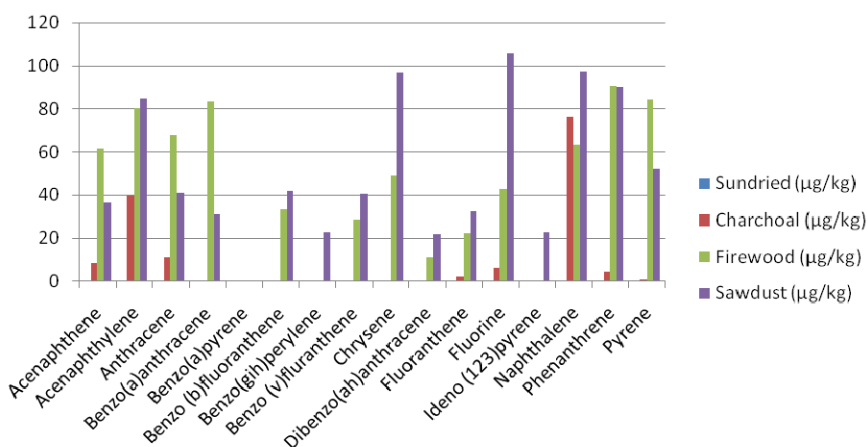


Figure 5. Concentrations of individual PAHs in *Blunt hwake* according to the smoking methods

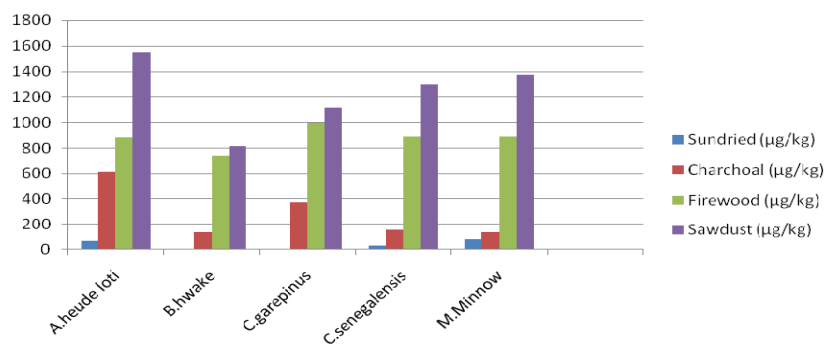


Figure 6. Concentrations of total PAHs in the fishes according to the smoking methods and sun drying

4. Discussion

4.1 Performance Characteristics of the Smoking Methods

Generally, the intensity of heat produced by the smoking material (Table 1) determines how fast the fish species dried fully and hence, the contact duration with the smoke. Among the traditional smoking methods used to smoke the fishes in this study, saw dust generated the highest smoke intensity but low temperature. This may be due to the fact that during the combustion of saw dust, only the outer layer makes direct contact with air, making it difficult for the inner part to burn. This leads to a very slow and incomplete burning with intense smoke but

less heat energy being produced. Consequently, the smoke has longer contact time with the fishes before they dry fully and become brittle. The long duration of drying may also have implication for the PAHs contamination of the smoked fishes. Firewood produced moderate intensity of smoke and heat during the combustion because a greater proportion of its surface is exposed to air which facilitates more burning and hence, generating higher heat energy. This led to faster drying up of the fishes than the saw dust smoke. Charcoal however, produced the least intensity of smoke and highest temperature because most of the numerous compounds found in wood and saw-dust which may inhibit burning have been burnt off during the initial process of charcoal production leaving a relatively pure carbon (charcoal). Thus, the charcoal combustion is almost complete giving off high amount of heat energy which made the fishes to dry very fast. The fish samples dried with the charcoal have therefore, less contact duration with the smoke. In this way, they may absorb less of the PAHs-bearing smoke generated during combustion and hence, low level of PAHs contamination of the fishes. This suggests that charcoal smoking is a safer traditional method of drying fish in terms of PAHs contamination of smoked fish.

4.2 Concentrations of Individual PAHs in the Dried Fishes

Most of the individual PAHs (Figures 1-5) were recorded in the smoke-dried fishes processed using saw dust and firewood smoke respectively. This may be attributed to the longer drying times as a result of the less heat and high smoke produced by saw dust and firewood leading to prolonged fish contact with the smoke, being the major source of the PAHs contamination. Generally, all the fish samples processed using charcoal smoke recorded less PAHs. This may be due to the short drying time as a result of the high heat and less smoke produced by the charcoal. In a similar study, Silva et al. (2011) also observed that at high temperatures, less smoke was produced and at lower temperatures, more smoke was produced during the smoking process. Among the sun-dried samples (control), only acenaphthylene, acenaphthene, naphthalene and fluorene were recorded in *Arius heude loti* and Mud minnow while only acenaphthylene and naphthalene were recorded in *C. senegalensis*. However, these PAHs were regarded as not very carcinogenic (Silva et al., 2011). The sun-dried samples of *Blunt hwake* and *Clarias gariepinus* did not record any of the individual PAHs. This suggests that smoke is actually the major source of PAHs contamination in the smoked fishes.

4.3 Concentrations of Total PAHs in the Smoked Fishes

The results of concentrations of total PAHs in the smoked fishes presented in Figure 6 revealed that all the samples dried using saw dust smoke recorded the highest levels varying between 815.75 µg/kg to 1550.28 µg/kg followed by the samples dried using firewood with total PAHs content varying between 738.14 µg/kg to 994.09 µg/kg. The samples dried using charcoal smoke recorded the lowest total PAHs ranging from 135.02 µg/kg to 614.42 µg/kg. The sundried samples (control) only recorded 79.96 µg/kg in the Mud minnow, 70.51 µg/kg in *Arius heude loti* and 28.94 µg/kg in *C. senegalensis*. The concentrations of PAHs in the fish varied with the smoke source. The trend of the concentrations of the total PAHs of the fishes based on the processing methods revealed the following order: saw dust smoking > firewood smoking > charcoal smoking > sun-drying. The levels of the PAHs recorded may be attributed to the intensities of the smoke and heat generated by the smoking material which determine the drying duration of the fishes and hence, their contact time with the smoke. This finding also corroborates the report of similar study by Silva et al. (2011) that smoked fishes processed by charcoal gave the lowest level of total PAHs, followed by firewood method, while the saw dust method gave the highest level of total PAHs in the smoked fishes. The concentration of total PAHs detected in the sun-dried samples of both *C. senegalensis* and Mud minnow may be attributed to contamination by PAHs from other sources such as oil spills into the fishes' water habitat and air deposition of smoke particles from other burning sources since the fishes' sun drying was carried out in the open.

Benzo(a)pyrene levels of 5.68 µg/kg and 5.44 µg/kg were detected in the saw dust smoked samples of *Arius heude loti* and Mud minnow respectively. These concentrations exceeded the EC's recommended maximum limit of 5 µg/kg for Benzo(a)pyrene which has been classified as carcinogens. To simplify assessment of toxicological risk measures coming from different effects of PAHs, benzo(a)pyrene has been accepted as the indicator of PAHs presence in food due to its most intense carcinogenicity (Dobříková & Světlíková, 2007).

5. Conclusion

The results of this study revealed that fish samples smoked using saw-dust had the highest concentrations of PAHs followed by firewood, charcoal and sun-drying. Although, sun-drying is the safest method of drying fish in terms of the product PAHs contamination; it is however, impracticable all year round due to unfavorable weather conditions and its ineffectiveness in drying big sized fish. For a sustainable drying method, charcoal smoking produced the healthiest smoked fish product in terms of PAHs contamination. Benzo(a)pyrene concentrations of 5.68 µg/kg and 5.44 µg/kg respectively were detected in the samples of *Arius heude loti* and Mud minnow

processed using saw dust smoke. These levels exceeded the EC regulatory limit of 5 µg/kg. This gives concern for the usage of saw dust in smoking fishes due to contamination by benzo(a)pyrene, being associated with the most intense human carcinogenicity. Therefore, it is imperative that regulatory bodies conduct awareness campaigns to educate both the smoked fish processors, traders and consumers on the need to discourage the use of saw dust in smoking fish and adopt safer and improved methods. This becomes necessary because smoked fish is a popular protein diet of many unsuspecting persons and fish processors are making use of all available smoke materials to process the fishes.

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