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# Proximate and PAH Compositions of Raw and Smoked Samples of Scomber Scombrus and Trachurus Trachurus

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**ABSTRACT**

Proximate and polycyclic aromatic hydrocarbon (PAH) compositions were analyzed in raw and smoked samples of two exotic fish species; mackerel (*Scomber scombrus*) and horse mackerel (*Trachurus trachurus*) obtained from a local market in Umuahia, Nigeria. The fish were procured in triplicate and split into two equal parts. One part was analyzed raw while the other was smoked with firewood before analysis. Moisture content was higher in the raw samples compared to their corresponding smoked samples. Crude protein was higher in horse mackerel samples for both raw and smoked categories while other parameters were generally higher in mackerel. Horse mackerel had the higher value of 44.784 mg/kg for total mean PAH (∑mPAH) but PAH4 (sum of the four indicators of PAH contamination; benzo[*a*]pyrene, benz[*a*]anthracene, benzo[*b*]fluoranthene and chrysene) was similar in raw mackerel and horse mackerel. Mean BaP concentrations were higher than the EU limits (2 µg/kg) while PAH4 values were within the EU limit (12 µg/kg) in raw samples of both species. For the smoked samples, Mean BaP concentrations and mean PAH4 exceeded the EU maximum limits in the muscle of smoked fish and public health authorities are urged to take appropriate action.



## Introduction

Nutritional security in Nigeria and other developing countries is greatly aided by fish consumption. Fish is a major source of protein for the increasing world population especially in the developing countries of Africa, Asia and South America[1]. However, local fish production hardly meets demand in Nigeria and the country spends about $700m to import an estimated 1.9 million metric tonnes of fish species like mackerel and horse mackerel to ameliorate the shortfall[2]. Such imported fish are sold all over the country in raw and processed (especially smoked) forms. The proximate composition of locally available foods/diets are used to estimate the adequacy of dietary intake of population groups, to enable the researcher access if the feed is within its normal compositional parameters or adulterated, to determine diet-disease relationships, health and nutritional status, and for achieving dietary intake goals[3].

Wood fuel is usually used to smoke fish in Nigeria but awareness of the possibility of the processes depositing harmful polycyclic aromatic hydrocarbons (PAHs) on the smoked fish is not widely known. PAHs are a large group of organic compounds composed of two or more fused aromatic rings and which are mainly formed by incomplete combustion or pyrolysis of organic matter[4]. Food can be contaminated by PAHs during heating, drying, smoking, grilling and roasting[5,6]. PAHs are classified among persistence organic pollutants (POPs) due to their relative chemical stability and low rate of biodegradation and some are suspected to be carcinogenic and mutagenic[7.8].Proximate and PAH compositions of raw and smoke samples of mackerel (Scomber scombrus) and horse mackerel (Trachurus trachurus) are analyzed in this study.

## Materials and Methods

### Sampling

Raw samples of two exotic marine species Atlantic Horse Mackerel (*Trachurus trachurus*) and Mackerel (*Scomber scombrus*), were procured from Ahiaeke local market in Umuahia, Nigeria and each was divided into two equal parts. One part was analyzed raw while the other was smoked before analysis. Triplicate samples of each fish species of similar weights were collected. The standard and total lengths of fish were measured with a meter rule while their weights were determined with a balance. The raw samples were stored at -20oC in a refrigerator (Haier-Thermocool, Port-Harcourt) prior to analysis. The lipidic extraction of fish muscle samples was done in the Chemistry Laboratory of Michael Okpara University of Agriculture, Umudike. The extracted solution was then sent to BGL laboratories Ltd, Elelenwo, Port Harcourt where the GC/MS analysis was carried out. Proximate analysis of samples was done at the National Root Crop Research Institute, Umudike.

### The fish smoking process

The raw samples were washed with clean tap water and rinsed with distilled water before they were brined with 10 % salt solution and placed on wire gauze placed on drum type smoking kiln. Wood served as fuel and a distance of 30 cm was maintained between fish and the flame. Smoking temperature was measured with a Mercury-in-glass thermometer and smoking was done for a period of 6 h and after which the fish was allowed to cool for 1 h and wrapped in polyethylene bags prior to analysis.

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**Proximate Analysis**

Proximate analysis of fish was done with standard method [9]. This includes the determination of moisture, crude fat, crude protein, crude ash, crude fiber, and nitrogen free extracts.

***PAH analysis***

**Soxhlet extraction method**

Homogenized fish muscle sample (10 g) was weighed and mixed thoroughly with 5 g of anhydrous sodium sulfate (Loba Chemie analytical grade) in a laboratory crucible/mortar until a complete homogenate was obtained. The extraction was carried out using a Soxhlet extractor (ADARSH Borosilicate Glass) apparatus which consists of a 250 $cm^3$ round bottomed flask, condenser and an extractor tube, seated in a temperature-controlled heating mantle. A Fischer brand rotary evaporator was used to evaporate the extract to the desired concentration. The homogenate was carefully transferred into the extraction thimble placed in the extraction chamber of a Soxhlet extraction unit. The extraction was carried out as recommended by USEPA 3540 method using 150 $cm^3$ dichloromethane (Riedel-de Haen 52790 analytical grade 67-63-0) for 16 h[10]. The extract was concentrated to 2 $cm^3$ using a rotary evaporator in a water bath that was pre-set to a temperature of 35 $^oC$ and was stored in an amber bottle and kept in a refrigerator to avoid oxidation of the extract prior to clean up. The same procedure was used for all fish samples.

**Sample purification**

The extracted samples were purified by passing them through a silica gel column prepared by loading 10 g of activated silica gel (100-200 Mesh, Loba Chemie analytical grade) onto a chromatographic column (1cm internal diameter) to about 5 cm. This was topped with 1 cm of anhydrous $Na_2SO_4$. It was then conditioned with dichloromethane. 2 $cm^3$ of the concentrated extract was loaded and eluted with 20 $cm^3$ of dichloromethane. This method is able to remove the very polar lipids off the extract. Prior to analysis with GC/MS, the extracts obtained were preserved in an amber bottle to avoid oxidation.

**Preparation of standard solution and analysis**

Sixteen PAH stock solutions (supplied by instrument manufacturer) were used to prepare calibration standards at different concentrations, 0.5, 1, 2, 3 and 5 mg/L using the dilution formula:

$C1V1 = C2V2$

Where C1 is the initial concentration

C2 is final concentration (stock)

V1 is initial volume to be taken from stock (unknown)

V2 is final volume (50 ml of volumetric flask)

from above, $V1 = \frac{C2V2}{C1}$

V1 was calculated, measured from the stock using micropipette and poured into a 50 ml volumetric flask and made up to mark with dichloromethane. A calibration curve was obtained by analyzing each of the standard PAHs solutions prepared on the GC/MS. The target PAH compound/internal standard peak heights were plotted against the PAH concentration to obtain a linear graph Y= mx + b, with an intercept (b) on the y-axis.

An Agilent 7890 Gas Chromatograph (Agilent, California) equipped with auto sampler connected to an Agilent 5975 MSD mass spectrometric detector was used. 1 µl of sample solution was injected in the pulsed spilt less mode onto a 30 m x 0.25 mm id DB5 MS coated fused silica column with a film thickness of 0.15 µm. Helium was used as the carrier gas and the column head pressure was maintained at 20 psi to give constant flow 1ml/min. Other operating conditions were pre-set, pulse time 0.90 min, purge flow 50 $cm^3$, purge time 1 min, and injection temperature 300 $^oC$. The column temperature was initially held at 55 $^oC$ for 0.4 min, increased to 200 $^oC$ at a rate of 25 $^oC$/min, then to 280 $^oC$ at a rate of 8 $^oC$/min and to a final temperature of 300 $^oC$ at a rate of 25 $^oC$/min and held for 2 min at transfer line of 320 $^oC$. The mass spectrometer (MS) condition was electron impact positive ion mode. The PAHs identification time was based on retention time since each of the PAHs has its separate retention time in the column. Those with lower retention times were identified first followed by those with longer retention times.

**Statistical Analysis**

The analysis was carried out on triplicate samples and the value of each determination is presented as mean ± standard error of mean. The data were subjected to analysis of variance (ANOVA) using SPSS statistical software (20.0). Comparison of means was done using the Duncan method and P values < 0.05 were considered statistically significant.

**Results**

Table 1 shows the mean standard and total lengths of fish and the mean weights of fish species used in the study. Mean values for weights and lengths were similar (P>0.05).

**Table 1. Mean weights and lengths of fish species**

| Parameter | Mackerel (*Scomber scombrus*) | Horse Mackerel (*Trachurus trachurus*) |
|---|---|---|
| Standard length (cm) | **6.54 ±0.83** | **6.92 ±1.05** |
| Total length (cm) | **7.82 ±0.68** | **8.11 ±0.70** |
| Weight (g) | 121.44±2.01 | 123.18±1.36 |

Table 2. shows the mean temperatures at which the fish species were smoked. There was no significant difference (P<0.05) in smoking temperatures for the species.

**Table 2. Mean temperatures of the Smoking Process for each species.**

| Samples | Sample ID | Average Smoking Temperature (°C) |
|---|---|---|
| Mackerel (*Scomber scombrus*) | C | 71.45 ±4.18 |
| Horse Mackerel (*Trachurus trachurus*) | D | 71.61±3.83 |

The result of mean concentrations for each PAH in raw fish samples are shown in table 3.

Naphthalene, acenaphthylene and acenaphthene were predominant in the raw samples. Naphthalene concentration was significantly higher (P<0.05) than those of other PAHs in both species. The result also shows that all the 16 targeted PAHs were detected in all the fresh samples except acenaphthylene, dibenz(a,h)anthracene, indenol[1,2,3-cd] Pyrene and benzo(g,h,i)perylene which were not detected in mackerel.

Mean PAH concentrations for each PAH in smoked fish samples are shown in table 4. All the PAHs analyzed were detected in all smoked samples except acenaphthylene in mackerel and horse markerel and dibenz[a,h]anthracene and benzo[g,h,1]perylene in mackerel. Naphthalene

concentrations were significantly higher (P<0.05) than the concentrations of other PAHs.

**Table 3. Mean PAH concentrations (mg/kg) in Fresh samples.**

| | | |
|---|---|---|
| Naphthalene | 30.908 ±3.613a | 44.442 ±1.978a |
| Acenaphthylene | 0.000 ±0.000 | 0.002 ±0.001b |
| Acenaphthene | 0.070 ±0.004b | 0.189 ±0.012c |
| Fluorene | 0.013 ±0.001c | 0.047 ±0.006d |
| Anthracene | 0.011 ±0.001c | 0.034 ±0.001d |
| Phenanthrene | 0.013 ±0.001c | 0.013 ±0.002b |
| Fluoranthene | 0.002 ±0.001c | 0.003 ±0.002b |
| Pyrene | 0.001 ±0.000c | 0.003 ±0.000b |
| Benz[a]anthracene | 0.002 ±0.001c | 0.002 ±0.001b |
| Chrysene | 0.002 ±0.001c | 0.003 ±0.000b |
| Benzo[b]Fluoranthene | 0.004 ±0.001c | 0.003 ±0.001b |
| Benzo[k]Fluoranthene | 0.004 ±0.001c | 0.004 ±0.001b |
| Benzo[a]Pyrene | 0.004 ±0.002c | 0.004 ±0.002b |
| Dibenz[a,h]anthracene | 0.000 ±0.000 | 0.011 ±0.001b |
| Indenol[1,2,3-cd] Pyrene | 0.000 ±0.000 | 0.009 ±0.004b |
| Benzo[g,h,i]perylene | 0.000 ±0.000 | 0.015 ±0.000b |
| ∑Mpah | 31.041 | 44.784 |
| ∑PAH4 | 0.012 | 0.012 |

Values along the same row with different letters are significantly different (P<0.05). Values are mean ± S.E.M for three replicates, (n=3)

**Table 4. Mean PAH concentrations (mg/kg) in smoked fish samples.**

| | | |
|---|---|---|
| Naphthalene | 48.862 ±3.719a | 48.790 ±3.692a |
| Acenaphthylene | 0.000 ±0.000 | 0.000 ±0.000 |
| Acenaphthene | 0.184 ±0.006b | 0.212 ±0.001b |
| Fluorene | 0.038 ±0.004c | 0.045 ±0.003c |
| Anthracene | 0.035 ±0.001c | 0.038 ±0.003c |
| Phenanthrene | 0.036 ±0.002c | 0.038 ±0.003c |
| Fluoranthene | 0.004 ±0.002d | 0.006 ±0.001d |
| Pyrene | 0.003 ±0.001d | 0.003 ±0.001d |
| Benz[a]anthracene | 0.002 ±0.000d | 0.002 ±0.001d |
| Chrysene | 0.004 ±0.001d | 0.002 ±0.001d |
| Benzo[a]Pyrene | 0.004 ±0.002d | 0.005 ±0.002d |
| Dibenz[a,h]anthracene | 0.000 ±0.000 | 0.000 ±0.000 |
| ∑mPAH | 49.191 | 49.177 |
| PAH4 | 0.014 | 0.013 |

Values along the same row with different letters are significantly different (P<0.05). Values are mean ± S.E.M for three replicates (n=3)

**Table 5. Result of proximate composition (%) of the analyzed Fish samples.**

| Proximate Analysis | Mackerel | | Horse mackerel | |
|---|---|---|---|---|
| | Fresh | Smoked | Fresh | Smoked |
| Moisture content | 63.80 ±0.02a | 51.97 ±1.74a | 71.01 ±2.03a | 62.05 ±2.60a |
| Crude protein | 21.52 ±0.81b | 29.61 ±0.98b | 25.67 ±1.51b | 37.23 ±0.90b |
| Crude fibre | 0.66 ±0.01c | 2.05 ±0.03c | 0.36 ±0.01c | 1.47 ±0.03c |
| Crude lipid | 11.84 ±1.04d | 23.40 ±1.80b | 8.23 ±0.13d | 14.31 ±0.78d |
| Ash | 1.13 ±0.06c | 5.29 ±0.40c | 1.01 ±0.01c | 5.09 ±0.22e |
| Nitrogen free extracts | 64.85 ±5.01a | 39.64 ±1.61d | 64.72 ±3.75e | 41.91 ±1.66b |

Means with different letters in the same row are significantly letters (P<0.05).

Table 5 presents the proximate composition of all the analyzed fish samples. The result reveal significant differences (P<0.05) in moisture content, crude protein, ash content, crude lipid and crude fibre among the different samples. Crude protein was significantly higher (P<0.05) in horse mackerel samples for both fresh and smoked categories while raw and smoked mackerel had significantly higher (P<0.05) crude lipid content. Ash content was lower in fresh horse mackerel. Generally, ash content was higher in the smoked samples compared to their corresponding fresh samples. This increase in ash content as observed in the smoked samples is attributed to loss of moisture and humidity level during the smoking process.

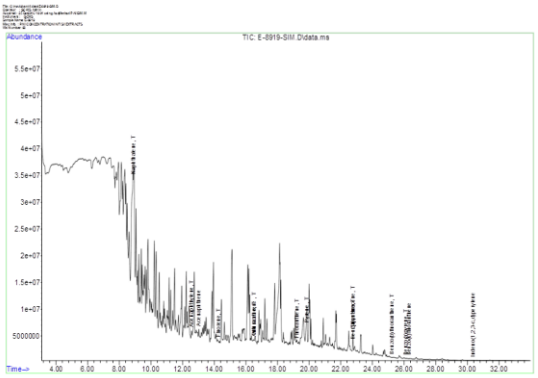

**Figure 1. Chromatogram for Raw mackerel Sample.**

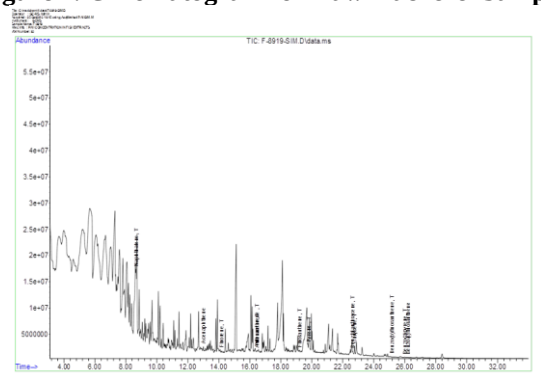

**Figure 2. Chromatogram for Smoked mackerel Sample.**

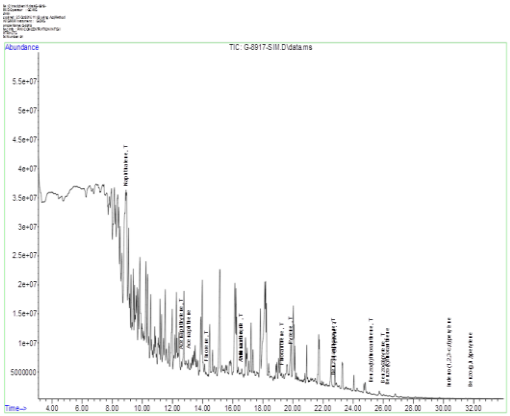

**Figure 3. Chromatogram for Raw Horse mackerel Sample.**

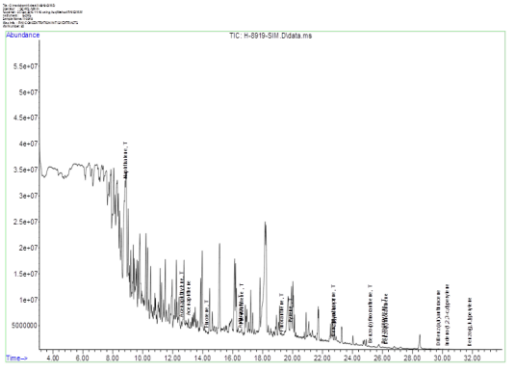

**Figure 4. Chromatogram for Smoked Horse mackerel Sample.**

**Discussion**

Benzo(a)pyrene was formerly used as an indicator of PAH contamination since it is a confirmed human carcinogen[11]. However, the European Food Safety Authority concluded that a more suitable indicator for PAH toxicity is PAH4 (the sum of benzo[*a*]pyrene, benz[*a*]anthracene, benzo[*b*]fluoranthene and chrysene)[4]. The EU maximum limit for benzo(a)pyrene and PAH4 is 2 and 12 µg/kg respectively[12]. Horse mackerel had the higher value of 44.784 mg/kg for total mean PAH (∑mPAH) but PAH4 was similar in raw samples of mackerel and horse mackerel. Mean BaP concentrations were higher than the EU limits while PAH4 values were within the EU limit in raw samples of both species.

Mean BaP concentrations and mean PAH4 exceeded the EU maximum limits in the muscles of smoked samples. For the mackerel sample, mean concentration of naphthalene were higher in smoked than in the raw sample. Indenol(1,2,3-cd)pyrene was not detected in raw sample but was detected in smoked sample. The concentration of benzo(a)pyrene were the same in the smoked and raw mackerel. The comparison between raw and smoked sample of horse mackerel showed that, all the 16 PAHs were detected. The mean concentration of acenaphthylene, fluorene, chrysene and dibenz(a,h)anthracene were higher in the raw sample than in the smoked. The mean concentration of pyrene, benzo(a)anthracene, benzo(k)fluoranthene and benzo(g,h,i)perylene remained the same, which shows they were not affected by the smoking process. Naphthalene, benzo(a)pyrene and others were higher in the smoked than in the raw sample. A study of PAHs in various foods in Abidjan obtained a BaP and PAH4 concentrations of 34.07 and 159.48 µg/kg respectively which are higher than the values obtained in this study[13]. Another study recorded a mean total PAH level of 63.43 µg/g and mean Bap concentration of 2.41 µg/g *Scomber scombrus*. BaP was not detected in the raw fish but was detected in the raw samples of this study[14].

A study of the effect of smoking on the proximate and mineral compositions of *Trachurus trachurus* obtained values of 66.68±1.19, 3.46±0.01, 4.93±0.68, 2.06±0.24 and 74.02±0.02 % for protein, fat, ash, fibre and moisture contents[15]. Also a related study that investigated the heavy metal, proximate and microbial profile of fresh samples of some commercial marine fish showed that mackerel had moisture, protein, ash, lipids and carbohydrate contents of 63.3866±0.5398, 23.0900±0.0100, 1.1133±0.0057, 10.2133±0.0057 and 2.5133±0.0152 % respectively[16]. The results suggested that the fish species could be used as a good source of minerals. The relatively high levels of crude protein observed in the analyzed samples indicate that the fish species are good sources of pure protein. However, the different protein and lipid content observed in the various species may be attributed to their individual consumption or absorption capabilities, and their relative potentials of converting essential nutrients from their diet of local environment into biochemical entities[17]. The range of ash content gave an indication that the fish samples may be good nutritional source of minerals such as calcium, potassium, zinc, iron and magnesium[18].

**Conclusion**

Mean BaP concentrations were higher than the EU limits while PAH4 values were within the EU limit for raw samples of both species. For smoked samples, Mean BaP concentrations and total mean PAH4 exceeded the EU maximum limits in the muscle of smoked fish. Public health authorities are therefore urged to take necessary action to ensure that wholesome fish devoid of PAH contamination are imported and also put in place enlightenment and control measures for the fish smoking process to reduce PAH contamination of consumed fish.

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