*Research Article*

# POLYCYCLIC AROMATIC HYDROCARBONS IN SOME NIGERIAN ROASTED PLANT FOODS

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## ABSTRACT

Thirteen polycyclic aromatic hydrocarbons (PAHs) were identified and quantified in three different roasted plant foods (*Zea mays, Dioscorea rotundata and Musa paradisiaca*) using a rapid method involving microwave assisted saponification and simultaneous extraction followed by solid-phase extraction (SPE), high-performance liquid chromatography (HPLC) separation and spectrofluorometric detection. The method applied had good recovery and repeatability characteristcs.

With respect to raw samples, roasted samples had higher contamination levels with a maximum benzo[a]pyrene (BaP) content of 0.6 μg kg$^{-1}$ dry weight. Roasted *Zea mays* had the highest low molecular weight- polycyclic aromatic hydrocarbons (LMW-PAH) load of 31.2 μg kg$^{-1}$ dry weight, which may be due to the fact that the charred portions after roasting are not usually subjected to scrapping, an exercise which is usually carried out on other roasted plant foods.

Anyways, PAHs exposure due to the consumption of roasted plant foods may not pose serious concerns for human health, especially as they are low in heavy molecular weight polycyclic aromatic hydrocarbons (HMW-PAHs).

**KEY WORDS:** polycyclic aromatic hydrocarbons (PAHs), microwave assisted extraction (MAE), HPLC, roasted plant foods, risk assessment.

## INTRODUCTION

Polycyclic Aromatic Hydrocarbons (PAHs) are a large group of environmental and food processing contaminants (formed by two or more condensed benzene rings), originated from incomplete combustion of organic matter, which have proved carcinogenic activity [1]. To date, more than one hundred PAHs have been characterized in nature, sixteen of which (2-6 ring compounds) have been placed under the 'priority pollutants' list by the United States Environmental Protection Agency, USEPA since the 1970s [2]. After 2005, a new list of 16 EU-priority PAHs (4-6 ring compounds), whose presence in foods should be monitored due to their carcinogenic and genotoxic properties, was introduced [3][4]. Both EPA- and EU-priority PAHs comprise 8 high-molecular weight compounds (PAH8) and a subgroup of 4 compounds (PAH4) that have been recently recognized as the most suitable indicators of the presence of carcinogenic and genotoxic PAHs in foods [5].

It is well known that food processing such as smoking, grilling, toasting and roasting may lead to high amounts of PAHs. The large consumption of smoked foods seems to be responsible for the higher incidence of primary liver and stomach cancer in countries where traditional smoking

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processes are still carried out such as in Nigeria [6][7]. Different studies highlighted the high PAHs intake by Nigerian population through the widely consumed smoked meat and fish products [7][8][9][10]. Recently, we demonstrated that the daily consumption of 100g of smoked fish and/or meat from the Nigerian market, lead to margin of exposure (MOE) values well below 10,000 (without considering the possible contribution of other food items), which according to EFSA [5], indicate a potential concern for consumer health [11]. Although plant foods are important contributors to the total PAH dietary intake, relatively few works deals with PAHs determinations in these food items [12][13][14]. Plant foods can be contaminated with PAHs as a result of environmental contamination and food processing. Raw vegetables may present large levels of PAHs in relation to the contamination of the environment where they grow. Atmospheric fallout is recognized as the major route of contamination, while more controversial is the possibility of sorption from direct contact with contaminated soil particles [15][16][17][18].

According to the last EFSA Opinion [5] both fresh and dried vegetables are generally characterized by relatively low PAHs contamination levels (BaP lower than 0.5 µg kg$^{-1}$). Nevertheless, BaP levels up to 400 µg kg$^{-1}$ were found in potatoes grown in contaminated soil [19] and up to 160 µg kg$^{-1}$ in smoked cereals [20]. BaP amounts higher than 100 µg kg$^{-1}$ were also reported in dried fruits [21]. Different authors found relatively high PAHs amounts in roasted coffee beans. Recently Houesson *et al* . [22] Observed that the formation of phenanthrene, anthracene, benzo[a]anthracene occurs at temperature above 220°C, whereas formation of pyrene and chrysene requires 260°C.

In tropical Nigeria, roasted plant foods such as *D. rotundata* (root and tuber group), *M. paradisiaca* (fruit group) and *Zea mays* on-the-cob (cereal group) are usually eaten as snack foods. It is therefore not unusual to see women roasting these plant foods, on commercial basis, along roadsides. Since no data on the level of PAHs in tropical roasted plant foods are available from the literature, the aim of this work was to assess the level of contamination of these roasted vegetables commonly consumed in Nigeria.

A simultaneous saponification and solvent extraction carried out by microwave assisted extraction (MAE) method, previously used by Akpambang and co-workers for PAH determination in fish and meat [11] followed by HPLC and spectrofluorometric determination, was applied for this purpose.

## MATERIALS AND METHODS

### Reagents and standards

All the solvents used were of HPLC grade (Sigma, St. Louis, MO, USA). Water was purified with a Milli-Q System (Millipore, Bedford, MA, USA).

The standard PAH mixture (610M) in 1 ml of methanol/dichloromethane (Supelco, Bellefonte, PA, USA) consisted of: acenaphthene (Ac) (1000 µg/ml), fluoranthene (Fl) (200 µg/ml), naphthalene (Na) (1000 µg/ml), benzo[a]anthracene (BaA) (100 µg/ml), benzo[b]fluoranthene (BbF) (200 µg/ml),benzo(a)pyrene (BaP) (100 µg/ml), benzo[k]fluoranthene (BkF) (100 µg/ml), chrysene (Ch) (100 µg/ml), acenaphthylene (Ap) (2000 µg/ml), anthracene (A) (100 µg/ml), benzo[g,h,i]perylene (BghiP) (200 µg/ml), fluorene (F) (200 µg/ml), phenanthrene (Pa) (100 µg/ml), dibenz[a,h]anthracene (DBahA) (200 µg/ml), indeno[1,2,3-cd]pyrene (IP) (100 µg/ml), pyrene (P) (100 µg/ml).

All the glassware was carefully washed and rinsed with distilled solvent (acetone and hexane) before use.

### Samples

About 2 kg of each raw plant was purchased from five commercial vendors in the Oba market in Akure. About 500g portion of each of the sample was prepared for roasting. *Zea mays* was roasted on-the-cob on wire gauze placed over a charcoal fire for 10 minutes while constantly changing its position in order to avoid charring. *Dioscorea rotundata* sample was cut in rectangular pieces of approximately 3x4 inches, unpeeled and roasted on hot hardwood charcoal fire for a total of 15 minutes on all sides. Ripe *Musa paradisiaca* was peeled and placed on hot hardwood charcoal fire. They were roasted for about 15 minutes with constant changing of its position for even roasting. The distance between wire gauze and hot coals was 10cm.

Approximately one kilogram of each roasted plant sample was purchased at random from six commercial vendors along roadside in Akure, Ondo state, southwestern Nigeria. The same roasting methods described for laboratory samples was used for commercial samples, with the difference that commercial vendors may keep the roasted vegetables for longer period beside the burning charcoal (until a buyer comes).

For each of the three plant samples analyzed, samples from different vendors were pooled together to obtain individual samples for raw (A), laboratory roasted (B) and commercially roasted (C) plant. An aliquot of the sample so obtained (about 100g) was lyophilized, milled in a domestic blender (Sharp Blender HR 2815), packed in aluminum foil wraps and stored in the freezer at -20$^{\circ}$C prior to analysis.

### Apparatus

A microwave extractor (Mars, CEM Corporation, Matthews, North Carolina, USA) able to process up to 14 sample simultaneously was used to extract PAHs from lyophilized samples.

A Varian model 9010 HPLC gradient pump (Varian, Palo Alto, CA, USA) equipped with a Rheodyne 7161 injector with a 20 µL loop was used for analytical determination. The column was a C18 reversed phase (Supelcosil LC-PAH), 250 x 3 mm ID, 5µm particle size (Supelco,

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Bellefonte, PA, USA) thermostatted at 38°C. A mobile phase consisting of acetonitrile and water (flow rate of 1 ml/min) was used for HPLC determination. The gradient elution program started with 40% acetonitrile (isocratic for 5 minutes), going linearly to 100% acetonitrile (total run time: 40 minutes).

The detector was a programmable spectrofluorometer (Jasco, model FP 1520, Cremella, Como, Italy) whose wavelength settings are reported elsewhere [23]. A second spectrofluorometer set at different wavelengths settings was connected in series with the former and used to confirm analyte identity (Varian spectrofluorometer, model 9070). Quantification was carried out by the external standard method.

**Sample preparation**

PAHs extraction and purification was carried out on lyophilized samples by applying the method described by Akpambang *et al.* [11] for PAHs determination in smoked/grilled fish and meat, which was a modification of the method proposed by Pena *et al.*[24]. Briefly, 400 mg of lyophilized sample were weighed directly in a Teflon-lined vessel (Green Chem plus, CEM Corporation), added with 1.6 mL of water, 8 mL of saturated methanolic potassium hydroxide and 20 mL of *n*-hexane. A microwave assisted saponification/extraction was carried out at 120 °C for 20 min. Once cooled, vessels were opened and a measured amount of the organic extract was withdrawn and concentrated to a few microlitres using a rotavapor and then a nitrogen flow. Sample purification was performed on a 500 mg silica cartridge (Supelco), previously conditioned with 2 mL of dichloromethane and 2 mL of *n*-hexane. After sample loading, the PAH fraction was eluted with 3 mL of *n*-hexane/dichloromethane (70/30). The fraction so obtained was gently concentrated to a few microlitres under a nitrogen flow and then left to evaporate spontaneously to dryness at room temperature. The sample residue was dissolved in 100 µL of acetonitrile and directly injected into the HPLC apparatus. Two different aliquots of each sample underwent the entire analytical procedure and each replicate was injected twice. Analytical blanks were analysed (in duplicate) every day together with the samples and average data obtained were subtracted from those obtained for real samples.

## RESULTS AND DISCUSSION

**Method Performance**

Linearity was verified elsewhere [11] as well as limits of detection (LOD, s/n=3) and quantification (LOQ, s/n=10). LOD and LOQ lower than 0.1 and 0.3 µg kg$^{-1}$ dry weights, respectively, were found for all PAHs.

A spiking procedure was used to estimate PAHs recovery for each of the relevant matrices. A measured amount of lyophilized raw plant was slurred with a little amount of *n*-hexane containing a known amount of PAHs standard mixture (to obtain a final BaP concentration of 2 µg/kg and other quantified PAHs concentrations ranging from 2 to 4 µg/kg). The mixture was efficiently mixed and the solvent was left to evaporate. After solvent removal the sample was left to age for 3 days in the dark before being analysed.

Mean recoveries (4 replicates), calculated by comparing the difference between spiked and unspiked sample with the known amount of PAHs added, ranged from 64 to 96 % (higher than 72% for PAH8) for *Zea mays* sample, from 59 to 93% (higher than 77% for PAH8) for *Dioscorea rotundata* and from 64 to 88 % (higher than 74% for PAH8) for *Musa paradisiaca*.

The precision of the method was estimated by four replicate analysis of fortified samples. Coefficients of variation less than 23 % were obtained for each single PAH (less than 11 % for PAH8)

**PAH content in raw and roasted vegetables**

To better evaluate the effect of roasting on PAHs content (in order to exclude the effect due to the different water content and loss of water occurring during roasting), data reported in Table 1 are expressed in µg kg$^{-1}$ dry weight. The dry weight of the samples was determined by oven drying at 103±2°C until a constant weight was achieved. PAHs amounts are the average of duplicate sample analyses (two replicates per sample). Data were not corrected for recoveries. Due to their high volatility with consequent low recoveries and high coefficients of variation, data concerning Na and Ac were not reported. The sum of five low molecular weight PAHs (LMWPAHs) (F, Pa, A, Fl and P), of 8 high molecular weight PAHs (PAH8, including BaA, Ch, BbF, BkF, BaP, DBahA, BghiP and IP), and total PAHs (T-PAHs) are also reported.

Raw samples had LMW-PAHs and PAH8 ranging from 4.7 to 10.7 and from 0.3 to 0.6 µg kg$^{-1}$ dry weight, respectively. The roasting process always lead to an appreciable PAH increase, with LMW-PAH and PAH8 ranging from 9.6 to 37.6 and from 2.5 and 6.9 µg kg$^{-1}$ dry weight, respectively. The most represented PAH in general was Pa, followed by P and Fl. The slightly higher amount of LMW-PAH observed for roasted *Zea mays* could have been due to the fact that burnt or charred portions are not usually scrapped off as it is the case with the other two roasted plant foods. Among PAH8, Ch was the most abundant one. BaP levels not exceeding 0.6 µg kg$^{-1}$ dry weights were found in roasted samples. No evident differences between laboratory and commercially roasted samples were observed.

PAHs levels considerably lower than those reported by the same authors [11] for smoked fish and meat were obtained. These results are in agreement with those obtained by Kuo et al.[25] Which studied PAHs exposure of different vendor groups in Taiwan. Particularly, the daily exposure dose of BaPeq for the fish vendor group during working hours was 22 times higher than that for control group, while daily exposure for roasted corn on-the-cob vendor group was comparable with the dose for control group.

Different reasons such as shorter processing times and lower fat content can help to explain the low PAHs content of roasted vegetables with respect to those found for traditionally smoked fish and meat.

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Table 2 reports (for only the roasted products), PAH8 and BaP concentrations expressed in $\mu g\ kg^{-1}$ of product, as well as BaP and PAH8 intake ($ng\ kg^{-1}\ bw\ day^{-1}$), calculated considering a daily consumption of 100g of roasted plant per person and assuming a reference body weight of 60kg.

No data about the real consumption of these food items in Nigeria are available. According to Aromolaran [26]yam meals and cereals supplied 17% and 18% of total calorie intake in South-Western Nigeria, respectively.

An average daily intake of 0.3 and 2.6 $ng\ kg^{-1}\ bw\ day^{-1}$ was obtained for BaP and PAH8 in commercially roasted samples, respectively. It represents about 10% of the median European dietary exposure calculated by the EFSA [5] for mean dietary consumers (3.9 $ng\ kg^{-1}\ bw\ day^{-1}$ for BaP and 28.8 $ng\ kg^{-1}\ bw\ day^{-1}$ for PAH8), for which margin of exposure (MOE) values higher than 10,000 were obtained for both BaP and PAH8 indicating a low concern for human health.

**Table 1:** *Polycyclic aromatic hydrocarbon (PAH) content ($\mu g\ kg^{-1}$ dry weight) in raw, laboratory and commercially roasted plants from Akure, south western Nigeria*

| | *Zea mays* | | | *D. rotundata* | | | *M.paradisiaca* | | |
|---|---|---|---|---|---|---|---|---|---|
| | **A** | **B** | **C** | **A** | **B** | **C** | **A** | **B** | **C** |
| **F** | 0,7 | 1,7 | 2,3 | 0,1 | 0,2 | 0,3 | 0,8 | 0,4 | 0,2 |
| **Pa** | 6,1 | 17,0 | 14,3 | 1,8 | 2,4 | 2,3 | 3,4 | 4,3 | 4,0 |
| **A** | 0,4 | 2,0 | 1,6 | 0,5 | 0,3 | 0,3 | 0,3 | 0,5 | 0,4 |
| **Fl** | 2,0 | 8,1 | 5,0 | 1,2 | 4,0 | 2,6 | 1,1 | 2,6 | 2,8 |
| **P** | 1,6 | 8,8 | 6,3 | 1,1 | 5,3 | 4,2 | 1,3 | 4,5 | 3,7 |
| **BaA** | 0,1 | 0,7 | 0,2 | 0,0 | 0,8 | 0,4 | 0,0 | 0,3 | 0,2 |
| **Ch** | 0,3 | 2,5 | 0,5 | 0,1 | 2,4 | 0,9 | 0,4 | 0,5 | 0,5 |
| **BbF** | 0,0 | 1,0 | 0,5 | 0,0 | 1,1 | 0,7 | 0,0 | 0,4 | 0,5 |
| **BkF** | 0,0 | 0,4 | 0,2 | 0,0 | 0,5 | 0,3 | 0,1 | 0,2 | 0,2 |
| **BaP** | 0,0 | 0,6 | 0,3 | 0,0 | 0,6 | 0,4 | 0,0 | 0,3 | 0,3 |
| **DBahA** | 0,0 | 0,5 | 0,2 | 0,0 | 0,2 | 0,2 | 0,0 | 0,2 | 0,2 |
| **BghiP** | 0,0 | 0,8 | 0,4 | 0,0 | 0,8 | 1,2 | 0,0 | 0,5 | 0,7 |
| **IP** | 0,1 | 0,4 | 0,3 | 0,0 | 0,6 | 0,4 | 0,0 | 0,2 | 0,2 |
| **LMW-PAHs** | 10,7 | 37,6 | 29,6 | 4,7 | 12,2 | 9,6 | 6,8 | 12,4 | 11,1 |
| **PAH8** | 0,6 | 6,9 | 2,5 | 0,3 | 6,9 | 4,6 | 0,6 | 2,7 | 2,8 |
| **T-PAHs** | 11,3 | 44,5 | 32,1 | 5,0 | 19,1 | 14,2 | 7,5 | 15,0 | 13,9 |

Note: Data are mean of two replicate analyses (each one injected twice). A, raw samples; B, laboratory roasted samples; C, commercially roasted samples. Fluorene (F), phenanthrene (Pa), anthracene (A), fluoranthene (Fl), pyrene (P), benz[*a*]anthracene (BaA), chrysene (Ch), benzo[*b*]fluoranthene (BbF), benzo[*k*]fluoranthene (BkF), benzo[*a*]pyrene (BaP), dibenz[*a,h*]anthracene (DBahA), benzo[*g,h,i*]perylene (BghiP), indeno[*1,2,3-cd*]pyrene (IP), LMW-PAHs = low molecular weight PAHs (F+Pa+A+Fl+P), PAH8 = BaA+Ch+BbF+BkF+BaP+DBahA+BghiP+IP, T-PAHs = total PAHs (LMW-PAHs + PAH8).

**Table 2:** *Daily intake*

| | Zea mays | | D.rotundata | | M.paradisiaca | |
|---|---|---|---|---|---|---|
| | **B** | **C** | **B** | **C** | **B** | **C** |
| **BaP ($\mu g\ kg^{-1}$ wet weight)** | 0.5 | 0.2 | 0.5 | 0.3 | 0.2 | 0.2 |
| **PAH8 ($\mu g\ kg^{-1}$ wet weight)** | 5.5 | 2.0 | 5.6 | 3.6 | 2.1 | 2.2 |
| | | | | | | |
| ***Daily human exposure[a]*** | | | | | | |
| **ng BaP $kg^{-1}$ bw $day^{-1\,[b]}$** | 0.8 | 0.3 | 0.8 | 0.6 | 0.3 | 0.4 |
| **ng PAH8 $kg^{-1}$ bw $day^{-1\,[b]}$** | 9.2 | 3.4 | 9.3 | 6.1 | 3.6 | 3.7 |

Notes: Data are mean of two replicates (each one injected twice).
B, laboratory roasted samples; C, commercially roasted samples.
[a] Daily human exposure was calculated on the basis of amounts of 100 g of roasted vegetable consumed daily.
[b] Assuming a reference body weight of 60 kg.

# CONCLUSIONS

Roasting of plant foods, when carried out with traditional systems, always lead to PAH generation. However, the consumption of roasted plant foods (*Zea mays, D.rotundata and M. paradisiaca*) which is very common in Nigeria does not expose the Nigerian population to high PAHs exposure levels, especially when compared with the median European exposure [5] and exposure levels due to the consumption of traditionally smoked fish and meat [11]. BaP levels always below $0.6$ µg kg$^{-1}$ dry weight were found in all roasted samples. Probably the short duration of the roasting process and the low fat content of the sample are responsible for these results.

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