Full Length Research Paper

# Evaluation of polycyclic aromatic hydrocarbons (PAHs) content in foods sold in Abobo market, Abidjan, Côte d'Ivoire

# Pierre MANDA<sup>1</sup>\*, Djédjé Sébastien DANO<sup>1</sup>, Ehouan Stephane-Joel EHILE<sup>1,3</sup>, Mathias KOFFI<sup>2</sup>, Ngeussan AMANI<sup>3</sup> and Yolande Aké ASSI<sup>2</sup>

<sup>1</sup>Laboratoire de Toxicologie et Hygiène Agro-industrielle, UFR des Sciences Pharmaceutiques et Biologiques, Université de Cocody, BPV 34 Abidjan, Côte d'Ivoire.

<sup>2</sup>Laboratoire Central pour l'Hygiène Alimentaire et l'agro-industrie, Laboratoire National pour le Développement Agricole, Abidjan Côte d'Ivoire.

<sup>3</sup>UFR des Sciences et Techniques des aliments, Université d'Abobo Adjamé, Abidjan Côte d'Ivoire.

# Accepted 2 April, 2012

This work was aimed to record the concentrations of eight polycyclic aromatic hydrocarbons (PAHs): (benzo[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, indeno[1,2,3-c,d]pyrene, benzo[g,h,i]perylene and chrysene) in meats and fishes sold in Abobo market in Abidjan, Côte d'Ivoire. The amount of PAHs present in each sample was quantified using high-performance liquid chromatography (HPLC) equipped with ultraviolet (UV) detector. PAHs were present in all samples in variable quantity. More over benzo[a]pyrene (B[a]P) was present in majority of samples, in quantity above the limit fixed by European Union. With regard to cooking processes, smoking produce more PAHs compared to frying or grilled cooking. Concerning the nature of the matrices, no significant differences were found between meat and fish except benzo[g,h,i]perylene. The study declared that PAHs contamination in the tested foods exceeded the acceptable limit. Health risks linked with the consumption of these foods is a real danger that requires further study.

**Key words:** Polycyclic aromatic hydrocarbons (PAHs), fishes, meats, high-performance liquid chromatography (HPLC).

# INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are a group of environmental contaminants that originate from the pyrolysis or incomplete combustion of organic matter (Costes and Druelle, 1997). They are universal contaminants of our environment and of the human food chain (Lacoste et al., 2003). In food, PAHs are formed during processing and food preparation, either industrial or domestic, especially during the processes of smoking, drying and cooking (Moret et al., 1997; Bardolato et al., 2006). Food contamination may also occur during periods of atmospheric pollution in which PAHs are deposited on seeds, fruits or vegetables, which are then consumed (Guillen et al., 1994; FAO/OMS 2008; Rey-Salgueiro et al., 2008). Experimental data related to PAHs in animals have shown that some of these compounds can induce many health effects such as systemic effects (hepatic, hematological and immunological effects and the development of arteriosclerosis) genotoxic and carcinogenic effects (Nisbet and Lagoy, 1992; Ramesh et al., 2004). Hence, special interest was given to studying the toxicity of PAHs by different international bodies: The Scientific Committee for Food (SCF) and Joint FAO/WHO Expert Committee on Food Additives (JECFA).

<sup>\*</sup>Corresponding author. E-mail: mandapierre@yahoo.fr. Tel: 225 05 69 87 26.

Based on reviews of profiles of PAHs in food and the results of the carcinogenicity study of two coal tar led by Culp et al. (1998), the SCF and JECFA suggested that B[a]P should be used as a marker for the occurrence and carcinogenic effect of PAHs in food. In 2005 and again in 2008, the European Commission has established maximum limits for PAHs in different foodstuffs (Regulation (EC) No 1881/2006). More recently, the Scientific Panel on Contaminants in the food chain (CONTAM Panel) of EFSA reviewed the available data on the occurrence and toxicity of PAHs. The CONTAM Panel concluded that B[a]P alone is not a valid indicator of the occurrence of PAHs in food. The group proposed four PAHs (benzo[a]anthracene, benzo[b]fluoranthene, benzo[a]pyrene, chrysene) eight PAHs or (benzo[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[g,h,i]perylene, benzo[a]pyrene, chrysene, dibenzo[a,h]anthracene and indeno[1.2.3-cd]pvrene) as best indicators of PAHs in food to preserve the health of consumers better.

In fact, according to Kluska (2003), food contributes significantly to human exposure to PAHs. Many studies have shown that cereals, vegetables (COT, 2002), oil and fat (De Vos et al., 1990; Moret et al., 2002) are the main contributors to the ingestion of PAHs. However, grilled or smoked fishes and meats show a relatively low contribution, except in specific cases or due to socio-cultural reasons that cause these foods to occupy a prominent place in the diet (Kazerouni et al., 2001; Jira, 2004; FAO/WHO, 2008).

PAHs are most often identified and quantified using several analytical techniques: either gas chromatography with flame ionization detection (GC/FID) or coupled to mass spectrometry (GC/MS), high performance liquid chromatography with ultraviolet (HPLC/UV) or fluorescence (HPLC/FL) detection or coupled to mass spectrometry (HPLC/MS). The European Food Safety Authority (EFSA, 2007) in the report on PAH, revealed that concerning the analytical method used, 4% indicated GC-FID, 28% the HPLC-FL, 26% the HPLC-UV/FL and 43% the GC-MS. Nowadays GC/MS and HPLC/FLU techniques are the most sensitive and therefore more currently used for the analysis of PAH in foodstuff.

Presently, the two main analytical techniques used for determining PAHs in foods are high performance liquid chromatography (HPLC) coupled to a fluorescence detector (FLD) and gas chromatography-mass spectrometry (GC-MS). Both methods are sufficiently sensitive for determining PAH concentrations usually found in foods. Earlier, HPLC with an ultraviolet (UV) or a photo-diode array (PDA) detector and GC with a flame ionization detector (FID) were also methods often applied.

In Cote d'ivoire, meats and fishes are principal protein sources. However, the available literature relates very few national studies on food contamination linked to PAHs. It is also important to note the work of YebouéKouamé et al. (2003), who examined the risk involved in fish smoking in Abidjan through urinary 1- Hydroxypyrene (1-OHP) dosage. Their results show that the professional exhibition to the fish smokers was weak. This absence or insufficiency of basic data regarding PAHs in food in Cote D'ivoire led to the present study. Our objective has been to promote production of food free of contamination for human consumption. This study tried to evaluate the level of PAHs contamination in the two most consumed foodstuff, which are fish and meat sold in the markets in Abidjan.

#### MATERIALS AND METHODS

#### Standards and reagents

All solvents used were of HPLC quality. All of the individual standard solutions used were from Chiron (Denmark).The standards consisted of benzo[a]anthracene (B[a]A) (5 mg/ml), benzo[b]fluoranthene (B[b]F 1 mg/ml), benzo[k]fluoranthene (B[k]F; 0.2 mg/ml), benzo[a]pyrene (B[a]P; 1 mg/ml), benzo[g,h,i] perylene (B[g,h,i] P; 1 mg/ml), chrysene (CHR) and indenol(1,2,3,c,d)pyrene (IP; 0.2 mg/ml) packaged in 1 ml of toluene and dibenzo[*a*,*h*]anthracene (DB[*a*,*h*]A; 1 mg/ml) in solid form. From these standard stock solutions, solutions of individual PAHs at different concentrations up to 300 µg/l in an appropriate solvent (acetonitrile, HPLC grade, Scharlau) and 50, 100, 200 and 300 µg/l mixed standard solutions of the 8 PAHs were prepared. All of these solutions were prepared and stored in amber bottles in the dark at a temperature of 4°C.

#### Materials used for solid phase extraction

Two types of cartridges were used, C18 grafted-phase cartridges (Mega Bond Elut SPE Be-C18, VARIAN) with a phase of 2 g and a capacity of 12 ml and transplanted Florisil phase cartridges (Mega Bond Elut SPE Be-FI VARIAN) with a phase of 1 g and a capacity of 6 ml.

## Samples

The biological materials studied were made of two matrices (meat and fish) commonly consumed by the population. The sampling point of each matrix was its transformation by the processes of smoking, frying and broiling. Foods samples collected were from those sold to the population. Meat matrices were made of smoked pork samples, fried chicken samples and cooked or grilled mutton samples. Fish matrices were made of smoked sardine samples, fried tuna samples and carp samples cooked on the grill or broiled. Food matrices sampled were in three separate campaigns by applying the Directive 2005/10/EC. Indeed, the number of shelves on which food were sold was counted. A method applied to determine the number of tables representing each lot was stratified sampling method. The displays were by a chancy selection technique without repetition. Thus, for each sampling campaign, the lot defined the amount of ways present on the selected tables. The elementary sample constituted the quantity of removal materials at divers' corners on the lot. The total sample corresponded to the aggregation of all elementary samples; it was the sample made available to the laboratory. The stock of the three campaigns was 18 samples and 3 kg per sample. Samples were collected in aluminum foil, transported using coolers and then after grinding,

mixing, weighing and labeling, kept in the freezer (-18°C) until analysis.

#### Sample preparation

Jira (2004) has described the extraction method used with some modifications. It consisted of a liquid extraction followed by two extraction-purifications (solid phase extraction) on two different cartridges. 10 ml of the acetone-acetonitrile mixture (60:40; v/v) was added to the sample (2.5 g). The samples were then vortexed for 30 s, followed by an extraction for 5 min in an ultrasound bath and a centrifugation at 4000 rpm/min for 5 min. The upper phase extracted and collected were in a Teflon conical tube. This process was repeated three times and the extracts were concentrated using a rotary evaporator (Rotavap, Butchi) at 35°C. The residue was taken up in 2 ml of acetone-acetonitrile mixture (60:40; v/v), stirred for 10 s by vortexing, centrifuged at 4000 rpm/min for 5 min and the remains were collected. The process was repeated three times. This extraction step was followed by a double purification. The first purification performed was on a grafted C18 cartridge. The cartridge previously conditioned with 2 ml methanol and 24 ml acetonitrile. The three extracts were transferred into the cartridge, the tube was rinsed with  $2 \times 2$  ml acetone-acetonitrile (60:40; v/v), which were subsequently transferred to the head cartridge. The elution was done with 5 ml acetonitrile-acetone (60:40; v/v) at atmospheric pressure. The eluate obtained was evaporated using a rotary evaporator at 35°C. The dry residue was taken up in 1 ml hexane.

The second purification was carried out on a bonded-phase Florisil cartridge. The cartridge was previously conditioned with 15 ml dichloromethane and 12 ml hexane. After vortexing for 15 s, the extract was transferred to the head cartridge; the conical tube was rinsed 2 times with 2 ml hexane-dichloromethane (75:25; v/v) and then transferred to the head cartridge. The elution was performed with 4 ml hexane-dichloromethane (75:25; v/v).The eluate evaporation occurred until 1 ml remained, and then 500 µl toluene (retainer) was added. Evaporation continued up to about 50 µl. The required volume of solvent (acetonitrile) was added to obtain 1500 µl and its volume was calculated according to Equation (1):

$$V \text{ added} = 1500 \text{-m/d} \tag{1}$$

where m = sample mass in mg and d = 0.8669 g/ml (density of toluene). Obtained samples were then transferred into vial for HPLC analysis.

#### HPLC apparatus and conditions

The HPLC system was equipped with a Shimadzu SIL-20 AC automatic injector (Kyoto, Japan), a Shimadzu LC-20 AD pump (Kyoto, Japan), a Shimadzu DGU-20A degasser (Kyoto, Japan), a specified HPLC column: Prevail column C18, 150 × 4.6 mm, 5  $\mu$ m particles, kept constant at 40°C by a Shimadzu CTO -20A oven (Kyoto, Japan) and a UV-visible SPD-20A Shimadzu detector (Kyoto, Japan) at 284 nm. The whole chain was controlled by a Shimadzu CBM-20A system controller.

The injection volume was 20  $\mu$ l. The mobile phase consisted of solvent A (water) and solvent B (acetonitrile). The speed was 1.5 ml/min. The gradient was binary with 72% solvent B, 28% solvent A at 0 to 4 min, then 100% solvent B at 15 min. The calculation of PAHs was obtained by the method of external normalization following Equation (2), with Ci corresponding to the PAH content in a sample calculated in  $\mu$ g/kg fresh weight; Ai, the area of the peak (average of 2 injections) of PAHs in the sample solution; Air, the peak area (mean dose) of PAHs in the standard solution; Cir, PAH concentration in the standard solution in  $\mu$ g/l; V, the volume of the

final extract and m, the sample mass in grams.

#### Validation of the method

The validation of the analysis method was achieved according to NF V03-110 (1998) Standard. This procedure consists of the determination of detection and quantification limits, the calculation of the coefficient of variation of tests of repeatability and reproducibility and the determination of the linearity domain, the rate of recovery, the sensitivity and the specificity.

#### Statistical analysis

Data were analyzed using SPAD version 4.01. The tests used for the comparison of the PAHs according to the nature of foods were the t-test for independent samples when variances were equal and the Mann-Whitney test for unequal variances. Concerning the comparison of cooking processes, ANOVAs (analysis of variance) were used when variances were equal. In contrast, the Kruskal-Wallis test was applied in cases of unequal variance. To detect levels of differences between cooking processes, the Kolmogorov-Smirnov test with two samples were used. Significance was accepted at a level of 5%.

# **RESULTS AND DISCUSSION**

#### Validation of the method

In our study, we used HPLC/UV to quantify PAHs. The method by HPLC/UV also provides good results. The maximum rate of PAHs and particularly that of B[a]P is set at 5 µg/kg. This value is well above the HPLC/UV limit of detection. To reduce interference due to the low sensitivity of UV detector, a double purification was performed: on C18 bonded phase cartridge and the bonded phase florisil cartridge. The method used presented good linearity, established from 0 to 200 µg/kg with a coefficient of determination  $r^2 = 0.9997$ . The coefficients of variation of repeatability and reproducibility were respectively 1.34 (n = 10) and 4.74 (n = 5). Recovery rates were obtained from the standard addition method. PAHs standards were added to a quantity of 5 g of each matrix powder. The average recovery rate obtained for all eight PAHs was 91% (that is 80 to 101%). The detection limits ranged from 0.03 µg/kg for benzo(a)pyrene, benzo(k)fluoranthene to 0.36 µg/kg for chrysene and benzo(k)fluoranthene. As for the limits of quantification, they ranged from 0.06 to 1.2 µg/kg. In the EFSA (2007), the limits of detection varied from 0.0002 to 1 µg/kg with a mean of 0.12 µg/kg for B[a]P by the GC/MS technique.

## PAHs concentration in samples

Table 1 reports the average concentration of individual

Sample	CHR	B[a]A	B[b]F	B[k]F	B[a]P	DB[a,h]A	IP	B[g,h,i]P
Smoked meats	10.36	12.7	25.41	ND	8.76	0.97	64	0.11
Fried meats	ND	2.86	0.69	0.59	2.32	0.66	51	4.42
Grilled meats	5.04	12.6	19.22	ND	7.15	0.73	32.31	ND
Smoked fishes	13.67	45.07	66.67	ND	34.07	1.37	37.31	2.03
Fried fishes	ND	0.5	0.41	0.36	6.54	0.7	74.19	19.29
Grilled fishes	0.7	6.7	1.56	0.15	2.56	0.14	37.41	0.59

Table 1. Average amount of PAHs (µg/kg) in samples.

ND, Below detection limit; CHR, chrysene; B[a]A, benz[a]anthracene; B[b]F, benzo[b]fluoranthene; B[k]F, benzo[k]fluoranthene; B[a]P, benzo[a]pyrene; DB[a,h]A, dibenz[a,h]anthracene; IP, indeno[1,2,3-cd]pyrene; B[ghi]P, benzo[ghi]perylene.

PAHs in the various food samples. PAHs are present in all samples in varying concentrations. The highest mean levels of individual PAHs were observed in samples of smoked fish with B[b]F (66.67  $\mu$ g/kg), B[a]A (45.07  $\mu$ g/kg) and B[a]P (34.07  $\mu$ g/kg). B[a]P and IP were present in all foods with concentration greater than 1  $\mu$ g/kg. Concerning matrices of smoked foods, meats contained an average amount of 8.76  $\mu$ g/kg B[a]P. This concentration is greater than the amount observed by Jira (2004), who measured an average concentration of 0.12  $\mu$ g/kg B[a]P in samples of smoked ham and sausage, and is higher than the European Commission standard fixed at 5  $\mu$ g/kg.

Concerning matrices of fried samples, fishes had 6.54 µg/kg B[a]P and fried meats had 2.32 µg/kg B[a]P. This concentration in fried meats is lower than the European Standard (5 µg/kg). However, it is higher than the average value of 0.05 µg/kg found in fried meats by Kazerouni et al. (2001). In broiled food matrices, fishes had an average of 2.56 µg/kg B[a]P and meats had an average of 7.15 µg/kg B[a]P. These amounts are higher than the European Standard fixed at 2 and 5 µg/kg respectively, for broiled fishes and meats. These values are also higher than the average concentration of 1.72 µg/kg found by Kazerouni et al. (2001). However, they are similar to those of Akpambang et al. (2009) who found that five of the six tested grilled food samples sold in Nigeria exceeded the limit of PAHs fixed by the European Union.

# Total sum of the amount of various contents of PAHs 4 and PAHs 8 in samples

Table 2 shows the sum of the concentrations of different components of PAHs 4 and PAHs 8. The panel recommended the calculation of the sum of the contents of PAHs 4 or PAHs 8 during the last evaluation of the toxicity of PAHs in food. The CONTAM Panel proposed that the two sums are the best indicators of the occurrence of PAHs in food instead of B(a)P. Thus, in our study, the sum of the contents of PAHs 4 was highest in samples of smoked fish (159.48 µg/kg) followed by samples of smoked meats (57.23 µg/kg). Samples of fried meats and fried fish presented the lowest amount 7.45 respectively. 5.87 µg/kg and µg/kg, About PAHs 8, also samples of smoked fish (200.19 µg/kg) and smoked meat (122.31 µg/kg) have the highest amounts. These values are higher than that observed by Akpanbang (2009) in samples of grilled fish in Nigeria. The proportion of B(a)P found in the amounts of the sum PAHs 4 and PAHs 8 are evaluated and presented in Table 2. The highest rates were found in samples of smoked fish (17%) and grilled meat (9.27%).

# Evaluation according to food nature

Table 3 shows the studied PAHs profile according to the nature of the food matrix considered. The IP has the highest average levels but equal in the two matrices: 49.10  $\mu$ g/kg for meat and 49.64  $\mu$ g/kg for fish. The lowest levels are observed in B(k)F with 0.20  $\mu$ g/kg for meat and 0.17  $\mu$ g/kg for fish. The statistical analysis reveals that except for B[g,h,i]P (P=0.004), the amount of the other PAHs in meats and fishes was statistically equal (P>0.05). Thus, contamination by PAHs does not only depend on the nature of the food but also on the binary combination of the nature and cooking process applied to the food or on a likely environmental contaminant.

# Evaluation according to cooking process

Table 4 shows the average amount of the individual PAHs in studied sample, according to the cooking process. PAHs appear in all samples because of the cooking processes. In the smoking process, the highest average amounts of individual PAHs were found in IP and B[b]F with 50.66 and 46.04  $\mu$ g/kg, respectively. The amount of B[k]F found was inferior to the limit of detection . The B[a]P has a non negligible amount of 21.41  $\mu$ g/kg. The lowest amounts of PAHs after smoking were 1.07  $\mu$ g/kg B[g,h,i]P and 1.17  $\mu$ g/kg DB[a,h]A. In the smoking

Parameter	Smoked meat	Fried meat	Grilled meat	Smoked fish	Fried fish	Grilled fish
PAHs 4	57.23	5.87	44.01	159.48	7.45	11.52
PAHs 8	122. 31	62.54	77.13	200.19	101.99	49.81
B[a]P / PAHs 4 (%)	15.3	39.52	16.24	21.36	87.78	22.22
B[a]P / PAHs 8 (%)	7.16	3.70	9.27	17.01	6.41	5.13

Table 2. Sum in  $\mu$ g/kg of PAHs 4 and PAHs 8 contents and its percentages in B[a]P.

Table 3. Average amount of PAHs (µg/kg) and comparisons according to the nature of matrix

Nature	CHR	B[a]A	B[b]F	B[k]F	B[a]P	DB[a,h]A	IP	B[g,h,i]P
Meat	5.13	9,39	15.11	0.20	6.08	0.79	49.10	1.54
Fish	4.79	17.42	22.88	0.17	14.39	0.74	49.64	7.30
Significance	NS	NS	NS	NS	NS	NS	NS	S

S, Significant comparatively; NS, not significant comparatively.

Table 4. Average amount of PAHs (µg/kg) according to cooking process and comparisons.

Nature	CHR	B[a]A	B[b]F	B[k]F	B[a]P	DB[a,h]A	IP	B[g,h,i]P
Smoking	12.01	28.88	46.04	ND	21.41	1.17	50.66	1.07
Frying	ND	1.68	0.55	0.47	4.43	0.68	62.59	11.85
Broiling	2.87	9.65	10.39	ND	4.85	0.43	34.86	0.33
Significance	S	S	S	NS	S	NS	NS	S

S, Comparatively significant; NS, comparatively not significant.

process, the use of traditional and rudimentary methods, such as dry wood and coconut wadding for fuel contribute to increased amounts of PAHs in foods (Lozada et al., 1998). In addition, the covering of foods with cardboards at the operation time could modify the photosensitive properties of the PAHs, causing their accumulation due to the presence or absence of light and/or oxygen modification of the PAHs content in foods (Simko, 2005). In addition, the long smoking times and uncontrolled temperatures would cause an increase in PAHs quantity. Indeed, according to Kazerouni et al. (2001), the quantity of B[a]P depends on the smoke temperature and the exposure time and has a tendency to concentrate PAHs in cooked products.

In the frying process, the highest amount of any individual PAH was IP (62.60  $\mu$ g/kg), followed by B(g,h,i)P (11.85  $\mu$ g/kg), B[a]P (4.43  $\mu$ g/kg), DB[ah]A (0.68  $\mu$ g/kg), B[b]F (0.55  $\mu$ g/kg) and no traces of CHR was found. High amounts of PAHs could be due to the quality of the oil used. Indeed, oil manufactured under good conditions does not contain PAHs (Larson et al., 1987). The use of active coal in the refinement contributes to the total elimination of PAHs in oils during their manufacture (Lacoste et al., 2003). The repeated use of oil during frying would contribute to an increase of PAHs in the oils.

In the process of grilled cooking, IP presented the highest amount (34.86  $\mu$ g/kg), followed by B[b]F (10.39  $\mu$ g/kg), B[a]P (4.86  $\mu$ g/kg), CHR (2,87  $\mu$ g/kg), DB[a,h]A (0.44  $\mu$ g/kg) and B[g,h,i]P (0.33  $\mu$ g/kg). B[k]F was present with the lowest concentration (0.07  $\mu$ g/kg). The application of sauce generally rich in fat could influence the amount of PAHs in foods cooked on a grill. Jägerstad and Skog (2005) pointed out that sauces smeared on meat increase the burnt surface of the meat and encourage the production of PAHs. In addition, during grilling, the greases melt and flow onto the heat source, causing their pyrolysis and the formation of PAHs.

According to Stolyhwo and Sikorski (2005), foods prepared under controlled conditions generally contain low amounts of PAHs. A lack of knowledge about PAH formation and the failure to employ restrictive parameters is the origin of the PAH contamination associated with the use of traditional methods could be incriminated in the formation of PAHs in all cooking processes.

# Detection limits of different cooking methods

To better identify the differences between the cooking processes in the genesis of PAHs, they were compared

РАН	Comparisons of the cooking method in pairs							
	Smoking/frying (Sm/Fr)	Smoking/grilled (Sm/Gr)	Frying/grilled (Fr/Gr)					
CHR	Sm > Fr	Sm > Gr	NS					
B[a]A	Sm > Fr	Sm > Gr	Fr < Gr					
B[b]F	Sm > Fr	Sm > Gr	Fr < Gr					
B[k]F	Sm < Fr	NS	NS					
B[a]P	Sm > Fr	Sm > Gr	NS					
DB[a,h]A	Sm < Fr	NS	NS					
IP	NS	NS	NS					
B[g,h,i]P	Sm < Fr	NS	Fr > Gr					

 Table 5. Differences in detection levels according to cooking process.

NS, Comparison not significant; Sm > Fr, smoking generate more PAHs than frying.

statistically in pairs (Table 5). This table shows the result of this comparison. The comparisons show that with the exception of B[g,h,i]P, smoking was the method that generated the most PAHs among the three practices, as measured by CHR, B[a]A, B[b]F, B[k]F and B[a]P. This result has been confirmed by Azeredo et al. (2006), who found that smoking generated significant amounts of B[a]P. Frying and broiling generates PAHs in equal proportions for CHR, B[k]F, B[a]P, DB[ah]A and IP.

## Conclusion

Smoked, grilled and fried meats and fishes sold in the Abobo market demonstrate contamination with different levels of PAHs. This contamination is marked by the presence of B[a]P in foods in quantity above European Standard. Smoking was revealed as the process that generated the most PAHs. Finally, according to the nature of the food, meats and fishes have high amounts of PAHs; however, there were no significant difference between the two types of matrices. Considering the potential carcinogenicity of PAH contamination and the importance of this food group in the Cote d'Ivoire food regime, the establishment of a regulation and surveillance plan should be considered as a high priority.

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