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Research Article

Levels of contamination of meat and offal (skins) by polycyclic aromatic hydrocarbons during grid cooking or following pre-treatment of tire stripping

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Abstract: The "choukouya", beef cooked on artisanal metal grills powered mainly by wood, is being a high consumption in Ivory Coast particularly in Abidjan, in the towns of Port-Bouet and Yopougon. It is the same for the "kplo" beef skin stripped to the fire made of used tires and debris of rubber, particularly in the communes of Abobo and Port-Bouët. These cooking process generate toxic compounds in foods among which are PAHs, carcinogenic or mutagenic. The objective of this study is to determine the levels of contamination of the meat and offal (skins) by PAHs. Thus, 30 samples of skins and 30 samples of meat were collected and assayed on the HPLC. The results indicate the presence of PAHs sought at various concentrations. In samples of skins from Abobo and Port-Bouet, the highest average concentrations are those of the chrysene. The average concentrations of benzo (a) pyrene and the sum of the concentrations of 4 PAHs of reference of the Council of Europe are beyond MRLs set at 2 µg / kg and 12 µg / kg respectively, according to the EC regulations number 835/2011 . Considering meat samples from Port-Bouet and Yopougon, the chrysene and the fluoranthene have the highest average

concentrations. As previously, the average concentrations of the benzo (a) pyrene and the sum of the concentrations of the 4 PAHs are higher than the MRLs.

Key words: meat; offal; skins; PAHs; choukouya; kplo; concentrations

INTRODUCTION

A significant amount of meat and offal is traded poorly but fairly dynamically in Côte d'Ivoire¹. Especially, grilled beef, commonly known as "choukouya" is very popular. However, the conditions of its cooking by the craftsmen leave doubts as to the hygienic quality of this food. Indeed, the various fuels used are not advisable. These are rubber woods, wood from evacuation operations often covered with paint, varnish, mothproofs etc. In addition, the metal barrels often painted or having contained various chemicals are used for the construction of the artisanal cooking ovens of this meat.

Pickled beef skin, commonly known as "kplo" is heavily consumed in Côte d'Ivoire. One of the practices increasingly in progress and specifically in Abidjan, is to strip the skins of slaughtered animals (oxen, sheep, goats ...) with fires made from used tires and rubber debris instead of wood too expensive and because of the high calorific value of rubber (PCI) (40 000 kJ / kg)². Indeed, the intensification of car traffic generates used tires that are available at low cost and sometimes free for craftsmen.

These techniques of meat preparation and tire stripping to obtain "kplo" are likely to generate or transfer toxic compounds in these foods during their preparation. Among these compounds are polycyclic aromatic hydrocarbons (PAHs), carcinogens or mutagens³. These pollutants could thus contaminate these foods, pollute the environment and ultimately constitute a risk for the health of large consumers.

Already, Ivorian data indicate 15,000 to 20,000 new cases of cancer per year of which 20% are related to food⁴. In response to this situation, the State has given priority to food safety through quality control by adopting maximum residue limits (MRLs) or health and hygiene standards. The objective of this study is to determine the levels of contamination of meat and offal (skins) by PAHs.

MATERIAL AND METHOD

Material:

Biological material: The biological material consists of:

- ox skin from stripping with tires or rubberwood;
- Beef tenderloin cooked at the grill.

Reagents and solvents: The solvents used for PAH extraction are all HPLC grades. Acetone and acetonitrile were supplied by Fluka with a purity of 98.5%. Dichloromethane, N-hexane, methanol of greater than 98% purity come from the company PROLABO. Toluene of 99% purity was supplied by MERCK. The standard solutions of 9 PAHs were provided by Fluka. Their purity varied between 98% and 99.9%.

Methods: The study took place in the district of Abidjan, in the Republic of Côte d'Ivoire. Three communes of the district were selected namely Abobo, Port-Bouet and Yopougon. This choice was motivated by the intensity of stripping and restoration activities in these municipalities. Indeed, they are highly human with a large number of restaurants and grilled meat outlets, particularly in Abobo and Yopougon. In addition, the town of Port-Bouët is home to the slaughterhouse of the city of Abidjan around which many commercial and gastronomic activities are developed. In the different

zones, sites are identified for the collection of meat and hides. These include restaurants, grilled meat outlets and pickling locations. **Figure 1** below shows the study areas.

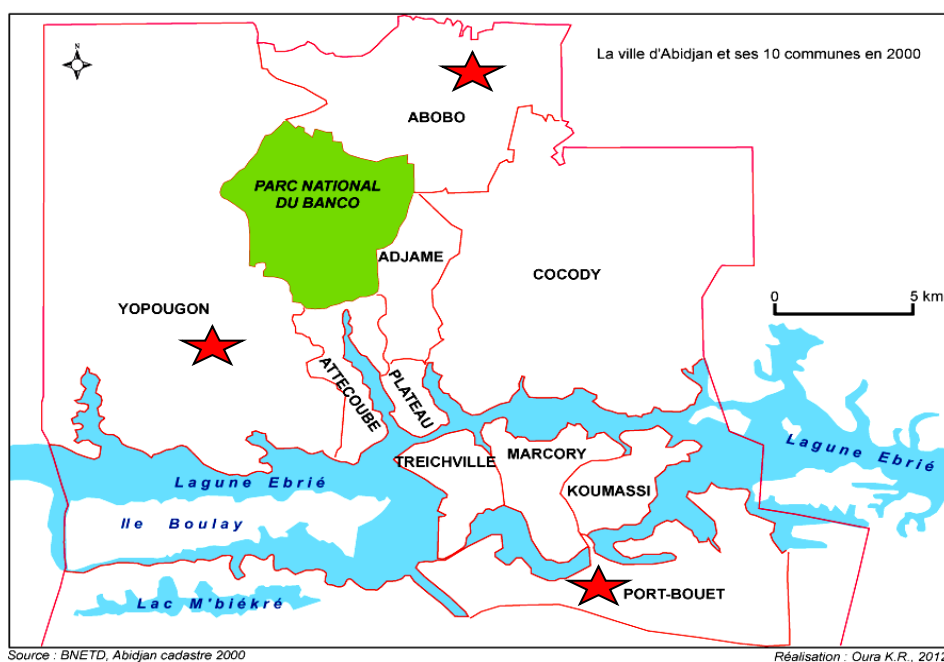


Figure 1: Study areas

Sampling skins: The skins sampling area is made up of the communes of Abobo and Port-Bouët. Two sites were chosen in these municipalities for their intense stripping activity. The site located in the town of Abobo is identified by the GPS coordinates $5^{\circ} 27'4''N$ and $3^{\circ} 58'29''W$ and that of the town of Port-Bouët for GPS coordinates $5^{\circ} 15'45'' N$ and $3^{\circ} 58'11''W$.

The sampling was random and was done in two campaigns. The first took place in March 2017 and focused on the collection of samples from Abobo. As for the second, it was done in August 2017 and it concerned the skin sampling in Port-Bouët.

In each commune, pieces of skin from pre-treatment to the tires are purchased with the artisans of the stripping and handed over to the restorers to be cleaned before cooking. Purchases of the skins are distributed over 5 foci (combustion areas) arbitrarily chosen on the site of the stripping.

A pre-treatment of skins with potash is mainly practiced by the restorers. According to these, it allows them to soften and facilitate their cooking. Due to the widespread nature of this type of cooking, the pieces of skin have undergone the same pre-treatment. Thus, 5 kg of beef skin was cleaned, cut and introduced into 10 L of water; 20 g of potash were added and the whole is heated to cooking for 5 hours. After cooking, the skins were cooled and approximately equal size piles were formed randomly. Each pile represents a sample. All the samples were then packaged in food bags to avoid any contamination and then transported to the laboratory and stored in the freezer at a temperature of $-4^{\circ} C$. By commune, 15 samples are made up of 30 samples on the whole of the two communes.

Sampling of meat: The municipalities of Port-Bouët and Yopougon constitute the meat sampling area. Six meat cooking sites were selected in these communes, because of three per commune and their GPS coordinates are shown in **Table 1** below:

Table 1: GPS coordinates of meat cooking sites

Sites	Port-Bouët	Yopougon
I	5°15'34''N et 3°58'5''W	5°21'18''N et 4°4'45''W
II	5°15'35''N et 3°58'9''W	5°21'14''N et 4°4'27''W
III	5°15'33''N et 3°58'8''W	5°20'47''N et 4°3'54''W

The sampling was random and took place over two campaigns. The first took place in February 2017 and focused on the collection of Yopougon samples. The second took place in July 2017 in the town of Port-Bouët.

Beef was bought and handed over to the grilling artisans to be cooked. After cooking and cooling, heaps of almost identical size are formed and put in food bags. Each of the piles is a sample. All the samples were then packaged in plastic freezer bags and stored in a cooler. Finally, it was transported to the laboratory and stored at a temperature of -4°C . By commune 15 samples are made; which makes a total of 30 samples for the two communes.

Analytical methods: The chromatographic method which comprises two main steps has been used. The first step was to prepare the samples to extract the samples and purify the extracts. The second step was instrumental analysis for the identification and quantification of PAH molecules. The assay involved 9 PAHs, 8 of which are classified as priority substances for food safety⁵. In addition to these 8 molecules, that of fluoranthene was retained because it is among the 16 PAHs classified by the USEPA⁶. In the end, the following PAHs were determined: benzo (a) pyrene; benzo (a) anthracene; benzo (a, h) anthracene; benzo (b) fluoranthene; benzo (k) fluoranthene; benzo (g, h, i) perylene; chrysene; fluoranthene; indeno (1,2,3-c, d) pyrene.

Methodological approach: PAHs were measured using the ISO / DIS 15753 chromatographic method. The choice of this method was motivated by its easy implementation, with little solvent used. In addition, it offers the possibility of determining 15 PAH molecules, which is considerable. After grinding the samples, the PAHs were extracted with an acetonitrile / acetone mixture and then purified on C18 grafted phase cartridges. Determination of the individual PAH content after separation is done on HPLC by measuring the fluorescence at different excitation and emission wavelengths.

Extraction of PAHs: Skin or meat samples removed from the freezer and thawed at room temperature (25°C) were first milled using a turbo-mill. Then, 2.5 g of the ground material was removed and mixed with 15 g of anhydrous sodium sulfate in a porcelain mortar. After a much finer grinding, the mixture was introduced into a centrifuge tube. To this content, 20 mL of acetonitrile / acetone (60:40) was added. The whole was stirred for 30 seconds with a vortex mixer and then placed in the centrifuge for 5 minutes at 4000 rpm. Using a micropipette, the upper phase was removed and stored in a flask. The extraction is repeated twice with 20 ml of acetonitrile / acetone (60:40). The solvent of the collected extracts was then evaporated on a rotary evaporator at 35°C .

Purification of extracts: First, the C18 grafted phase cartridge (LRC Bond Elut 500 mg, 10 mL) was conditioned with 12 mL of methanol and 12 mL of acetonitrile. Then, it was rinsed with 15 mL of

dichloromethane and then with 12 mL of hexane. The residues obtained after evaporation of the solvent from the extracts were collected in a flask with 1 mL of hexane / dichloromethane solvent (75:25) and transferred into the conditioned cartridge. The flask is rinsed 2 times with 2 ml of this same mixture. PAHs were eluted with 4 mL of hexane / dichloromethane (75:25) and 0.5 mL of toluene was added to the collected solution. The solvents were evaporated and the residues were recovered with 200 μ L of acetonitrile and stored in a vial ready for HPLC analysis.

Instrumental analysis: The chromatographic analysis conditions are mentioned in the following **Table 2**.

Table 2: Chromatographic Analysis Conditions

Column with reversed phase polarity HAP Mobile phase	Mobile phase	Flow rate (mL / min)	Injection volume (μ L)
Chromspher PAH-5 μ m - 250 mm x 4.6 mm	acetonitrile- water gradient	0.6	25

The absorption and emission wavelengths of the different molecules are shown in **Table 3** below:

Table 3: Excitation and emission wavelengths of PAH molecules

PAH molecules	Excitation wavelength (nm)	Emission wavelength (nm)
B(a)P ;B(a)A; B(b)F; B(k)F; B(g,h,i)P	365	420
B(a)P ; B(b)F; B(k)F; B(g,h,i)P; DB(ah)A	296	405
B(b)F; B(k)F I(c,d)P;CHR	300	500

Identification and quantification: The identification consisted of the injection of 25 μ L of the mixture to be separated at the inlet of the column; the identification of each compound of the mixture is made thanks to its retention time.

Quantification was done by determining the areas of the peaks; two measurements were made per sample. Thus, the average concentration obtained from these two measurements was selected for the sample.

RESULTS AND DISCUSSION

Level of contamination of the skins: **Figure 2** shows the average levels of PAHs in skin samples from the two communes (Abobo and Port-Bouët).

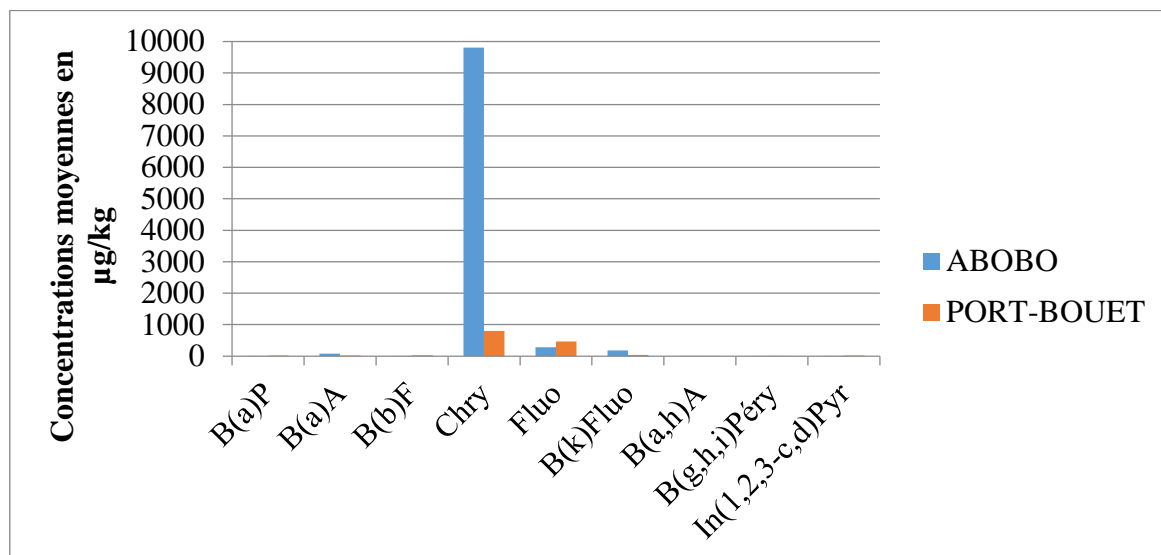


Figure 2: Mean levels of PAHs in skin samples

According to this figure, the desired PAH molecules are present in skin samples at various concentrations. These results could be explained by the fuels used mainly consisting of used tires and rubberwoods.

Chrysene has the highest average concentrations of 9810.924 µg / kg and 803.224 µg / kg respectively in Abobo and Port-Bouët. B (a) P, which is the reference PAH for toxicity, was detected in the Abobo and Port-Bouët samples at the mean concentrations of 5,738 µg / kg and 12,355 µg / kg, respectively, above the MRL set at 2 µg / kg. The sum of the average concentrations of the 4 reference PAHs is 9902.058 µg / kg and 851.631 µg / kg respectively in Abobo and Port-Bouët. According to EC Regulation No. 835/2011, these values are well above the MRL set at 12 µg / kg⁷. It should be noted that these 4HAPs are benzo (a) pyrene, benzo (a) anthracene, benzo (b) fluoranthene and chrysene.

The high concentration of chrysene can be explained on the one hand by its presence in the resin coming from rubber woods. Indeed, according to Hansen *et al.*⁸, some plants such as conifers can create, for example, phenanthrene or chrysene present in the resin. Similarly, its formation during the combustion of rubber would also be at the origin of its abundance. This result is consistent with that obtained in a study of pollutant emissions during tire fire⁹. Among the products generated then in the PAH group, chrysene had the highest average concentration which was about 0.4 mg / m³.

In addition, according to INERIS¹⁰, chrysene is present at higher concentrations than most other PAHs in fossil fuels such as crude oil and lignite. It is one of the predominant PAHs in particulate emissions from household waste incinerators, natural gas appliances and domestic heating appliances, particularly those using wood combustion.

The average chrysene content in Abobo is much higher than in Port-Bouët. This result could be explained by the relatively higher use of fuels such as rubber wood and plastics from aircraft carcasses in the Abobo area. Indeed, resin and thinner likely to contain PAH are also involved in the assembly of these devices.

Level of contamination of meat: Figure 3 shows the average PAH levels in the meat samples from the two communes (Port-Bouët and Yopougon).

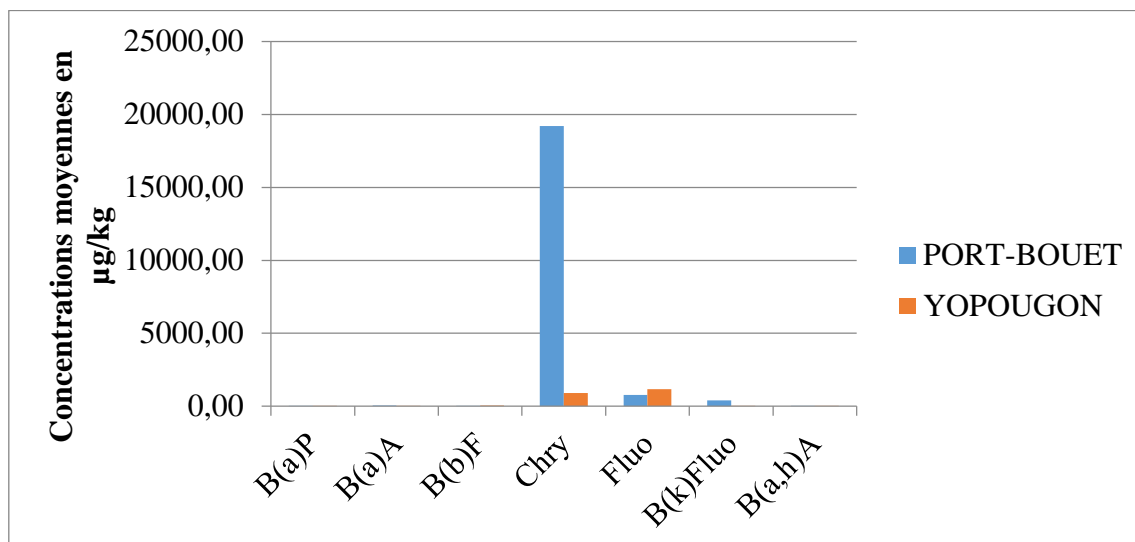


Figure 3: Average levels of PAHs in meat samples

It appears that the PAHs sought were detected in the meat samples at different concentrations. These results could be explained by the fuels used mainly made of rubberwood. In addition, the PAH molecules could come from metal barrels used for the construction of craft ovens often covered with paint, varnish or containing various chemicals.

Chrysene and fluoranthene have the highest average concentrations in both municipalities. B (a) P was found at concentrations of 9.866 µg / kg and 13.474 µg / kg respectively in the Port-Bouët and Yopougou samples. These values are higher than the MRL. The cumulative concentrations of the 4 PAHs of reference are respectively for the Port-Bouët and Yopougou samples of 19294.814 µg / kg and 974.48 µg / kg. They are well beyond the MRL.

The results also show that the chrysene content in Port-Bouët is much higher than that obtained in Yopougou. This could be explained by the fuels used especially the wood of rubber trees and also those resulting from evacuation operations often covered with paint, varnish, mothproofs etc. The contents of fluoranthene are of the same order in the two communes. Moreover, combustion is also an important source of fluoranthene emission. This PAH is also the most emitted in the atmosphere in France through combustion in domestic appliances, the tertiary sector and road transport¹¹.

CONCLUSION

The desired PAHs were detected at various concentrations in the various matrices analysed. In skin samples (kplo) from Abobo and Port-Bouët, the highest mean concentrations are chrysene (9810.924 µg / kg and 803.224 µg / kg respectively). The average concentrations of benzo (a) pyrene and the sum of the average concentrations of the 4 reference PAHs of the European Council are above the MRLs set at 2 µg / kg and 12 µg / kg, respectively, according to Regulation EC No 835 / 2011. With regard to grilled meat samples (choukouya) from Port-Bouët and Yopougou, chrysene and fluoranthene have the highest average concentrations. As previously, the average benzo (a) pyrene concentrations and the sum of the average concentrations of the 4 reference PAHs are above the MRLs.

In view of the fact that the MRLs have been exceeded and the carcinogenic or mutagenic properties of PAHs, it will be necessary to assess the risks associated with the consumption of grilled meat and pickled skins.

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