Influence Of Post-Harvest Technology Treatments on Polycyclic Aromatic Hydrocarbons Formation in Cocoa Beans and Derived Products

ABSTRACT

Aims: This study aimed to determine influence of post-harvest treatments on PAH's formation in raw cocoa.
Study design: A total of 370 samples were analyzed during 2014 and 2015 cocoa harvesting.
Methodology: A method based on a solid-liquid and liquid-liquid extraction followed by an HPLC-Fluorescence assay was optimized and validated for PAHs analysis in cocoa.
Results: Whatever the post-harvest treatment, cocoa PAH's contents were under 6.19 ± 0.30 $\mu g.kg^{-1}$. Then, after traditional warehouse storage of raw cocoa, PAHs contents ranged from 35.11 ± 3.30 to 39.53 ± 0.75 $\mu g.kg^{-1}$ above EU standards. Artisanal smoked dried cocoa recorded the highest PAH contents comprising between 35.06 ± 0.42 and 172.22 ± 14.79 $\mu g.kg^{-1}$. Also, monitoring of PAH concentrations during make process of cocoa into derived products has shown that shelling reduces the initial contamination of cocoa beans by more than 90 %. **Conclusion**: The use of artificial drying methods and storage conditions in the presence of smoke-induced and increasing PAHs to a critical level. Particularly, drying techniques using smoke caused considerable accumulation of PAHs in raw cocoa.

Keywords: Cocoa beans and Derived products, Post-harvest treatment, PAHs, Côte d'Ivoire, HPLC, Validated Method

1 INTRODUCTION

Polycyclic aromatic hydrocarbons (PAH) constitute a large class of chemical compounds of high toxicity and are widespread in the environment **[35]**. They are formed during the pyrolysis and pyrosynthesis of organic matter during domestic, industrial, or geothermal processes **[18; 43]**. These compounds are very lipophilic and can induce mutations within DNA **[43,17]**. The experimental data available in animals have shown that certain PAHs are carcinogenic, teratogenic, and genotoxic **[19; 46].** Due to their mode of formation, human exposure to these compounds seems inevitable and the main route of exposure is food **[35; 4; 43]**. PAHs affect various foodstuffs, mainly smoked meat products **[2; 4]**, plant products such as cereals, and oilseeds **[4; 3]** such as cocoa beans **[48]**. Due to their high-fat content of around 60 %, cocoa beans are very prone to PAH contamination **[37; 48]**. Indeed, the formation of PAH in cocoa beans would be linked to artificial cocoa drying techniques using wood fires **[47; 30]**, to drying supports such as bitumen areas **[34; 32]** and the storage of merchant cocoa near the smoke from wood fires. Cocoa beans are processed into three main derived products including cocoa liquor, cocoa powder, and cocoa butter. This latter is an essential ingredient in the chocolate industry due to its specific rheological, functional, and chemical characteristics **[21; 5; 28]**. The quality of this fat can be altered by levels of PAHs outside international specifications **[48]**. The impossibility of refining cocoa butter like

other vegetable oils **[15; 8]** would pose an acute public health problem concerning the high levels of PAHs that could occur in chocolate. In order to protect consumer's health of foodstuffs likely to be incriminated, in particular chocolate, maximum levels of PAHs allowed in cocoa have been set **[13; 15]**. These regulations invite all cocoa exporting countries to adopt measures to reduce the PAHs content of their cocoa production. Côte d'Ivoire as the world's leading producer of cocoa beans is directly impacted by these European measures. To preserve Ivorian cocoa from contamination with PAHs on the one hand and on the other hand, to avoid enormous financial losses to the country and to small cocoa producers while guaranteeing the health of chocolate consumers, it is urgent and necessary to investigate the factors of formation of PAHs in this product. This study aims to analyze the causes and conditions of the formation of PAHs in cocoa beans during post-harvest processing to reduce their critical content in chocolate and other derived products.

2 MATERIAL AND METHODS

2.1 Material

2.1.1 Vegetal material

Beans extracted from healthy and/or damaged cocoa pods used consisted of cocoa beans of all comers and harvested during the major cocoa harvesting seasons (November 2014 and 2015) in a peasant plantation were used. This plantation, with an area of 5 hectares, is located 142 km from Abidjan in the Department of Akoupé located in the southeast of Côte d'Ivoire between 6.38° North latitude and 3.87° West longitude more precisely at GPS coordinates N 06°30.806' and W 003°57.001' (**Fig. 1A-B**). This geographic area is characterized by a large and small rainy season in the periods of May to June and September to December, respectively **[25]**. Healthy pods are visibly intact, with integrity, and without bruising. Damaged pods were either partially attacked by rodents, affected by rot and/or fungal agents on the cocoa plant, accidentally damaged during harvesting, or deliberately injured in depth by machete cutting after harvest.

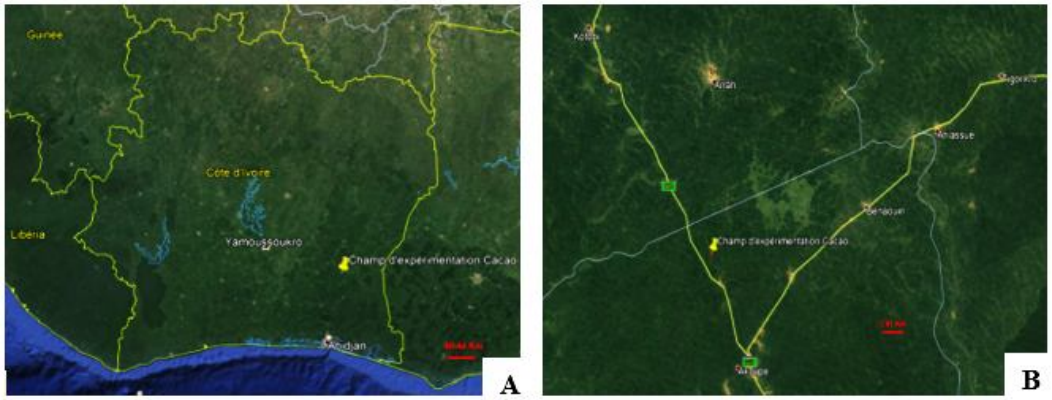

Fig. 1A-B. The geographical location of the farmer's plantation for agricultural experimentation in the department of Akoupé (South-East of Côte d'Ivoire). A) Situation in Côte d'Ivoire, B) Situation in the department of Akoupé

2.2 Methods

2.2.1 Preparation of cocoa samples

2.2.1.1 Pod opening

The preparation of the first series of cocoa bean samples required 2400 pods, of which 1200 were healthy and 1200 damaged. Each category of pods was divided into two parts of 600

pods each. The pods of one of the parts were opened without delay while those of the other parts were opened with a delay of 7 days of post-harvest storage (**Fig. 2**). Preparation of the second set of samples required 3500 pods opened without delay (**Fig. 3**). In all cases, pods opening was carried out manually using clubs and machetes. The extracted cocoa beans were carefully removed from the placenta or rachis, before being put into fermentation.

2.2.1.2 Fermentation

Cocoa beans from the first set of samples (2014) were subdivided into three equal parts of approximately 50 kg each. The beans from each share were fermented for 6 days without stirring using three fermentation techniques: wooden crate fermentation (CB), plastic crate fermentation (CP), and heap fermentation in banana leaves (FB) **[20; 25; 27]**. Beans from the second series (2015) were fermented in heaps in banana leaves for 6 days without stirring. At the end of fermentation, the processed cocoa beans were divided into 4 batches of 56 kg each.

2.2.1.3 Drying

Each batch of fermented beans from the first set of samples was subdivided into 4 fractions of the same weight of approximately 12.5 kg each. The cocoa beans were then sun-dried for 6 days on 4 different areas including a cemented area (A), a bitumen area (B), a bamboo rack woven (C), and a plastic tarpaulin (D). During the drying process, the cocoa beans were mixed every 2 hours. Those dried on the bamboo rack woven were the control. Cocoa beans from the second set of samples were dried using 4 combined drying modalities sometimes practiced in cocoa basins in Côte d'Ivoire **[29; 30]**. These modalities were declined in several drying technics, including wood fire drying (8 h/day for 2 days), solar drying on a plastic tarpaulin (8 h/day for 5 days), solar drying on a plastic tarpaulin (8 h/day for 2 days) followed by drying over a wood fire (8 h/day for 2 days) and solar drying on a plastic tarpaulin (8 h/day for 2 days) followed by artificial drying by a peanut roasting mill (1 h 30 min). For this series of samples, the cocoa beans sun-dried on a plastic sheet constituted the control.

2.2.1.4 Storage

Five (5) samples of 250 g each were formed from each batch of cocoa beans from the first set of samples (2014). One batch of two samples of cocoa beans was stored for four (4) weeks in a kitchen where food is cooked over a wood fire and the second batch of two samples in a non-smoking store usually used as a storage place for merchantable cocoa at the producers. The last sample of cocoa beans that was not stored either in the kitchen or in the store was immediately packed and served as a control. For the second set of samples (2015), five samples of cocoa beans of 250 g were formed from cocoa beans dried on a plastic tarpaulin. Two batches of two samples were stored respectively in the kitchen and stored in the cocoa producer. The remaining sample was the control at time T0. In all sample sets, the fermented and dried cocoa beans were packed in jute bags before being stored for four weeks. One sample of cocoa beans was taken from each batch every two weeks during storage and then wrapped in aluminum foil. In addition, two samples were taken from the beans of each drying technique and wrapped in aluminum foil. Finally, about twenty samples of beans of an unknown technical itinerary of preparation except for their origin in the warehouses of cocoa in the port of Abidjan were taken for the implementation of the validated method of analysis.

2.2.2 Complete experimental design of cocoa bean sampling

In summary, sample preparation was carried out during the 2014 and 2015 cocoa harvest campaigns according to two experimental designs schematized in the diagrams described in **Fig. 2 and 3**. To determine the impact of each post-harvest technological treatment on the

formation of PAHs in cocoa, the content of major PAHs in fermented and dried cocoa beans from each post-harvest technical route before and after storage was determined.

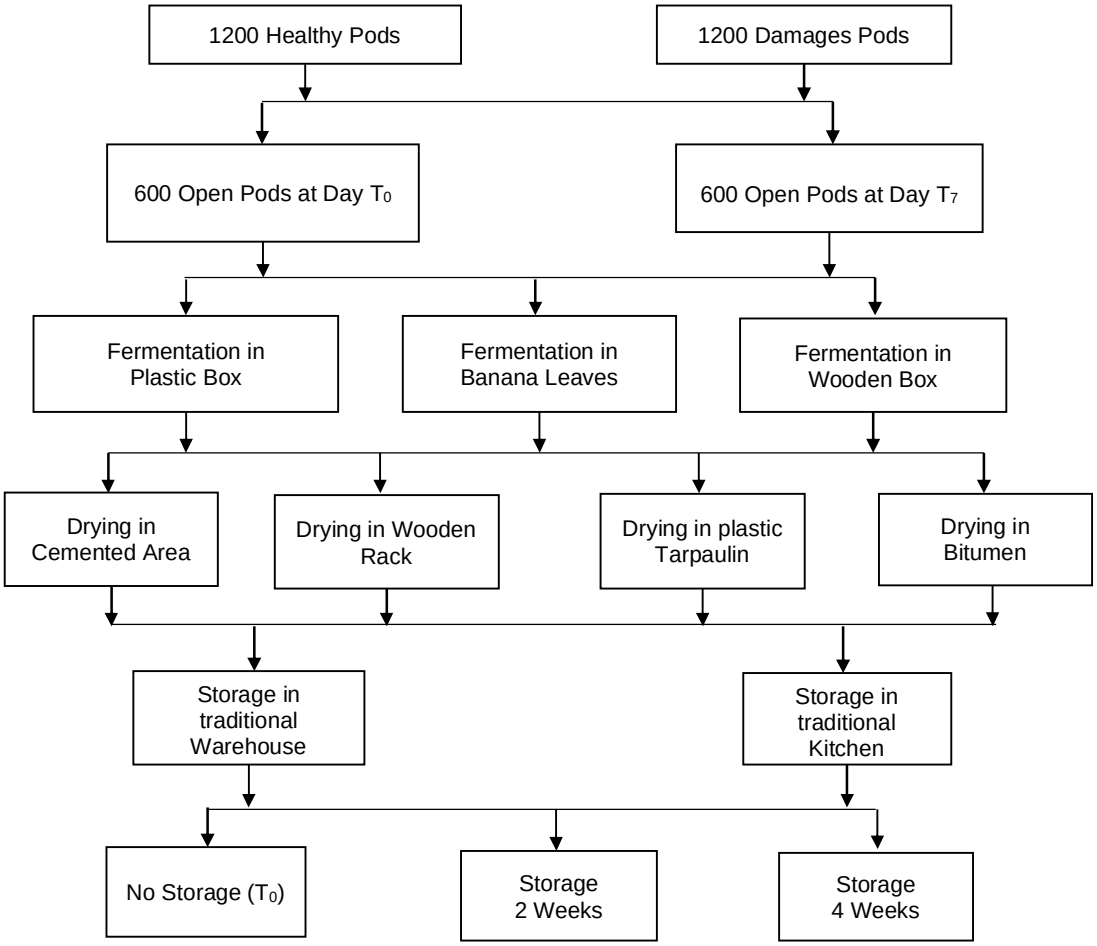

Fig. 2. Diagram of preparation of the first cocoa beans samples series

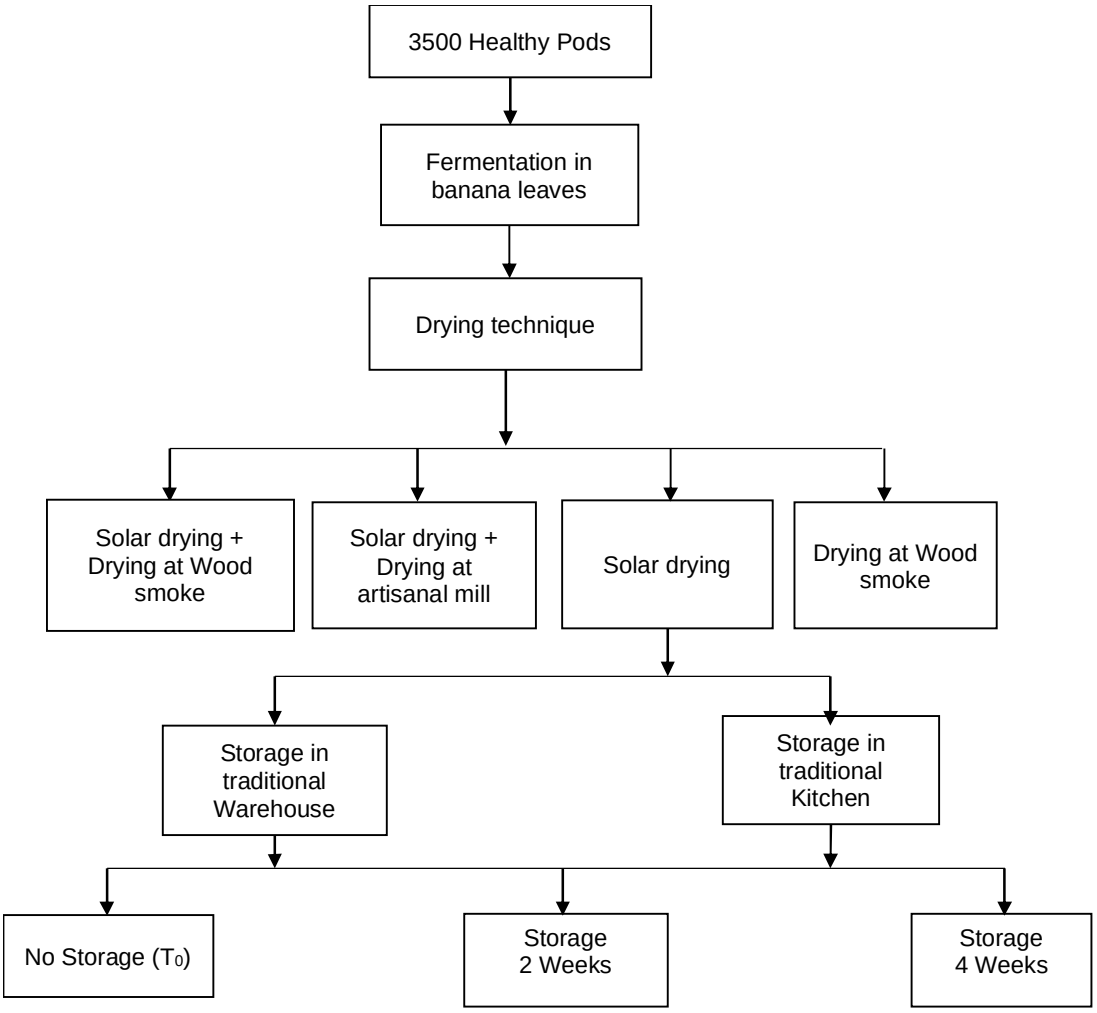

Fig. 3. Diagram of preparation of the second cocoa beans samples series

2.2.3 Manufacturing process of products derived from cocoa beans

The manufacturing process is described in **Fig. 4**. Approximately 232 g of cocoa beans were roasted for 25 minutes at 125 °C in a ventilated oven. The water loss rate of the cocoa beans was calculated by weighing before and after roasting the beans. The roasted beans were coarsely ground using an ice crusher to facilitate shelling. The shells were removed by ventilation in a catador. The coarsely crushed cocoa kernels, also called grits, were weighed before being ground. The grits were fed in small quantities into a heated porcelain mixer. Grinding 175-200 g of grits for 20-30 minutes resulted in the cocoa mass. White powdered sugar was added to 50 % of the cocoa mass. The grinding process was continued until a smooth and homogeneous paste called cocoa liquor was obtained, which was then subjected to refining. Refining consists of slowly turning over the cocoa liquor between the back roller and the intermediate roller of the refiner. The refined cocoa liquor was weighed and then stored in a refrigerated bank before the conching step. The conching temperature was programmed to 70 °C at least 2 hours before the conching began. Once the conching temperature was reached the machine was started at its minimum speed (≈ 33 rpm). The sweetened and refined cocoa liquor was gradually added to the conche. After the system stabilized, the conche was operated at its maximum speed (≈ 93 rpm). Then lecithin (0.01 % of the cocoa liquor mass) was added to the refined cocoa liquor and cocoa butter mixture.

The mixture was again subjected to conching for one hour. The cocoa liquor containing all the ingredients was collected, weighed, and placed in a refrigerated bank (+4 °C) before tempering was initiated in a tempering machine to 42 °C. The tempering process resulted in dark chocolate. The final product was then molded into bars of 70 g each and stored in refrigerate bank.

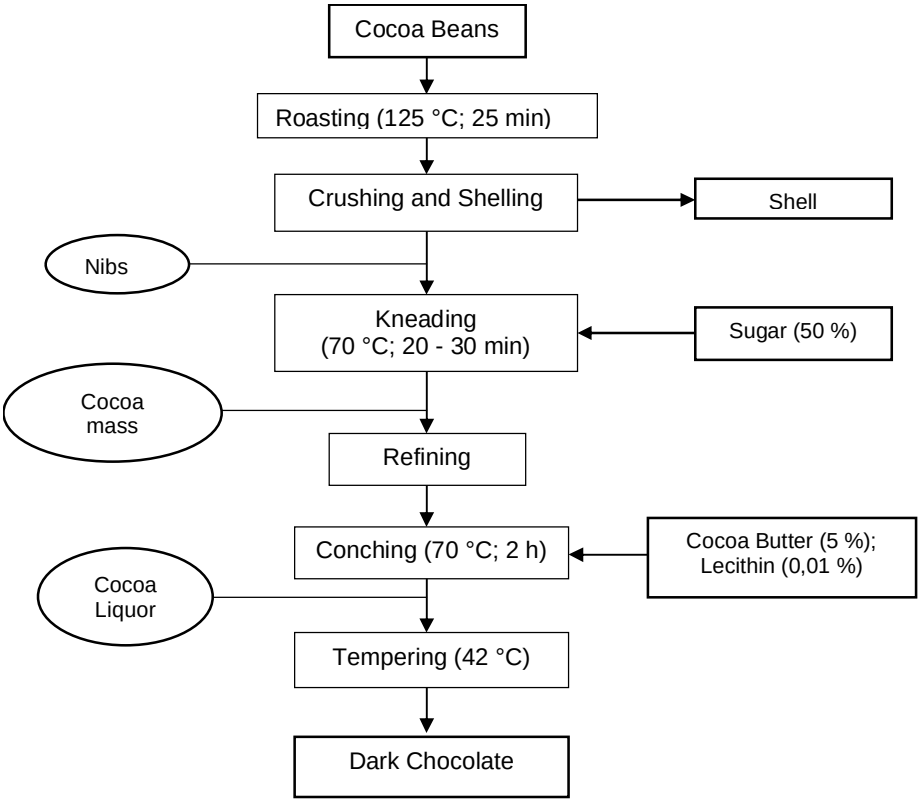

Fig. 4. The manufacturing process of cocoa bean products

2.3 Determination of PAHs contents in cocoa and derived products samples

2.3.1 Determination of moisture content

The moisture content of the cocoa samples was determined according to the gravimetric method **[23],** using a ventilated Chopin oven thermostatted at 103 ºC. The moisture content of each cocoa bean sample was calculated according to the following equation:

$$\textit{Moisture content}\ (\%) = \frac{m_1 - m_2}{m_1 - m_0} \times 100$$

Where:

m0 (g): mass of the empty dish and its lid;

m1 (g): sum of masses of the dish, its lid, and the cocoa powder sample before drying;

m2 (g): sum of masses of the dish, its lid, and the cocoa powder sample after drying.

2.3.2 Determination of the fat content

Fat extraction was performed at 110 °C for 1 hour from 3 g of cocoa powder using a SOXTEC Avanti 2050 semi-automatic programmable extractor **[39; 41]**. The fat content of each sample was determined according to the following equation:

$$\boldsymbol{FAT}(\%) = \frac{(Mp - Mv)}{Me} \times 100$$

Where:

FAT (%): Fat content expressed on a dry matter basis;
Mp (g): total mass of cup containing extracted fat;
Mv (g): mass of the empty cup;
Me (g): mass of cocoa powder test sample.

2.3.3 Method of determination of PAHs

The determination of PAHs in cocoa beans was performed according to the method validated by **[42]**. This method followed the performance criteria of the European Union regulations **[15; 16]** and the French standard **[31]**. The linearity of the method was determined in a five (5) point calibration range of concentrations between 2 and 32 ng.ml^{-1}. The PAHs limits of detection and quantification, the coefficients of variation for repeatability tests and intermediate precision; and finally, the recovery rates during an accuracy test were determined.

2.3.3.1 Method of PAHs extraction

The validated method in-house used a reference cocoa butter that contained PAHs (benzo(a)anthracene (BaA); chrysene (Chr), benzo(a)pyrene (BaP), benzo(b)fluoranthene (BbF)) at know concentrations. (**1**) 1 g of this reference material is weighed in a tube to centrifuge out of glass, to which 6 ml of ethanolic potash (KOH) at 1 M and a bar magnet there were added. (**2**) The unit is put to saponify in a hot bath at 80°C for 1 hour under agitation with 450 rpm. (**3**) The tube is then withdrawn from the hot bath and 6 ml of cyclohexane is added there. (**4**) The unit is put back to saponify in a hot bath at 80 °C for 5 min under agitation with 450 rpm. (**5**) At the end of saponification, the tube is withdrawn from the bath and 4 ml of ultrapure water is added there. (**6**) The whole is vortexed for 1 min to 1500 rpm and to centrifuged for 5 min at 3000 rpm. (**7**) The higher phase is recovered with a Pasteur pipet and transferred to another tube out of glass. (**8**) 3 ml of cyclohexane are added to the remaining phase to repeat processes (**6**) and (**7**). (**9**) The operation (**8**) is repeated twice to the times of continuation and the recovered supernatants are evaporated dry under nitrogen flow. (**10**) The dry extract is taken again in 1 ml of acétonitrile (ACN), vortexed at maximum speed, and filtered with a filter-syringe PTFE to 0.22 µm in an amber HPLC tube of 2 ml.

2.3.3.2 Method of PAHs quantification

PAHs are proportioned by using HPLC chain DIONEX of type ULTIMATE 3000 coupled to a fluorescent detector RF 2000. The wavelengths are programmed as follows: 0-29 min, 270 / 385 nm; 29-34 min, 270 / 420 nm; 34-60 min, 381 / 405 nm. A curve of calibration at 5 points of concentration (2 ng.ml^{-1}, 4 ng.ml^{-1}, 8 ng.ml^{-1}, 16 ng.ml^{-1}, 32 ng.ml^{-1}) carried out starting from a standard solution of PAHs mix made it possible to establish an adequate correlation between the surfaces of the peaks and concentrations found after analysis of the extracts of samples. The determination of PAH content in each sample was carried out in triplicate and the average concentrations were calculated.

2.4 Statistical analysis

The mean concentrations of the parameters (moisture, lipids, BaA, Chr, BbF, and BaP) were calculated with their standard deviation. Statistical processing was done with Microsoft EXCEL 2013 and IBM SPSS Statistics 23.0 and the significance level was 0.05. Comparisons between the different parameters were made with SPSS 23.0.

3 RESULTS AND DISCUSSION

3.1 Results

3.1.1 Influence of different technical itineraries of pods preparation to fermentation on PAHs contamination levels

For this part, the bitumen area was the drying surface chosen to discriminate cocoa samples because it was likely to generate a high level of PAH contamination of the cocoa beans. The levels of major PAHs (Σ4PAHs) in cocoa beans from various post-harvest primary preparation technical itineraries were tested and the integration of the sanitary state of pods, shelling time, fermentation techniques, and solar drying on bitumen were determined. The levels of major PAHs in cocoa beans studied were relatively close to 5 $\mu g.kg^{-1}$ (**Fig.5**). The results obtained showed that Σ4PAHs content in all cocoa beans analyzed ranged from 4.21±0.30 to 6.19±0.16 $\mu g.kg^{-1}$ regardless of the technical itinerary applied. The average value of Σ4PAHs contents was 5.27±0.73 $\mu g.kg^{-1}$. Beans from damaged pods and then fermented in plastic crates with no shelling delay (CPab0) had the highest content of Σ4PAHs at around 6.19±0.16 $\mu g.kg^{-1}$. However, the lowest Σ4PAHs contents of 4.21±0.30 $\mu g.kg^{-1}$ were measured in beans from damaged pods and then fermented in banana leaves with no shelling delay (FBab0).

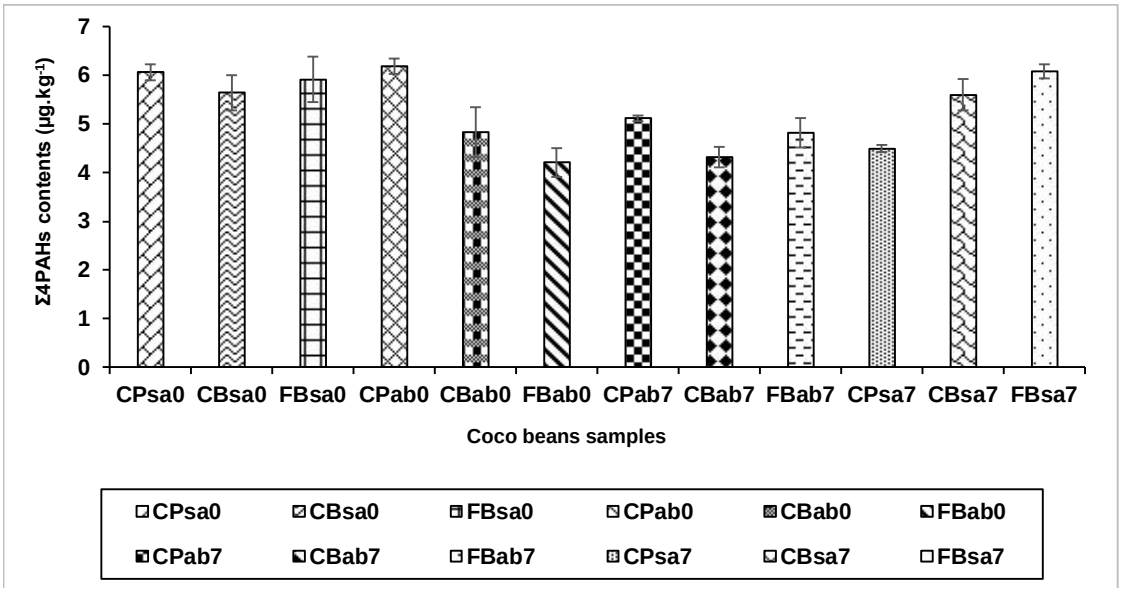

Fig. 5. Sum of 4 PAHs (Chr, BaA, BbF, BaP), (Σ4PAHs) contents of cocoa beans from various post-harvest technical itineraries and dried on bitumen area. With "CP" = Fermentation in Plastic Boxes, "CB" = Fermentation in Wooden Boxes, "FB" = Fermentation in Banana Leaves, "sa" = Healthy pods, "ab" = Damaged pods), "0" =0 days of pod opening time, "7" =7 days of pod opening time.

3.1.2 Influence of drying techniques

Fig. 6 shows the sum of 4 PAHs (Σ4PAHs) contents in cocoa beans dried by different techniques. The results obtained show that the butter extracted from sun-dried cocoa beans

was characterized by Σ4PAHs contents of 5.92±1.20 µg.kg^{-1}. When the cocoa beans were dried by solar technique followed by roasting in a peanut oven, the Σ4PAHs content of the resulting butter was 36.80±4.74 µg.kg^{-1}. However, Σ4PAHs contents in cocoa butter were 114.92±21.99 µg.kg^{-1} when the cocoa beans were dried first in the sun and then by the use of a wood fire. Finally, when the cocoa beans were dried by wood fire alone, the major PAH content of the derived butter was around 148.86±25.17 µg.kg^{-1}.

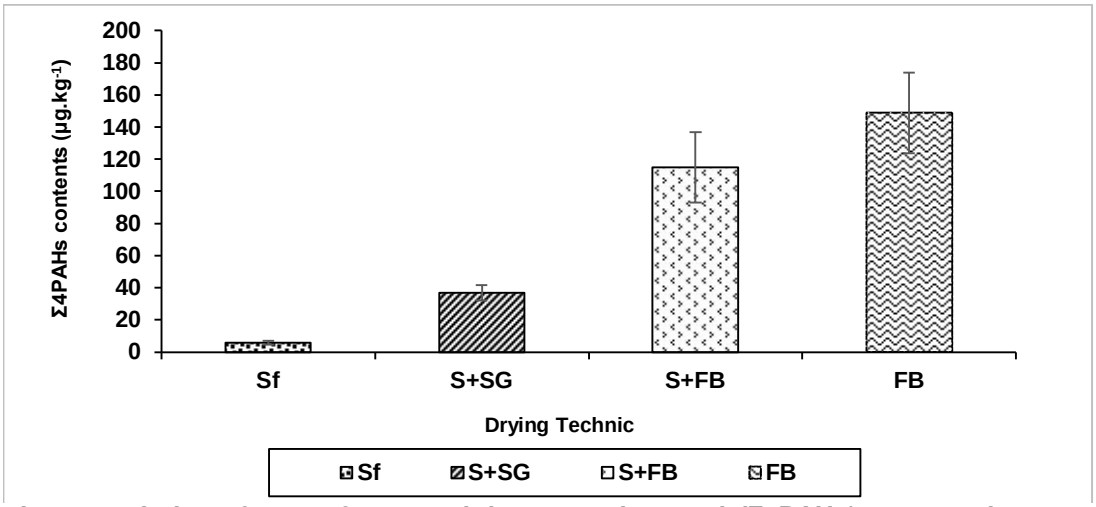

Fig. 6. Evolution of Sum of 4 PAHs (Chr, BaA, BbF, BaP) (Σ4PAHs) contents in cocoa beans dried by different techniques. FB: Wood-fired drying; S+FB: Solar drying followed by wood-fired drying; S+SG: Solar drying followed by peanut roasting oven drying, Sf: Solar drying of fermented cocoa beans.

3.1.3 Influence of storage conditions

Fig. 7 shows the evolution of major PAHs content (Σ4PAHs) in sun-dried cocoa beans according to different supports: racks, plastic tarpaulin, cemented area, and bitumen. The results obtained show that the PAH content of butter from cocoa beans drying on a rack was 5.64±0.01 µg.kg^{-1} before storage. After two weeks of storage, the Σ4PAHs content of butter from cocoa beans stored in a farmer's kitchen increased from 5.64±0.01 to 20.48±1.76 µg.kg^{-1} while butter extracted from beans stored in a warehouse had a Σ4PAHs content of 6.26±0.56 µg.kg^{-1}. After four weeks of storage, the levels of Σ4PAHs in the butter produced from the beans stored in a warehouse stagnated at around 5.98±0.30 µg.kg^{-1} while that of the butter extracted from the cocoa beans stored in the farmer's kitchen increased by nearly 44.86 %, namely a Σ4PAHs content of 37.14±2.79 µg.kg^{-1}. At the same time, butter from cocoa beans dried on plastic tarpaulin had Σ4PAHs contents of 5.12±0.27 µg.kg^{-1} immediately after drying. After two weeks of storage, this content reached 27.19±1.85 µg.kg^{-1} for the butter from the beans stored in the kitchen while that of the butter extracted from the beans stored in the store remained constant at around 5.83±1.21 µg.kg^{-1} for cocoa beans stored in the store. After four weeks of storage, the Σ4PAHs content in the butter obtained from the beans stored in the store did not change (5.57±0.61 µg.kg^{-1}) while that of the butter derived from cocoa beans stored in the farmer's kitchen increased to 35.11±1.30 µg.kg^{-1}, an increase of 20.28 %. For cocoa beans dried in cemented area, their initial Σ4PAHs content was 6.14±0.30 µg.kg^{-1}. In two weeks of storage in a warehouse, it changed very little (5.62±0.16 µg.kg^{-1}) while that of the butter extracted from beans stored in the kitchen increased by 78.80 % reaching 28.97±0.71 µg.kg^{-1}. After four weeks of storage, the Σ4PAHs content in the butter extracted from the beans stored in the store increased little to a value of 7.16±0.45 µg.kg^{-1}. However, the Σ4PAHs content of butter from cocoa beans stored in a kitchen increased to 39.53±0.75 µg.kg^{-1}. Finally, the Σ4PAHs content of the butter from the

bitumen-dried cocoa beans before storage was 5.27±0.73 µg.kg^{-1} at the end of drying. After two weeks of storage, it reached 6.27±0.78 and 21.81±3.22 µg.kg^{-1} for beans stored in the store and kitchen respectively. At the end of four weeks of storage, the levels of Σ4PAHs in the butter extracted from the beans kept in the store were 8.03±1.58 µg.kg^{-1} while that of the beans stored in the kitchen reached a value of 35.70±0.21 µg.kg^{-1} namely an increase of 38.91 %.

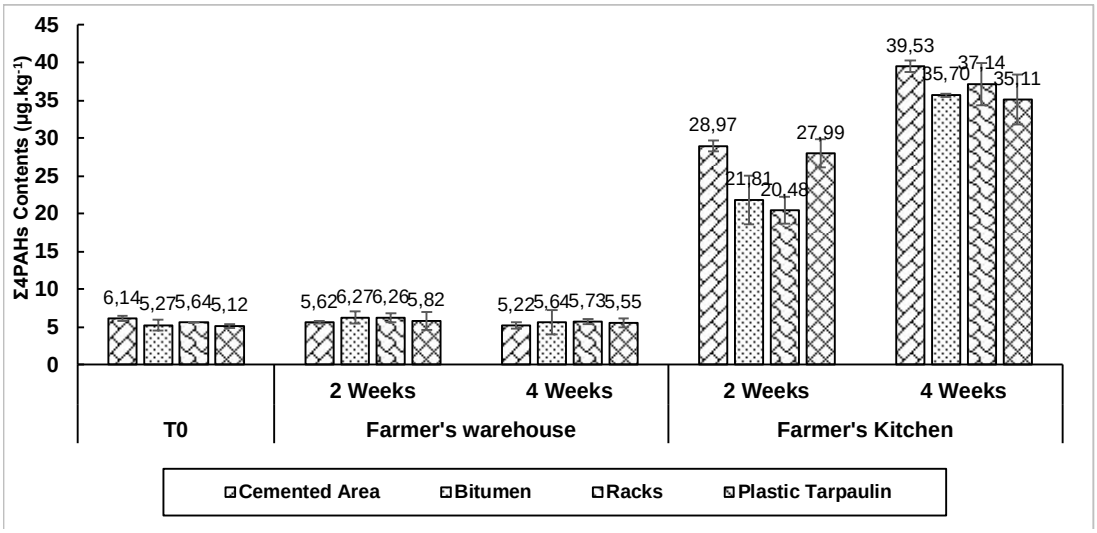

Fig. 7. Evolution of Sum of 4 PAHs (Chr, BaA, BbF, BaP), (Σ4PAHs) contents in butter extracted from cocoa beans dried on Cemented area, Bitumen, Racks, and Plastic sheeting during storage in a farming environment

3.1.4 Evolution of the PAH content of cocoa during the transformation into derived products.

Fig. 8 summarizes the contents of major PAHs (Σ4PAHs) in cocoa butter, determined at the different stages of the manufacturing process of chocolate derived from cocoa beans treated in this study. The results obtained show that the content of Σ4PAHs in the butter extracted from market cocoa beans before roasting was 97.10±1.48 µg.kg^{-1}. When the cocoa beans were roasted, the content of major PAHs drops sharply to 67.01±3.99 µg.kg^{-1}. However, after shelling the cocoa beans, the results show that the Σ4PAHs content in fat extracted from the bean shells was 91.40±8.96 µg.kg^{-1} while that of the butter obtained from the nibs was only 5.76±0.27 µg.kg^{-1}. After grinding the cocoa nibs, the Σ4PAHs content of the butter extracted from the resulting cocoa mass was 6.74±0.26 µg.kg^{-1}. The transformation of the cocoa mass into cocoa liquor and then into a chocolate induced Σ4PAHs contents of 7.79±0.26, 8.05±0.05 µg.kg^{-1} respectively. In total, there was a 91.71 % reduction in PAHs during the processing of cocoa beans into chocolate.

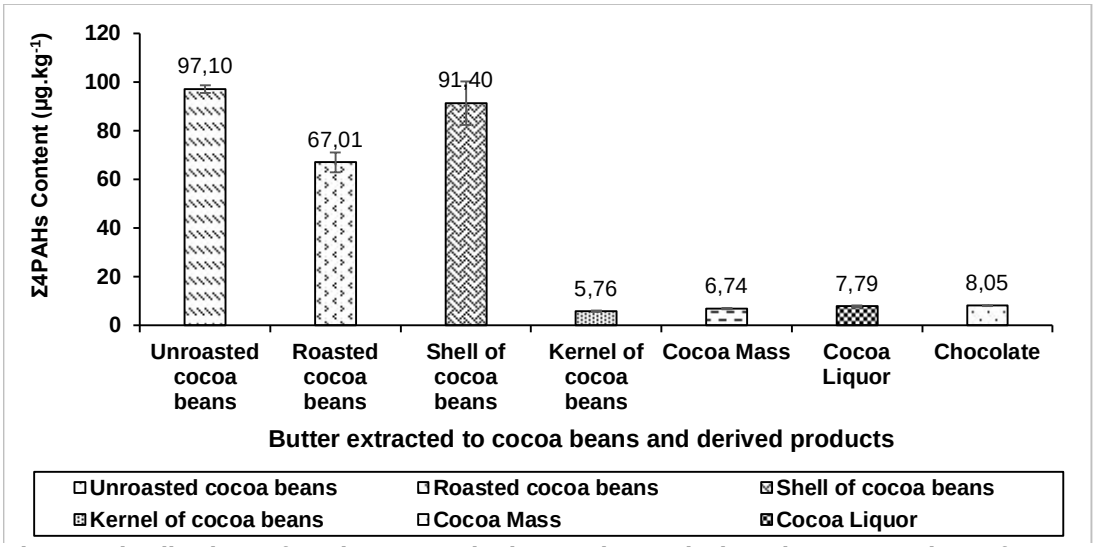

Fig. 8. Distribution of major PAHs in by-products during the processing of cocoa beans into by-products.

3.2 Discussion

A validated method for the detection and quantification of 4 PAH namely benzo(a)anthracene (BaA), chrysene (Chr), benzo(b)fluoranthene (BbF) and benzo(a)pyrene (BaP) in cocoa butter **[42]** were used of this study. This method in accordance with the standards of Commission Regulation (EU) No. 836/2011 for efficient and reliability measurement of sum of 4 PAH (Σ4PAHs) contents. The results of determination of the contents of major PAH of the butter extracted from cocoa beans obtained whatever the drying support (**Fig. 2**) used showed very negligible values lower than 7 µg.kg^{-1}. Specifically, bitumen, which was supposed to induce an important migration of PAHs towards the cocoa beans, also presented contents of major PAHs approximately 5 times below the maximum limits fixed by the European criteria **(Fig. 5)**. Contrary to several similar studies where significant levels of PAHs were found in foodstuffs dried on bitumen **[1; 14]**. This is because the bitumen surface used in our study was an old pavement with very little practice. Hydrocarbon particles that may come from this bitumen and/or exhaust from regular road traffic were not available to contaminate cocoa beans **[4]**. Although at very low concentrations, the presence of PAHs in cocoa beans seems to come from environmental sources during drying. Indeed, the soils of the plantations, the drying areas, and/or the plant material used in this study, could be contaminated by PAHs from the burning carried out during the clearing and by various mobile sources **[18]**. Also, PAHs could come from the environment itself polluted by dust and various fumes **[14; 18; 10]**. In the samples of cocoa beans dried on bitumen, the lowest content of PAHs was recorded at FBab0. This low presence of PAHs could be related to the biological nature of the fermentation medium (banana leaves) which would favor the growth of microorganisms capable of metabolizing PAHs **[6; 44]**. On the other hand, the presence of PAHs at levels around 6.19±0.16 µg.kg^{-1} can be explained by the development of microorganisms degrading these contaminants during the fermentation of cocoa beans, which would probably be limited by the nature (polyethylene) of the plastic crates. Whatever the technical itinerary of primary preparation applied, the post-harvest technological treatments preceding drying have no real impact on the formation of PAHs. However, the use of plastic materials for the fermentation of cocoa beans should be avoided because it would limit the growth of microorganisms that metabolize PAHs, in addition to having the disadvantage of producing poorly fermented cocoa **[20]** and promoting the secretion of ochratoxin A **[26]**. Considering the diversity of

cocoa drying techniques implemented, it was wise to study the impact of each technique on the formation of PAHs in cocoa beans **(Fig. 6)**. The butter derived from cocoa beans dried by sun recorded the lowest content of PAHs while that from cocoa beans treated only by wood fire is the most contaminated in PAHs with content 4-5 times higher than the European specification and nearly 30 times higher than the content of PAHs in the butter produced from cocoa beans dried by the solar mode. This explosion of the content of major PAHs is due to the migration of PAHs from the smoke resulting from a resinous wood fire to the cocoa beans. Indeed, as several studies had previously described, the incomplete combustion of organic matter including wood generates the formation of various types of PAHs **[11; 38; 33]**. Also, this abundance of PAHs could be explained by the fact that they appear to form via a carbonization process, where the initial matrix undergoes chemical transformation and rearrangement to a more condensed polycyclic aromatic structure **[22]**. In addition, to determine the influence of storage conditions at the producer level on PAH contamination of the beans, two warehouses were selected **(Fig. 7)**. In total, the content of major PAHs in the butter extracted from cocoa beans stored in a warehouse far from all sources of smoke remained almost constant, whereas the content of butter extracted from cocoa beans stored in a farmer's kitchen only increased regardless of the post-harvest technical itinerary used. These results in **Fig. 7** clearly show that when cocoa beans are stored under smoky conditions, the PAH content of the resulting butter increases during storage. This increase is related to the contamination of cocoa beans by PAHs from the smoke produced by the wood fire lit during the preparation of family meals **[30; 29; 34]**. Butter extracted from cocoa beans is highly contaminated with PAHs both if the cocoa beans were dried over a wood fire and if they were stored in smoked locations or in proximity to wood fire smoke. These observations allow us to conclude that the post-harvest technical itinerary involving at any stage a source of PAHs induces the contamination of cocoa beans with high concentrations of major PAHs. Therefore, the only way to preserve merchantable cocoa beans from alarming contamination of PAHs is to avoid artificial drying with smoke and storage in places near a smoke source. Finally, the only exposure of cocoa beans to smoke does not seem to be the only factor of contamination in PAHs. Indeed, this could be promoted by its fat richness as demonstrated in most fatty foodstuffs **[12; 45; 36; 24; 37]**. This observation was confirmed when studying the distribution of PAHs from cocoa beans in by-products during the processing of cocoa into chocolate **(Fig. 8)**. The results of this study found that roasting cocoa beans reduce the content of PAHs. This decrease in PAH content could be explained by the oxidation of PAHs at low temperatures (125 °C) in the presence of air-steam in the ventilated oven used for roasting **[7]**. Furthermore, in the post-roasting phase, the results indicate a discriminating distribution of major PAHs in the contaminated cocoa beans with a higher concentration in the shells compared to the kernels. This observation can be explained by the fact that the shell and the thin envelope covering the cocoa beans constitute a double barrier against PAHs migration **[9;34]**. Indeed, PAHs in contact with cocoa beans would be deposited in a first step on the shells where their migration towards the fat begins. Once this first barrier is crossed, the presence of the thin envelope around the cotyledons of the cocoa bean will also limit the passage of PAHs from the shells to the fat inside the cotyledons. After hulling the roasted beans and removing the shells, the butter extracted from the successive cocoa by-products such as cocoa mass and liquor as well as the resulting chocolate has almost the same lower PAH contents (about 4-5 times below the critical limits) as those of the butter from the shells of the beans. Thus, more than 90 % of the PAHs in the fermented and dried cocoa beans are eliminated during the process of transformation of cocoa into chocolate. This observation makes it possible to conclude that the contamination of the beans in PAHs presents a very limited sanitary problem despite the high toxicity of the PAHs. Indeed, the sanitary risk that can cause the cocoa products except the fibers produced from the shells is very considerably reduced at the end of the industrial transformation of cocoa. Unless cocoa processing is poorly controlled, the derived products do not present any danger to consumers in terms of PAHs.

4. CONCLUSION

The results of this study showed that the post-harvest technical itinerary from pods opening to cocoa beans drying is not a determining factor in increasing critic PAH level as long as the drying is natural. However, solar drying on a bitumen area can cause PAH migration in cocoa beans. On the other hand, artificial drying involving the fire of wood appears as one of the major causes of the presence of PAHs in cocoa beans. This role of the smoke generally rich in PAHs was confirmed by a high increase of the PAHs content of butter extracted from cocoa beans stored near a smoke source. Although low, the roasting (at 125 °C in a ventilated oven) of cocoa beans seems to reduce the PAH content of the butter. However, the contamination of cocoa beans by PAHs does not constitute a real health problem for consumers of derived products such as butter, mass, cocoa liquor, and chocolate, when the shelling stage is well done. Indeed, more than 90% of PAHs contaminating cocoa beans are concentrated in the shells. Their non-incorporation in cocoa products is a health guarantee for consumers. Considering the multiple uses of shells and their increasing consumption in the form of derived fibers, it is imperative to control the presence of PAHs in cocoa beans

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COMPETING INTERESTS

"Authors have declared that no competing interests exist.".

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. Author Sess-Tchotch Didier-Axel designed the study, wrote the protocol, fitted the data and wrote the first draft of the manuscript. Author Kouame N'Guessan checked the first draft of the manuscript and achieved the submitted manuscript. Authors Tagro Simplice Guehi, Fontana-Tachon Angelique, Grabulos Joël, Boulanger Renaud, Durand Noël, and Kone Koumba Maï performed the statistical analysis, managed the literature and assisted the experiments implementation. Author Tagro Simplice Guehi and Durand Noël expertized the results interpretations. All authors read and approved the final manuscript.

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