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POLYCYCLIC AROMATIC HYDROCARBONS AND AFLATOXINS (B1, B2, G1 & G2) CONTAMINATION IN HONEY FROM KORHOGO, CÔTE D'IVOIRE

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ABSTRACT

Honey, is a very popular ingredient of the Ivorian populations who use it in gastronomy, cosmetics and pharmacology. However, its mode of extraction and preservation makes it a nest for Polycyclic Aromatic Hydrocarbons (PAHs) and mycotoxins. In order to evaluate the quality of the honey produced in Cote d'Ivoire generally and that in the Korhogo region, particularly, PAHs and aflatoxins levels were determined in honey samples from the markets of Korhogo town. So, 26 honey samples were randomly collected from the markets of Korhogo, in north of Cote d'Ivoire. The analyses were performed using high performance liquid chromatography (HPLC). 19 samples analyzed are not contaminated by PAH, but 6 samples contained Benzo (k)fluoranthene (BkF) ranged from 12.55 to 27.28 µg kg^{-1}, while only 1 sample is contaminated with Benzo(a)pyrene (BaP) at 5.29 µg.kg^{-1}. In contrary all samples analyzed are contaminated by aflatoxins with levels ranged from 0.067 to 0.813 µg kg^{-1} for total aflatoxins (B1 + B2 + G1 + G2). However, in all samples levels of aflatoxin B1 and of the sum of the aflatoxins (B1 + B2 + G1 + G2) are less than 2 µg.kg^{-1} and 4 µg.kg^{-1} respectively. Moreover, Benzo(a)pyrene, a known carcinogenic molecule for humans, was almost not detected in all samples. So, according to this results, it can be concluded that the consumption of honey from Korhogo does not present any risk to human health.

Keywords : *honey, polycyclic aromatic hydrocarbons, aflatoxins, Korhogo, Côte d'Ivoire.*

RÉSUMÉ

Le miel est un ingrédient très populaire en Côte d'Ivoire. Il est fréquemment utilisé en gastronomie, cosmétique et en pharmacologie, mais son mode d'extraction et de conservation en fait un nid pour les Hydrocarbures Aromatiques Polycycliques (HAP) et les mycotoxines. Afin d'évaluer la qualité du miel produit en Côte d'Ivoire en général et celui de la région de Korhogo en particulier, les teneurs en HAP et en aflatoxines ont été déterminées sur des échantillons de miel provenant des marchés de la ville de Korhogo. Ainsi, 26 échantillons de miel ont été prélevés au hasard sur les marchés de Korhogo, au nord de la Côte d'Ivoire. Les analyses ont été effectuées en utilisant la chromatographie liquide à haute performance (CLHP). 19 échantillons analysés n'ont présenté aucune contamination par les HAP, 6 échantillons contenaient du Benzo(k)fluoranthène (BkF) dont les consentrations variaient de 12,55 à 27,28 $\mu g.kg^{-1}$ et un seul échantillon était contaminé par du Benzo(a)pyrène (BaP) à 5,29 $\mu g.kg^{-1}$. Contrairement aux HAP, tous les échantillons analysés contenaient des aflatoxines à des concentrations allant de 0,067 à 0,813 $\mu g.kg^{-1}$ pour les aflatoxines totaux (B1 + B2 + G1 + G2). Cependant, dans tous les échantillons, les niveaux d'aflatoxine B1 et de la somme des aflatoxines (B1 + B2 + G1 + G2) ont été respectivement inférieurs à 2 $\mu g.kg^{-1}$ et à 4 $\mu g.kg^{-1}$ (valeurs seuils fixées par l'Union Européenne). De plus, le benzo(a)pyrène, une molécule connue comme cancérogène pour l'homme, n'a presque pas été détecté dans tous les échantillons. Ainsi, au regard de ces résultats, nous pouvons conclure que la consommation de miel de Korhogo ne présente aucun risque pour la santé des populations.

Mots-clés : *miel, hydrocarbures aromatiques polycycliques, aflatoxines, Korhogo, Côte d'Ivoire.*

I - INTRODUCTION

Honey has antioxidant, antibacterial, immunity-enhancing, and other physiological activities. It has been reported that honey can be used an as environmental marker due to its ability to contain harmful pollutants such us PAH and Mycotoxins [1, 2]. The presence of these chemicals in honey may be caused by the incorrect procedures during the honey extraction and conservation phases [3, 4]. Indeed, polycyclic aromatic hydrocarbons are organic compounds which contain at least two aromatics bonds formed by carbon and hydrogen. These compounds derived from the incomplete combustion of organics materials such as wood, oil, etc. [5, 6] and are considered in many country as priority pollutants due to their carcinogenic, mutagenic and toxic properties [7 - 10]. While, mycotoxins are naturally-

occurring toxins which come from certain fungi that can grow on food such as dried fruits, nuts, cereals, legumes and spices. These substances are secondary metabolites of microscopic fungi which are not indispensable to the fungi's life but show toxic effects on human beings, animals, plants and microorganisms [11]. One of the most commonly observed mycotoxins that found are aflatoxins (B1, B2, G1 and G2) and ochratoxin-A. Aflatoxins directly damages DNA and have been shown to cancer contribution to food contamination, including mycotoxins. Like the PAH, Aflatoxins are carcinogenic, toxigenic, teratogenic and mutagenic [3]. Unfortunately, few authors in Africa have generally addressed the contamination of honey by residues, although the modes of extraction of honey, among which smoking, and the mode of conservation still remain artisanal in many countries. These studies have been done in Nigeria [12], in Algeria [13] and in Egypt [3]. In Cote d'Ivoire studies on consumer products contamination by chemical pollutants focused on fish contamination by heavy metals [14, 15], on cow's milk contamination by organochlorine pesticide residues [16], on groundwater contamination by nitrates [17], on physico-chemical and microbiological quality assessment of drinking waters [18], on trace metals and pesticides in market garden crops (*Abelmoschus sp*, *Corchoruus tridens*, *Basela alba*, *Solanum aethiopicum*) [19], on honey contamination by pesticides [20]. Data on Cote d'Ivoire's honey contamination by PAHs and mycotoxins are limited. Therefore, the present study aimed to investigate the concentration of polycyclic aromatic hydrocarbons and aflatoxins (B1, B2, G1 and G2) in honey.

II - MATERIAL AND METHODS

II-1. Honey sampling

A total of 26 samples of bee honey were collected at certain local markets from Korhogo (Cote d'Ivoire) during february 2018. Not less than 500 grams of honey were taken for each sample in jar labeled by name of market and number then transferred to the Laboratory [3]. Each jar was carefully washed, rinsed and dried before sampling and filled, capped and kept in an ice freezer until to the laboratory to avoid exterior contamination. All this samples were stored at room temperatures (20 ^{o}C) in the laboratory until the analyzing [21].

II-2. Chemicals and reagents

All solvents used in this work were of analytical grade. Aflatoxins and PAH standards were purchased from Dr. Ehrenstorfer GmbH, Germany. Acetonitrile were purchased from PROLABO, France. Methanol and Sodium Chloride from SCHARLAU, France, while Phosphate Buffered Saline (PBS) and Ummino Aflapred columns were purchased respectively from OXOID, England and PURI-FAST, France.

II-3. Sample extraction and analysis

For PAHs, the honey sample (10 g) was mixed with anhydrous sodium sulphate, and 40 mL of dichloromethane were added and mixture was extracted by means of ultrasonic bath and Ultra Turrax. After filtration the solvent was evaporated to dryness (Büchi, Flawil, Switzerland), redissolved in 10 mL of acetonitrile, filtered (0.45 µm nylon membrane filter) and analysed by HPLC [22]. Concerning aflatoxins (B1, B2, G1, and G2). Samples were analyzed using a HPLC following AOAC [23] with some modifications. 25g of samples were extracted with methanol:water:n-hexane (240:60:100, v/v/v). The mixture was shaken for 30 min on a mechanical shaker. The solution was left to sediment and filtered through a Whatman Filter 45 µm. The samples were diluted with water and after filtered. Ummino Aflapred column (UAC) was used for samples to clean up. First, 10 mL phosphate buffer saline (PBS) was passed through the UAC. Then, 75 mL of the filtrate was passed through the UAC at a flow rate of 1 mL/min, washed with water and dried using vacuum. Finally, aflatoxins was eluted firstly with 0.5 mL of methanol and after 1 min, with 0.75 mL of methanol. Eluted aflatoxins were diluted with water and analyzed using HPLC [24].

III - RESULTS AND DISCUSSION

III-1. PAH in honey

Limit of Detection (LOD) and limit of Quantification (LOQ) of PAH in honey obtained are 0.017 µg·kg-1 and 0.06 µg·kg^{-1} respectively. The LOD and LOQ of a method are respectively the lowest detectable concentration and the lowest quantifiable concentration with acceptable uncertainty under the experimental methods conditions [25]. These low LOQ values of our method allow us to determine the low concentrations of PAHs in our samples. However, we find that almost none of our samples are contaminated with PAHs, but only six samples contain residues of Benzo(k)fluoranthene, Benzo(a)Pyrene or Benzo(g,h,i)Pyrene (Table 1, 2). Concentrations of individual PAH in all samples from little and great markets of Korhogo ranged from Not Detected (ND) to 27.28 µg·kg^{-1} and from ND to 12.55 µg·kg^{-1}, respectively. PAHs in honey samples were detected at concentrations of the order of 3 to 30 µg·kg^{-1} (Table 1, 2). The individual PAH with highest concentration were Benzo(k)fluoranthene in Korhogo's little market (Table 1). Also, Benzo(a)pyrene, classed by IARC [26, 27] and US EPA [28] like probably cancerous for human being were detected in only one sample, either a detection level of 3.85 %. Although benzo[a]pyrene is considered to be a representative marker of total PAH in food [29] and probably cancerous for human being [26 - 28], it was almost not detected in the studied honey samples. These results mean

that the consumption of honey from the Korhogo region is therefore not a risk for the Ivorian populations, since our study area supplies honey to almost all regions of Cote d'Ivoire. Moreover, total PAH concentration in honey samples analyzed ranged from ND to 36.23 $\mu g \cdot kg^{-1}$ in Korhogo's little market and from ND to 12.55 $\mu g \cdot kg^{-1}$ in Korhogo's great market. These very low occurrences of PAHs in our samples indicate their good quality and extraction practices used. Indeed, there is no legislation regarding the permitted maximum level of PAH in honey, but the maximum level of Benzo(a)pyrene in oils and fats intended for human consumption or as ingredients in foodstuffs is 2 $\mu g \cdot kg^{-1}$ [30]. So, regarding level of benzo[a]pyrene, it can be concluded that the consumption of studied honey samples from Korhogo do not present any risk to human health. Comparable results were obtained by [4, 29] in Roumania who found levels of PAH contamination in honey well below the level of 2 $\mu g \cdot kg^{-1}$.

III-2. Aflatoxins (B1, B2, G1 and G2) in honey

The aflatoxins (B1, B2, G1 and G2) concentration in honey samples are presented in table 3 and table 4. Limit of Quantification (LOQ) of Aflatoxins B1, B2, G1 and G2 in honey are 0.0047 $\mu g \cdot kg^{-1}$, 0.0045 $\mu g \cdot kg^{-1}$, 0.0050 $\mu g \cdot kg^{-1}$ and 0.01878 $\mu g \cdot kg^{-1}$ respectively. All samples of honey analyzed were found to contain aflatoxins (Table 3 and 4). The concentrations of individual aflatoxins varied between Below the Limit of Quantification (< LOQ) to 0.806 $\mu g \cdot kg^{-1}$ in Korhogo's little market and between < LOQ to 0.538 $\mu g \cdot kg^{-1}$ in Korhogo's great market. While, those of total aflatoxins ranged from 0.067 to 0.813 $\mu g.kg^{-1}$, and from 0.102 to 0.5738 $\mu g.kg^{-1}$ in Korhogo's little and great markets, respectively. Among aflatoxins determined, only aflatoxin B1 was found in all our samples, whereas it proved to be the most toxic at significant doses. The presence of this mycotoxin in all our samples is to be taken seriously, because it comes from the way of conservation of honey sold on the markets of Korhogo. This compound is also the most prevalent and poisonous molecule between the four aflatoxins analyzed and it is categorized as group 1 human carcinogen by International Agency for Research on Cancer (IARC) [24, 31]. All samples of honey analyzed were found to contain aflatoxins. This results are similar to those obtained by [24] in Iran and Swalieh and Abdulkhaliq [30] in Palestine, who found aflatoxins also in all their samples, but there are differents from those obtained by [32] in Lisbon (Portugal) who found aflatoxins in none of their samples. In view of the fact that aflatoxins could pose a significant threat to human health because they are toxigenic, carcinogenic, mutagenic and teratogenic, it is necessary to draw the attention of producers, traders and consumers of honey to improve the way honey is stored in order to minimize the presence and proliferation of aflatoxins in the honey produced in the Korhogo region. indeed, although none of our honey

samples contained level of aflatoxins higher than 2 µg.kg^{-1} for aflatoxin B1 and 4 µg kg^{-1} for total aflatoxins (B1, B2, G1 and G2) According to EU regulations maximum permitted level [29, 30], it is no less necessary to work to obtain honey free from any contamination with aflatoxins. Compared to our results, those of Aleksandar ŽKostić obtained in Serbian regions [33] were more contaminated because all his samples analyzed contained level of aflatoxins B1 higher than 2 µg.kg^{-1}. Finally, according to the level of aflatoxins in our honey sample, which is below EU maximum permitted level, it can be concluded that the consumption of honey from region of Korhogo (Cote d'Ivoire) do not present any risk to human health.

Table 1 : *Concentrations of Polycyclic Aromatic Hydrocarbons (µg·kg^{-1}) in honey samples collected from little market of Korhogo*

Honey samples	HAP (µg/kg)								Total
	Flu	Pyr	BkF	BaP	InP	BgP	BaA	BbF	
1	ND	ND	27.28	ND	ND	8.95	ND	ND	36.23
2	ND	ND	3.26	ND	ND	ND	ND	ND	3.26
3	ND	ND	ND	ND	ND	ND	ND	ND	ND
4	ND	ND	ND	ND	ND	ND	ND	ND	ND
5	ND	ND	ND	ND	ND	ND	ND	ND	ND
6	ND	ND	ND	ND	ND	ND	ND	ND	ND
7	ND	ND	ND	5.29	ND	ND	ND	ND	5.29
8	ND	ND	ND	ND	ND	ND	ND	ND	ND
9	ND	ND	19.47	ND	ND	ND	ND	ND	19.47
10	ND	ND	ND	ND	ND	ND	ND	ND	ND
11	ND	ND	ND	ND	ND	ND	ND	ND	ND
12	ND	ND	ND	ND	ND	ND	ND	ND	ND
13	ND	ND	ND	ND	ND	ND	ND	ND	ND
14	ND	ND	ND	ND	ND	ND	ND	ND	ND
15	ND	ND	ND	ND	ND	ND	ND	ND	ND
Levels range	ND	ND	ND - 27.28	ND - 5.29	ND	ND - 8.95	ND	ND	ND - 36.23

Flu : Fluoranthene, Pyr : Pyrene, BkF : Benzo(k)fluoranthene, BaP : Benzo(a)Pyrene, InP : Indeno(1,2,3-cd)Perylene, BgP : Benzo(g,h,i)Pyrene, BaA : Benzo(a)anthracene, BbF : Benzo(b)fluoranthene
ND : Not Detected.

Table 2 : *Concentrations of Polycyclic Aromatic Hydrocarbons (µg·kg^{-1}) in honey samples collected from great market of Korhogo*

Honey samples	HAP (µg/kg)								Total
	Flu	Pyr	BkF	BaP	InP	BgP	BaA	BbF	
16	ND	ND	ND	ND	ND	ND	ND	ND	ND
17	ND	ND	ND	ND	ND	ND	ND	ND	ND
18	ND	ND	ND	ND	ND	ND	ND	ND	ND
19	ND	ND	12.55	ND	ND	ND	ND	ND	12.55
20	ND	ND	ND	ND	ND	ND	ND	ND	ND
21	ND	ND	ND	ND	ND	ND	ND	ND	ND
22	ND	ND	ND	ND	ND	ND	ND	ND	ND
23	ND	ND	ND	ND	ND	ND	ND	ND	ND
24	ND	ND	3.16	ND	ND	ND	ND	ND	3.16
25	ND	ND	3.48	ND	ND	ND	ND	ND	3.48
26	ND	ND	ND	ND	ND	ND	ND	ND	3.48
Levels range	ND	ND	ND - 12.55	ND	ND	ND	ND	ND	ND - 12.55

Flu : Fluoranthene, Pyr : Pyrene, BkF : Benzo(k)fluoranthene, BaP : Benzo(a)Pyrene, InP : Indeno(1,2,3-cd)Perylene, BgP : Benzo(g,h,i)Pyrene, BaA : Benzo(a)anthracene, BbF : Benzo(b)fluoranthene
ND : Not Detected.

Table 3 : *Concentrations of aflatoxins (µg·kg^{-1}) in honey samples collected from little market of Korhogo*

Honey samples	Aflatoxins (µg/kg)				Total
	AFB1	AFB2	AFG1	AFG2	
1	0.089	< LOQ	< LOQ	< LOQ	0.089
2	0.319	< LOQ	0.055	< LOQ	0.374
3	0.225	0.0048	0.005	< LOQ	0.2348
4	0.314	< LOQ	0.005	< LOQ	0.319
5	0.217	< LOQ	0.007	< LOQ	0.224
6	0.152	< LOQ	0.030	0.0171	0.1991
7	0.252	< LOQ	0.017	< LOQ	0.269
8	0.806	< LOQ	0.007	< LOQ	0.813
9	0.201	0.0072	0.017	< LOQ	0.2252
10	0.766	< LOQ	< LOQ	< LOQ	0.766
11	0.058	< LOQ	0.009	< LOQ	0.067
12	0.804	< LOQ	< LOQ	< LOQ	0.804
13	0.111	< LOQ	0.011	< LOQ	0.122
14	0.225	< LOQ	0.007	< LOQ	0.232
15	0.433	0.0046	0.015	0.0377	0.4903
Levels range	0.058 - 0.806	< LOQ - 0.0072	< LOQ - 0.055	< LOQ - 0.0377	0.067 - 0.813

AFB1 : Aflatoxin B1, AFB2 : Aflatoxin B2, AFG1 : Aflatoxin G1, AFG2 : Aflatoxin G2. < LOQ: Below the Limit of Quantification.

Table 4 : *Concentrations of aflatoxins ($\mu g \cdot kg^{-1}$) in honey samples collected from great market of Korhogo*

Honey samples	Aflatoxins (µg/kg)				Total
	AFB1	AFB2	AFG1	AFG2	
16	0.280	< LOQ	< LOQ	< LOQ	0.28
17	0.088	0.0055	0.026	< LOQ	0.1195
18	0.361	< LOQ	< LOQ	< LOQ	0.361
19	0.198	< LOQ	0.023	< LOQ	0.221
20	0.299	< LOQ	0.039	0.0265	0.3645
21	0.222	0.0056	0.005	< LOQ	0.2326
22	0.386	< LOQ	0.009	0.0021	0.3971
23	0.167	< LOQ	0.012	< LOQ	0.179
24	0.094	< LOQ	0.008	< LOQ	0.102
25	0.406	< LOQ	0.019	< LOQ	0.425
26	0.538	0.0158	0.020	< LOQ	0.5738
Levels range	0.088 - 0.538	< LOQ - 0.0056	< LOQ - 0.026	< LOQ - 0.0265	0.102 - 0.5738

AFB1 : Aflatoxin B1, AFB2 : Aflatoxin B2, AFG1 : Aflatoxin G1, AFG2 : Aflatoxin G2. < LOQ: Below the Limit of Quantification.

IV - CONCLUSION

Several honey samples from the Korhogo markets were investigated in order to determine the level of PAH and aflatoxins. The results obtained constitute a database on the level of contamination and quality of honey produced in the Korhogo region and frequently consumed by Ivorian populations. The results showed that level of PAHs and aflatoxins in honey sold on the Korhogo markets are lower than the threshold values set by the EU. So honey samples studied are free from real contamination by PAHs and aflatoxins. However, PAH and aflatoxin levels in Korhogo honey deserve to be monitored with special focus to his production and conservation, which are sources of contamination by these substances. So, further investigation is necessary to know the origin of the honey contamination by aflatoxins and the strategies for reducing aflatoxins contamination in honey with special focus on post-harvest conservation practices and climatic conditions.

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