FUNGAL CONTAMINATION AND MYCOTOXINS' OCCURRENCE IN PEANUT BUTTERS MARKETED IN ABIDJAN DISTRICT (CÔTE D'IVOIRE)

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Abstract: *This study was carried out in order to assess the detection of fungi contamination and mycotoxins (Aflatoxin B1 and Ochratoxin A) levels in peanut butters sold in retail markets in Abidjan District. Samples were aseptically collected and analyzed by using agar method dilution for fungi enumeration and HPLC for AFB1 and OTA quantification. The fungal isolates included Mucor, Alternaria, Helmintosporium, Geotrichum, Fusarium, Cladosporium, Penicillium and Aspergillus genera. The predominant fungi belonged to Aspergillus genus (20.22 – 51.65 %) followed by Helmintosporium (0 – 47.44 %) and Penicillium (0 – 41.54%). The mycotoxigenic fungi were isolated with a frequency of 14.81; 13.95; 9.1 and 21.62 % for Aspergillus versicolor, Aspergillus ochraceus, Aspergillus flavus and Aspergillus parasiticus, respectively. Mycotoxins (AFB1 and OTA) were detected in the marketed peanut butters. Their contents varied from 0.23 to 2.49 µg/kg for AFB1 and 0.53 to 2.23 µg/kg for OTA . The coexistence of aflatoxins and ochratoxin A (OTA) in the analyzed peanut samples was also established. The results showed that peanut butters sold in Abidjan District were highly contaminated and therefore unacceptable for human nutrition. So, good manufacturing processing including sorting and good hygiene practices would help to minimize mycotoxin occurrence in order to obtain good sanitary peanut butters.*

Keywords: *peanut butter, fungal contamination, aflatoxin B1, ochratoxin A*

1. Introduction

Fungal contamination of seeds before and after harvesting remains a major problem of food safety in most parts of Tropical Africa. Problems associated with this contamination include loss of germination, mustiness, moldy smell and mycotoxin production [1,2]. Mycotoxins, secondary metabolites produced by fungi, are toxic to both animals and humans and their occurrence in the food chain may have public health effects [3]. These toxic metabolites have attracted worldwide attention due to the significant losses associated with their impact on human and animal health, and consequent national economic implications [4].

Based on detailed study of the distribution of fungi in nature, the five agriculturally important toxins from fungi are aflatoxins, ochratoxin A fumonisins, zearalenone and deoxynivanelol [5].

Aflatoxins are mainly produced by the fungi *Aspergillus flavus*, *Aspergillus parasiticus* and Aspergillus *nominus* [6]. Aflatoxins (B1, B2, G1 and G2) are very powerful hepatocarcinogen and have been classified as a class 1 human carcinogen [5].

The European Union uses an action level of 2 µg/kg as the maximum residue limit allowed in food for human consumption [7]. Ochratoxin A (OTA) is produced by different species of *Aspergillus* and *Penicillium*, though it was first isolated from cultures of *Aspergillus ochraceus* [2]. OTA is immunosuppressive, teratogenic, genotoxic and mutagenic and IARC has classified it in-group 2B as possibly carcinogenic to human [5].

The Joint Expert Committee on Food Additives (JECFA) of the WHO and FAO set a provisional maximum intake of 100 ng/kg body weight (bw) while the Scientific Committee on Food of the European Union proposed that the maximum daily intake of OTA should not exceed 3 µg/kg [8]. Fumonisins produced by *Fusarium verticillioides* and *Fusarium proliferatum* are recently receiving increasing attention in scientific literature because they have been implicated in a number of animal diseases, such as porcine pulmonary oedema and rat liver cancer [9]. It can also cause hepatotoxicity and nephrotoxicity in many animals [10]. The United States FDA has proposed a guideline of tolerance level of 2 mg/kg total fumonisins for human consumption [11]. Zearalenone, an oestrogenic mycotoxin, causes problems with the reproductive organs of farm animals, especially swine and is often found together with deoxynivalenol (vomitoxin) [12]. Among the food crops susceptible to contamination by mycotoxigenic fungi, peanut (*Arachis hypogea* Linn) plays an important role [13]. Indeed, peanut which constitutes a major annual oilseed crop is liable to fungal contamination during handling, storage and transportation [14]. Peanut seeds are eaten raw, boiled or roasted, made into butter or paste and are used for thickening soups [15]. Peanut butter is traditionally made by grinding dry roasted groundnuts into a paste [14]. In Côte d'Ivoire, the use of marketed peanut butter in the confection of sauce is very popular among the urban population. Poor harvesting practices, improper storage and less than optimal conditions during transport and marketing of peanut butter could contribute to fungal growth and mycotoxins production. Therefore, the present work aimed to determine the level of mycotoxins (Aflatoxin B1 and OTA) and associated fungi species in peanut butters marketed in Abidjan in order to explore their sanitary quality.

2. Material and methods

2.1. Samples collection

Peanut butters were collected during the period (June – October 2012) in the main markets of the 9 communes of Abidjan (Côte d'Ivoire): Abobo, Adjamé, Attécoubé, Cocody, Koumassi, Marcory, Port-Bouet, Treichville, Yopougon identified as AB, AD, AT, C, K, M, P, T and Y. A total of 45 sellers (5 sellers per commune) were investigated and three (3) peanut butters samples (500g each) per seller were randomly and aseptically purchased. The collected peanut butters samples (135) were immediately transported in icebox (4°C) to the laboratory and aseptically mixed together to constitute a minimum of five (5) composite samples (500g each) per commune. The composite samples obtained were stored at 4°C until further analysis.

2.2. Mycological analysis

The isolation of fungi was carried out according to the agar dilution method [16]. Ten (10) gram from each sample, were homogenized with 90 mL of buffer peptone water (AES Laboratory, France) and serial decimal dilutions (10^{-1} to 10^{-4}) were performed. Fungal species were isolated on the semi selective Dichloran Rose Bengal Chloramphenicol (DRBC) agar (Biokar Diagnostics, France).

The medium was poured into sterile Petri dish and 0.1 mL of each sample suspension was spread-plated onto the DRBC agar in triplicate. The plates were incubated for 5 to 7 days at 25°C. Fungal isolates were sub-cultured on Malt Extract and Czapek Yeast medium agars (Oxoid, UK) and incubated for 5 to 7 days at 25°C for purification. Fungi were identified by using taxonomic schemes based on microscopic observation and culture appearance including colonies colours, texture, reverse colour, hyphae arrangement, conidia shape and nature of spores [17]. For the differentiation between *Aspergillus parasiticus* and *Aspergillus flavus* colonies, AFPA agar (Oxoid, UK) supplemented with chloramphenicol, was used. The total fungal count for each plate was expressed as colony-forming units per gram of sample (CFU/g). Each genus or specie identified was then expressed as percentage (%) of the total isolated fungi.

2.3. Aflatoxin B1 extraction and purification

Aflatoxin B1 (AFB1) was extracted and cleaned up following official method [18]. 25 g of peanut butter sample was mixed with 100 mL of methanol-water (80:20, v/v). The mixture was transferred to a conical flask and shaken for 30 min at 300 rpm. The extract obtained was then filtered through Whatman No. 4 filter paper and diluted (1:10) in phosphate buffered saline (PBS). An aliquot (30) mL of this mixture was passed through immunoaffinity column (Vicam, Watertown, MA, USA). After washing with 10 mL of PBS, the column was eluted with acetonitrile (1.5 mL) at a flow rate of 0.5 mL/min. The eluate was then evaporated to dryness under a stream of nitrogen at 40ºC and the residue was dissolved in 500 μL of methanol prior to HPLC analysis.

2.4. Ochratoxin A extraction and purification

Extraction and purification of ochratoxin A were performed according official method [18]. 25 g of peanut butter sample was mixed with 100 mL of methanol-sodium hydrogenocarbonate (1:1, v/v). The mixture was transferred to a conical flask and shaken for 30 min at 300 rpm. The extract obtained was then filtered through Whatman No. 4 filter paper and diluted (1:10) in phosphate buffered saline (PBS). An aliquot (20) mL of this mixture was passed through immunoaffinity column (Vicam, Watertown, MA, USA). After washing with 10 mL of PBS, the column was eluted with 1.5 mL of methanol-acetic acid (98:2, v/v) at a flow rate of 5 mL/min. The eluate was then evaporated to dryness under a stream of nitrogen at 40ºC and the residue was dissolved in 500 μL of methanol prior to HPLC analysis.

2.5. HPLC analysis

Final extracts were filtered through a 0.45 μm PTFE membrane and 20 μL were injected into a high performance liquid chromatography column, using a Shimadzu liquid chromatograph (Kyoto, Japan) equipped with a fluorescence detector (λexc 360 nm; λem 440 nm), a C_{18} column (Torrance, CA, USA) (4.6 × 150 mm, 4 μm) and a Shim-Pack pre-column (4 × 10 mm, 5 μm CLC G-ODS). The isocratic mobile phase consisted of methanol/water/acetonitrile (60:20:20, v/v/v) with a flow rate of 0.5 mL/min. Calibration curves were prepared using standard solutions of aflatoxin B1 and OTA (Sigma, St Louis, MO, USA). Detection limits (LOD) of aflatoxin B1 and OTA were 0.005 and 0.05 μg/g, respectively while their quantification limits (LOQ) were 0.02 and 0.2 μg/g

Statistical analysis

All analyses were carried out in triplicates and data expressed as means ± standard deviation. One way analysis of variance (ANOVA) and Duncan's multiple

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range test (DMRT) were carried out to assess significant differences between means (p<0.05) using STATISTICA 7.1 (StatSoft).

3. Results and discussion

The fungal count of peanut butters, collected from markets, is given in Table 1. Eight fungi genera were isolated from analyzed peanut butters. They included *Mucor*, *Alternaria*, *Helmintosporium*, *Geotrichum*, *Fusarium*, *Cladosporium*, *Penicillium* and *Aspergillus* genus (Table 1). The predominant fungi belonged to *Aspergillus* (20.22 – 51.65 %) included the following mycotoxigenic ones (Fig. 1): *Aspergillus parasiticus* (0 – 21.62 %), *Aspergillus flavus* (0 – 10.81 %), *Aspergillus ochraceus* (0 – 13.95 %) and *Aspergillus versicolor* (0 – 14.81 %). The peanut butter samples collected from Port-Bouet were more contaminated with *Helmintosporium* (44.44 ± 3.00 %) and *Geotrichum* (25.95 ± 2.00 %) while those collected from Treichville were more contaminated with *Alternaria* (31.11 ±

2.50 %) and *Fusarium* (26.66 ± 1.00 %). The highest occurrence of *Mucor* (30.23 ± 2.50 %) was noted for the samples collected from Cocody.

Other studies made in Kenya, Benin and Mali, have also revealed the occurrence of *Aspergillus*, *Fusarium* and *Penicillium* in peanut butters and other peanut products [19,20,21]. The presence of these fungi in relatively large amounts is a clear indication of poor handling, e.g. poor hygiene and storage, which exposes peanuts to favorable conditions for fungal growth and mycotoxin production. Indeed, *Aspergillus* sp. mainly *A. flavus* and *A. parasiticus* are considered as the major producers of aflatoxin while *A. ochraceus* is involved in OTA production [22].

As concern *Fusarium* strains, they can produce mycotoxins such as fumonisins, trichothecenes and zearalenones which are associated with mycotoxicoses in humans or domestic animals. In addition, toxigenic *Penicillium* species produce mycotoxins such as ochratoxin, citrinin, and patulin [23].

Table 1

Total fungi isolated from peanut butter samples sold in retail markets of Abidjan.

Fungi isolates (%)	Communes investigated								
	AB (n=5)	**AD (n=5)**	**AT (n=5)**	**C (n=5)**	**K (n=5)**	**M (n=5)**	**P (n=5)**	**T (n=5)**	**Y (n=5)**
Mucor	6.00[a] ±1.00	10.80[b] ±0.80	7.70[c] ±0.80	30.23[d] ±2.50	0.00[g] ±0.00	10.80[b] ±0.80	7.41[c] ±0.80	13.34[e] ±0.80	9.10[f] ±0.80
Alternaria	17.00[a] ±1.00	21.05[b] ±1.00	11.11[c] ±0.80	0.00[g] ±0.00	18.29[d] ±1.00	0.00[g] ±0.00	0.00[g] ±0.00	31.11[e] ±2.50	20.45[f] ±1.00
Helmintosporium	6.00[a] ±0.00	0.00[d] ±0.00	13.67[b] ±1.00	6.98[a] ±1.00	0.00[d] ±0.00	0.00[d] ±0.00	44.44[c] ±3.00	0.00[d] ±0.00	7.95[a] ±1.00
Geotrichum	0.00[g] ±0.00	12.63[a] ±1.00	15.38[b] ±1.00	0.00[g] ±0.00	0.00[g] ±0.00	8.12[c] ±1.00	25.93[d] ±2.00	6.67[e] ±1.00	3.41[f] ±0.00
Fusarium	5.00[a] ±0.00	0.00[d] ±0.00	0.00[d] ±0.00	0.00[d] ±0.00	15.85[b] ±1.00	0.00[d] ±0.00	0.00[d] ±0.00	26.66[c] ±1.00	0.00[d] ±0.00
Cladosporium	3.00[a] ±0.00	0.00[d] ±0.00	6.84[b] ±1.00	0.00[d] ±0.00	7.32[c] ±1.00	0.00[d] ±0.00	0.00[d] ±0.00	0.00[d] ±0.00	0.00[d] ±0.00
Penicillium	28.00[a] ±1.00	33.68[b] ±2.50	18.80[c] ±1.00	39.54[d] ±2.00	31.71[b] ±2.00	32.43[b] ±2.00	0.00[f] ±0.00	0.00[f] ±0.00	27.27[e] ±2.00
Aspergillus	35.00[a] ±2.50	22.11[b] ±1.00	26.50[c] ±1.00	23.25[d] ±1.00	26.83[e] ±1.00	48.65[f] ±3.00	22.22[g] ±2.00	22.22[g] ±2.00	31.82[h] ±2.00

Data are represented as means ± SD (n=3). Mean with different letters in the same row are statistically different (p < 0.05) according to Duncan's test. **AB: Abobo, AD: Adjamé, AT: Attécoubé, C: Cocody, K: Koumassi, M: Marcory, P: Port-Bouet, T: Treichville, Y: Yopougon.**

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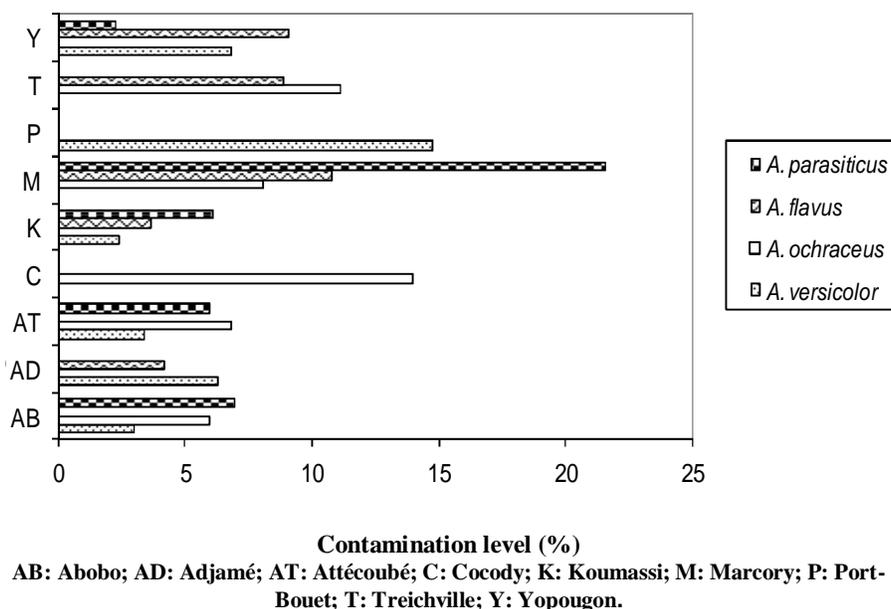
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Contamination level (%)
AB: Abobo; AD: Adjamé; AT: Attécoubé; C: Cocody; K: Koumassi; M: Marcory; P: Port-Bouet; T: Treichville; Y: Yopougon.

Figure 1: Level of mycotoxigenic *Aspergillus* species isolated from peanut butter samples sold in retail markets of Abidjan.

The occurrence of other detected fungi strains such as *Mucor* sp. can be used as indicators of potential presence of aflatoxins [24]. Aflatoxins remain the most dangerous mycotoxin in the world, recognized as a cause of liver cancer and various additional important toxic effects [25].

Ochratoxin A (OTA) is an important nephrotoxic and nephrocarcinogenic mycotoxin and has been associated with the development of urinary tract tumours in humans [26].

The contents of AFB1 and OTA in the marketed peanut butters are given in Table 2 and their retention times are depicted in Figure 2.

The retention time of AFB1 was 2.53 min while that of OTA was 8.99 min. There was variation ($p < 0.05$) in aflatoxin B1 and ochratoxin A levels among peanut butter samples ranging from 0.23 to 2.49 µg/kg for AFB1 and from 0.53 to 2.23 µg/kg for OTA.

Samples collected from Attécoubé and Adjamé were characterized by the highest values (2.49 and 2.23 µg/kg) of AFB1 and OTA, respectively. The values obtained for AFB1 contents in this study are lower than those reported in other areas of the world (Nigeria: 20 to 455 µg/kg; Mali: 4 to 35.19; Kenya: 0 to 2377.1; Mozambique: 3 to 5500 µg/kg; Pakistan: 24 to 800 µg/kg; and Brazil: 5 to 22500 µg/kg) [27,20,21]. Except the communes of Attécoubé, Abobo and Adjamé, AFB1 contents are lower than the maximum residue limit (2 µg/kg) allowed in food for human consumption [7].

These relatively lowest values of AFB1 contents could be explained by the manufacturing processing of peanut butter including roasting step. Thus, positive correlations were reported between loss of aflatoxins in the peanut seeds and the roasting conditions [28].

Indeed, seeds dry-roasted at 140°C for 40 min resulted in 58.8 % reductions in AFB1. Those roasted at 150°C for 25 min resulted in 68.5 % reductions in AFB1. Roasting at 150°C for 30 min led to 70.0% reduction in AFB1.

In addition the role of processing on potential reduction of toxin in peanut butter has been underlined [29]. However, there was a negative correlation between the incidence of *Aspergillus* spp. and the AFB1 level per commune. Indeed, *A. flavus* was not detected in peanut butters collected from Attécoubé while the occurrence level of *A. parasiticus* was 5.98% (Fig 1).

Moreover, the mycotoxins produced by *Aspergillus* spp. are of greatest significance in peanuts and peanut products include aflatoxins and ochratoxin A (OTA) [30]. In this study, the amounts (0.53 - 2.23 µg/kg) of OTA detected in peanut butters are lower than the limit value (3 µg/kg) recommended by European Union [7]. However consumers must pay attention to the excessive consumption of marketed peanut butters in order to avoid immunosuppressive, teratogenic, genotoxic and mutagenic effects on human [5].

Table 2

Aflatoxin B1 and Ochratoxin A levels in peanut butter samples sold in retail markets of Abidjan.

Communes	AFB1 (µg/kg)		OTA (µg/kg)	
	> LOD	Value	> LOD	Value
AB (n = 5)	5	$2.02^a \pm 0.46$	5	$2.13^a \pm 0.32$
AD (n = 5)	5	$1.61^b \pm 0.33$	5	$2.23^a \pm 0.55$
AT (n = 5)	5	$2.49^a \pm 0.98$	5	$2.12^a \pm 0.25$
C (n = 5)	4	$1.91^b \pm 0.00$	5	$1.55^b \pm 0.55$
K (n = 5)	4	$0.93^d \pm 0.07$	5	$1.92^b \pm 0.18$
M (n = 5)	5	$1.01^c \pm 0.08$	5	$1.76^c \pm 0.90$
P (n = 5)	3	$1.08^c \pm 0.08$	4	$0.53^e \pm 0.05$
T (n = 5)	2	$0.23^e \pm 0.08$	5	$0.76^d \pm 0.05$
Y (n = 5)	5	$2.20^a \pm 0.80$	5	$1.74^c \pm 0.41$

Data are represented as means $\pm$ SD (n=3). Mean with different letters in the same column are statistically different ($p < 0.05$) according to Duncan's test. **AB: Abobo, AD: Adjamé, AT: Attécoubé, C: Cocody, K: Koumassi, M: Marcory, P: Port-Bouet, T: Treichville, Y: Yopougon.** LOD: limit of detection: 0.05 µg/g.

As observed for AFB1, there was a negative correlation between the incidence of *A. ocharceus* and the OTA level per commune. The coexistence of AFB1 and OTA in the studied peanut butters should be taken into consideration as claimed by the European community [31]. This is particularly important in regard to possible synergism and additive effects of these mycotoxins. Such co-contamination has been previously observed with other food samples such as wheat or olives [32,33]. Therefore, special attention should be paid to certain critical points in the chain of processing, including sorting. Furthermore, sorting can remove a major part of aflatoxin contaminated units, but levels of mycotoxins in contaminated commodities may also be reduced through food processing procedures that may involve washing, wet and dry milling, grain cleaning, dehulling, roasting, baking, frying, and extrusion cooking [34]. These methods and their impact on mycotoxin reduction were previously reviewed [35].
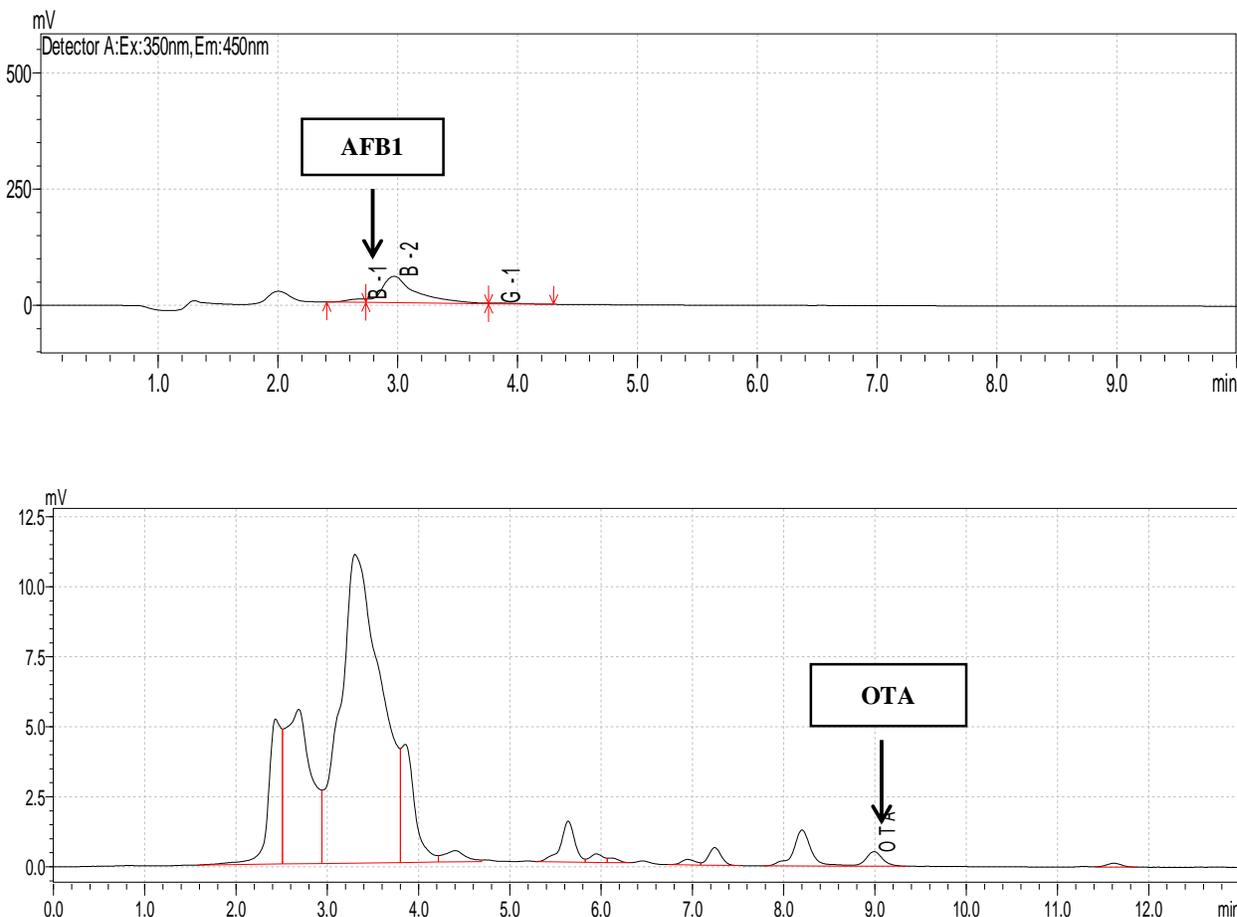
Figure 2: Retention times of aflatoxin B1 (AFB1) and ochratoxin A (OTA) detected in peanut butter samples sold in retail markets of Abidjan.

4. Conclusions

Peanuts butters sold in Abidjan are generally prepared in a traditional way and under conditions of unhealthy hygiene. The conditions of production and storage of these peanut butters and especially their exposure to the air and packaging used constitute potential sources of contamination by mycotoxigenic fungi. Indeed, the presence of toxigenic fungi as *Aspergillus flavus*, *Aspergillus ochraceus* and *Aspergillus parasiticus* and the detection of AFB1 and OTA highlight a potential public health problem concerning their consumption.

Therefore, the need to educate both vendors and consumers on processing, food handling procedures and personal hygiene would help to minimize fungal contamination in order to obtain good sanitary peanut butters.

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