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Potential Production of Mycotoxins by the Fungal Flora Isolated from "Garba" (Côte D'ivoire)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Garba, an Ivorian dish composed of attiéké (a fermented cassava couscous), fried tuna, and condiments, is increasingly consumed by the population, particularly in the streets of Abidjan. However, *garba* could pose a health risk to consumers. This study, aimed at preserving public health, sought to identify the toxigenic fungal flora present in *garba* sold on the streets of Abidjan. To this end, 300 *garba* samples were collected from four districts of Abidjan. Fungal strains contaminating the garba were enumerated and identified using classical mycological methods. Mycotoxins (aflatoxins and ochratoxins) were quantified using High-Performance Liquid Chromatography (HPLC). Results showed that the average mold counts ranged from 0 to 1.8 x

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10^3 CFU/g. The isolated mold strains belonged to ten species grouped into four genera: *Aspergillus, Mucor, Penicillium* and *Paecilomyces*, with *Mucor spp.* (36.89%) and *Aspergillus niger* (30.09%) being the most predominant. The mycotoxins detected in *garba* were aflatoxins (B1, B2, G1, and G2) and ochratoxin A, with average levels ranging from 0.42 to 8.07 µg/kg. Approximately 37% and 23% of *garba* samples had total aflatoxin and aflatoxin B1 levels exceeding regulatory limits.

The presence of potentially mycotoxigenic fungal strains in garba could pose a health risk to consumers. Compliance with good hygiene practices during the production and sale of *garba* will therefore be necessary to considerably reduce contamination and protect consumer health.

Keywords: Street food; garba; fungal flora; HPLC; mycotoxins.

1. INTRODUCTION

The consumption of street food is a very common and widespread phenomenon in Africa (Neffati & al., 2004, Nkosi & al, 2021.). These foods, most often sold by street vendors in streets and other public places, play an important socio-economic role in developing countries (Amoah & al., 2006). They provide populations with affordable and accessible meals. However, these foods raise serious concerns about food safety. Their contamination by chemical and microbiological agents contributes significantly to the occurrence of foodborne illnesses (WHO/FAO, 2010). Several authors (Todd & al., 2007) have attributed the outbreak of certain diseases to the consumption of street food. The work of Ghosh & al. (2007) has highlighted the presence of high levels of total coliforms and pathogenic bacteria (*Salmonella spp*., *Staphylococcus aureus*, *Clostridium perfringens* and *Vibrio cholerae*) in several street foods in India. According to the WHO/FAO (2010), the contamination of street food is linked to the conditions prevailing on the public highway, in particular to the increase in pollution levels due to dust and road traffic.

In Côte d'Ivoire, street foods are numerous and varied. Among these foods is *garba*, which occupies a place of choice in view of the craze it arouses today. It is indeed one of the most popular local dishes among Ivorian populations (FAO, 2012). It is a food made from attieke (cassava flour) of the 2nd grade (Gbané & al., 2012) and fried tuna, accompanied by vegetables (tomato, onion and fresh pepper...), oil, sometimes seasoned with culinary broth. Garba, although a source of energy, nutrients and accessible to many consumers (Ohiokpehai, 2003; Heuberger, 2005; Djeni, 2009, Ekissi &al. 2021), could, like other street foods, be contaminated by toxin-producing microorganisms. Among these microorganisms, mycoflora occupies an important place because it could have serious consequences both on the quality of the food and on the health of the consumer. Indeed, molds reduce the technological quality (gluten content) and sanitary (allergies, toxic agents responsible for serious human and animal intoxications) of contaminated products (Gacem, 2012). The main molds involved are mycotoxin-producing fungi. They are ubiquitous in nature and have a very varied enzymatic arsenal, allowing them to grow on various substrates.

This study aims to investigate the prevalence of toxigenic fungal flora in *garba* sold in the streets of Abidjan. By identifying the specific mycotoxin-producing fungi present in *garba*, this research will contribute to a better understanding of the risks associated with consuming street food in Côte d'Ivoire and inform strategies for mitigating mycotoxin contamination.

2. MATERIALS AND METHODS

2.1 Study Area

This study was conducted in the city of Abidjan, primarily focusing on four communes: Abobo (Northeast), Cocody (Center-East), Port-Bouet (Southeast), and Yopougon (Northwest) (Fig. 1).

2.2 Sampling of *garba*

Sampling was conducted from July to August 2017 from *garba* sellers in the communes of Abobo, Cocody, Port-Bouet, and Yopougon. Samples were taken under the usual selling conditions and during peak hours (8 AM, 12 PM, and 5 PM). A garba sample consisted of 200g of food made up of attiéké, fried tuna, and condiments. In total, three hundred (300) samples were taken from the sales containers (plastic bags) used by *garba* sellers, then stored in an isothermal cooler and transported to the

laboratory within 2 hours. Thus, for each commune, 25 *garba* samples were taken at 8 AM, 12 PM, and 5 PM, for a total of 75 *garba* samples taken per commune.

2.3 Mycological Analyses

2.3.1 Mold count

The mold count was performed according to the ISO 21527-1 (2008) method. To do this, 0.1 mL of the mother suspension and successive dilutions were transferred to the surface of Dichloro-Rose-Bengale Chloramphenicol (DRBC) (OXOID, England) agar contained in Petri dishes. The Petri dishes were then incubated at 25±1°C for 72 hours. After incubation, colonies in the form of filamentous and veiled brown-red, yellow-green, and black were considered to be molds.

2.3.2 Identification of Isolated Mold Strains

The identification of molds was based on the macroscopic and microscopic characteristics of these organisms obtained in pure cultures according to the identification key proposed by Pitt and Hocking (2009). At the macroscopic level, the growth time, the appearance of the colonies, the color of the reverse, the existence of ridges or deep arborization of the agar were observed. Microscopic observation was carried out using a Laborlux k (Leitz) optical microscope at ×40 magnification. This observation was based on the morphology of the different fungal organs, such as the type of thallus (septate or not), the shape of the spores and their origins, the color of the hyphae (dark or light), and the shape of the heads (brush or aspergillar).

2.4 Mycotoxin Search

2.4.1 Aflatoxin dosage

The dosage of aflatoxin was performed according to the ISO 16050 (2003) international method. The test sample was extracted with a mixture of water and methanol. The sample extract was filtered, diluted with water, and deposited on an immunoaffinity column Aflaprep (R-Biopharm, France) containing antibodies specific to aflatoxins B1, B2, G1, and G2. Aflatoxins were isolated, purified, and concentrated on the column, then released from the antibodies with methanol. Aflatoxins were quantified by high-performance liquid chromatography (HPLC) in reverse phase with fluorescence detection and post-column derivatization.

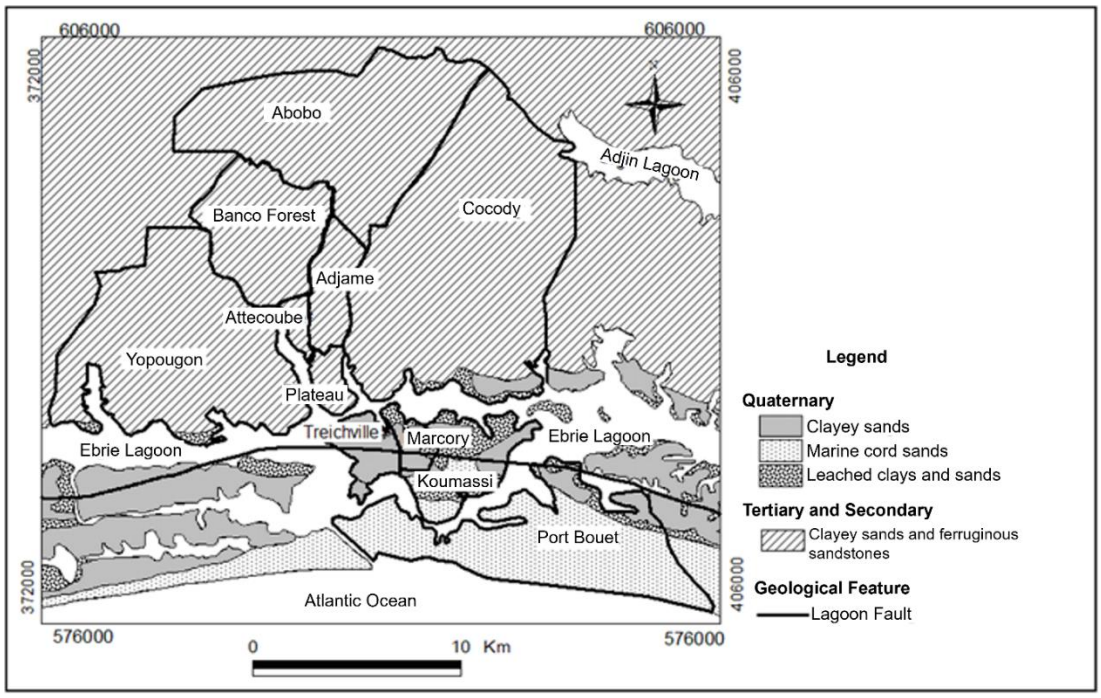


Fig. 1. Study Area

2.4.2 Ochratoxin A dosage

The dosage of ochratoxin A (OTA) was performed according to the NF EN 14133 standard. Ochratoxin A was extracted from the sample by mixing with methanol and sodium bicarbonate (Prolabo, Belgium). The extract was purified by passage through an immunoaffinity column Ochraprep (R-Biopharm, France). Ochratoxin A was separated by high-performance liquid chromatography in reverse phase and quantified by fluorimetry.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Evolution of average mold counts in garba over the course of a day

The average mold counts in *garba* samples from different districts ranged from 0 to 1.8 x 10^3 CFU/g. The average mold counts varied significantly from one sampling time to another and for samples taken within each district (Fig. 2). Fig. 3 shows the appearance of mold cultures on agar medium.

3.1.2 Frequency of identification of mold strains in *garba* samples

A total of 103 mold strains were isolated from all *garba* samples collected (300) from the various communes. These mold strains belong to 10 species grouped into 4 genera: *Mucor*, *Penicillium*, *Aspergillus* and *Paecilomyces*. These 10 species are as follows: *Mucor spp., Penicillium spp., Aspergillus niger, Aspergillus flavus, Aspergillus spp., Aspergillus fumigatus, Aspergillus glaucus, Aspergillus nidulans, Aspergillus terreus* and *Paecilomyces spp*. The most frequently isolated species were *Mucor spp.* and *Aspergillus niger* with frequencies of 36.89% and 30.09%, respectively (Table 1).

3.1.3 Mycotoxin levels in *garba* samples

The mycotoxins detected in *garba* samples from different regions were aflatoxins G1, G2, B1, and B2, as well as ochratoxin A, with average values ranging from 0.42 µg/kg (ochratoxin A) to 8.07 µg/kg (aflatoxin G1) (Table 2). Aflatoxin B1 was found in 56.6% of samples, with concentrations ranging 0.02 µg/kg and 35.78 µg/kg,while aflatoxin B2 was detected in 23.33% of garba samples analyzed, with levels ranging from 0.10 µg/kg to 23.95 µg/kg, The quantities of aflatoxin G1 found in. 46.6% of the samples were between 0.56 µg/kg and 69.32 µg/kg. As for aflatoxin G2, it was detected in 40% of the samples with levels ranging from 0.04 µg/kg to 13.33 µg/kg. Ochratoxin A (OTA) was found in 63.3% of the *garba* samples with values ranging from 0.06 µg/kg to 1.83 µg/kg (Table 3).

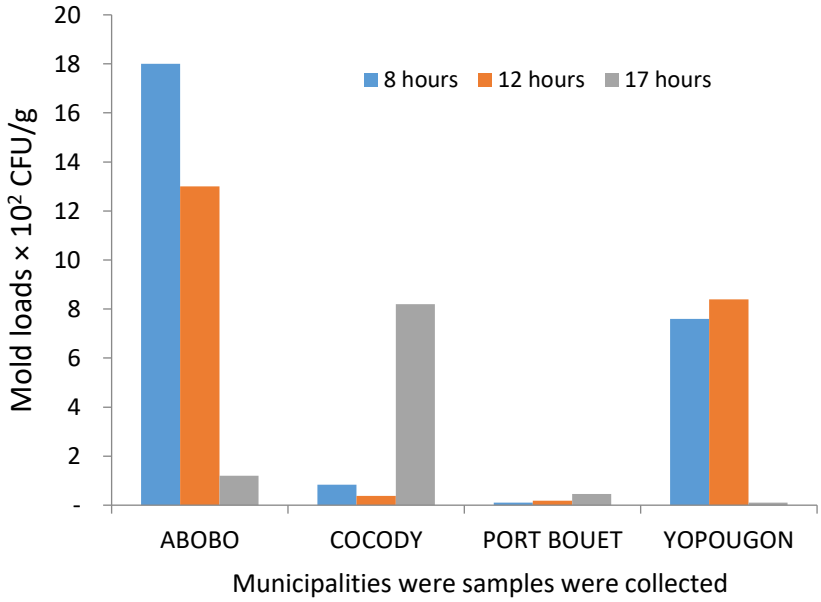
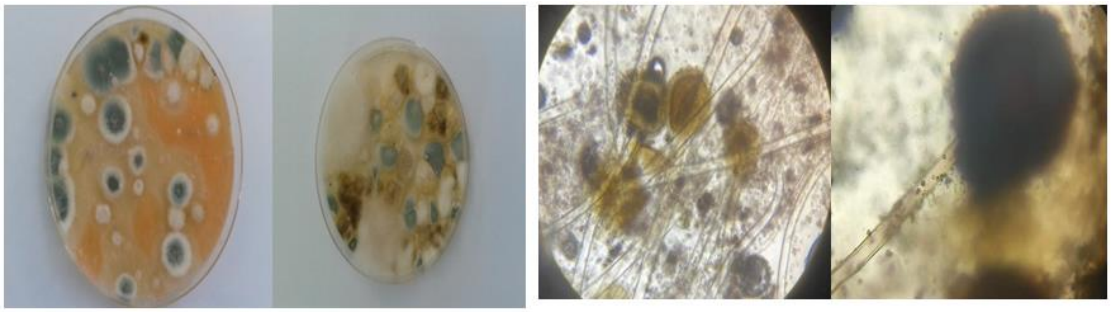


Fig. 2. Average mold counts in *garba* samples over the course of a day



A B

Fig. 3. Macroscopic (A) and microscopic (B) aspects of isolated mold strains

Table 1. Different mold species isolated from *garba* samples

Isolated molds				
Genera	**Species**	**Number of occurrences**	**Occurrence (%)**	**frequency**
Mucor	*Mucor spp.*	38	36,89	
Penicillium	*Penicillium spp*.	10	9,71	
Paecilomyces	*Paecilomyces spp*.	2	1,94	
Aspergillus	*Aspergillus niger*	31	30,09	51,44
	Aspergillus fumigatus	10	9,71	
	Aspergilus glaucus	3	2,91	
	Aspergillus terreus	3	2,91	
	Aspergillus spp.	3	2,91	
	Aspergillus nidulans	2	1,94	
	Aspergillus flavus	1	0,97	
Total		**103**	**100**	

Table 2. Mycotoxin levels in *garba* samples (µg/kg)

Mycotoxin	**Minimum Value (mg/kg)**	**Maximum Value (mg/kg)**	**Average Value**	**Alert Limit**
Aflatoxine B1	0,02	35,78	3,44 ± 8,11	8
Aflatoxine B2	0,1	23,95	1,89 ± 5,68	
Aflatoxine G1	0,56	69,32	8,07 ± 16,95	
Aflatoxine G2	0,04	13,33	0,56 ± 2,43	
Aflatoxine totale	0,01	39,85	13,95±17,96	15
Ochratoxine A	0,06	1,83	0,42 ± 0,56	

Table 3. Pourcentage of garba samples containing mycotoxins

Mycotoxins	**Number of positive samples**	**Maximum permitted level (EC/1881/2006)**	**Frequency of positive samples (%)**	**Number of positive samples exceeding the maximum permitted level**
Aflatoxine B1	170	8 µg/kg	56,60	40
Aflatoxine B2	70	-	23,33	-
Aflatoxine G1	140	-	46,66	-
Aflatoxine G2	120	-	40,00	-
Aflatoxines totales	290	15 µg/kg	96,67	107
Ochratoxine A	190	-	63,30	-

3.2 Discussion

Garba sold on the streets of Abidjan has shown high levels of fungal contamination. This contamination can be explained by the fact that these ubiquitous microorganisms are present in the production, storage, and sale environments of *garba*. Additionally, this trade is often carried out by individuals who do not adhere to good hygiene practices. It should be noted that the presence of excessive mold loads raises the risk of preformed toxins in *garba*.

Based on fungal identification, 103 mold strains were isolated from all garba samples. These isolated mold strains belonged to ten (10) species grouped into four genera: *Aspergillus* (*Aspergillus niger, Aspergillus flavus, Aspergillus spp., Aspergillus fumigatus, Aspergilus glaucus, Aspergillus nidulans* and *Aspergillus terreus*), *Mucor* (*Mucor spp*.), *Penicillium* (*Penicillium spp*.) and *Paecilomyces* (*Paecilomyces spp*.). These genera of molds are known to secrete toxins dangerous to human health (Chapeland-Leclerc et al., 2005; Ouattara & al., 2022). The genus *Aspergillus* was the dominant genus with an isolation frequency of 51.44% followed by the genus *Mucor* (36.89%). The dominance of the genus *Aspergillus* in the flora contaminating food has been reported in several studies (Riba et al., 2005; Riba & al., 2015, Ouattara & al., 2022). The high frequency of contamination by molds of the genus *Aspergillus* in *garba* samples could be explained by the fact that *Aspergillus* is a very common fungus in soil and air through its spores (Abdoullahi et al., 2016, Abdoullahi et al., 2019). This suggests that contamination of these products probably occurred either through spores that were initially present at the production site, or later during storage or during handling of the samples during sale. Among the *Aspergillus* strains isolated from the garba samples analyzed, the species *Aspergillus* niger (30.09%) was the predominant species. These results confirm those of Muhammad et al. (2010) who identified in foods intended for human consumption mold strains with a predominance of *Aspergillus niger* (37.7%). This fungal species is present in most poorly preserved or poorly dried foods (Abdoullahi et al., 2019).

In the present study, more than half of the samples contain aflatoxin B1. This mycotoxin is known to be very dangerous for humans and is responsible for liver cancer. Indeed, this aflatoxin is at the root of acute liver destruction and liver cirrhosis, as well as the development of tumors or other genetic effects (Kirk et al., 2006; Wild and Gong, 2010). Ingestion of a high content of aflatoxin B1 can also cause growth retardation in children (Okoth and Obringo, 2004). The presence of aflatoxin B1 in *garba* can be attributed to attiéké. Indeed, this food, which has a relatively high moisture content estimated at more than 40% (Gbané et al., 2012; Krabi et al., 2015), is susceptible to being contaminated by molds. Previous work has already mentioned the presence of molds in attiéké (Darboux and Ahounou, 2004; Darman et al., 2007; Kouamé et al., 2012). Moreover, several studies have already mentioned the presence of aflatoxins in foods with a high moisture content. This is the case of the work of Muthomi et al. (2012), Kang'ethe and Lang'at (2009), Offifah and Adesiyun (2007) and Lewis et al. (2005) who detected the presence of aflatoxins in pasteurized products, cheese, peanut butter, alcoholic beverages based on cereals, infant foods and corn.

The admissible levels of mycotoxins in food are not regulated in Côte d'Ivoire. Moreover, in European countries where they are, they vary according to the type of mycotoxin and food. According to European regulation (EC/1881/2006) which defines the maximum admissible quantities of mycotoxins in food, the highest values are 15 µg/kg for the sum of the 4 aflatoxins versus 8 µg/kg for aflatoxin B1. The aflatoxin B1 levels of the samples analyzed range from 0.02 to 35.78 µg/kg with an average value of 3.43 µg/kg. Forty (40) samples of garba (23.52%) contaminated with this mycotoxin have values that exceed the European regulatory limit (8 µg/kg). According to the 2001-2004 surveillance plan in France, the average concentrations of aflatoxins in cereals range from 0.2 to 0.3 µg/kg with only three products (rice, semolina and corn flour) whose concentrations in aflatoxin B1 exceed the regulatory limit (8 µg/kg). The average aflatoxin B1 in all food products was then 5.31 µg/kg (Afssa, 2009).

Regarding mycotoxins, 23% and 37% of garba samples respectively have values in aflatoxin B1 and total aflatoxins above the regulatory limit. For ochratoxin A, the quantities determined remain below the regulatory limits.

In view of these results, *garba* would therefore be a food dangerous to the health of consumers with regard to mycotoxins, especially aflatoxin B1.

4. CONCLUSION

This study revealed high levels of fungal contamination in *garba* sold on the streets of Abidjan's communes. Furthermore, a diversity of fungal strains belonging to ten (10) species grouped into 4 genera (*Aspergillus, Mucor, Penicillium* and *Paecilomyces*) was isolated, with a predominance of *Mucor spp*. (36.89%) and *Aspergillus niger* (30.09%). Mycotoxins detected in garba were aflatoxins (B1, B2, G1, and G2) and ochratoxin A, with average levels ranging from 0.42 to 8.07 µg/kg. Approximately 23% and 37% of *garba* samples had aflatoxin B1 and total aflatoxin levels, respectively, exceeding regulatory limits. The presence of potentially mycotoxigenic fungal strains in *garba* could pose a health risk to consumers. A better control of processing, storage, and conservation techniques, as well as sales methods and compliance with good hygiene and manufacturing practices, will be necessary to reduce fungal contamination and protect consumer health.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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COMPETING INTERESTS

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

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