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Microbiological Safety and Quality Assessment of Maize (*Zea mays L*) Produced and Stocked from Rural Conditions in Côte D'ivoire

Kouamé Désiré[1], Niamketchi Gilles Léonce[2*], Konan Constant[3], Konan Ysidor[4] and Biego G. Henri Marius[1,5]

[1]Laboratory of Biotechnology, Agriculture and Valorisation of Biological Ressources, Training and Research Unit of Biosciences, Félix Houphouët-Boigny University, 22 BP 582, Abidjan 22, Côte d'Ivoire.
[2]La Me Research Station for Palm Oil, National Center of Agronomic Research (CNRA), 01 BP 1740, Abidjan 01, Côte d'Ivoire.
[3]Eco Epidemiology Unit, Department of Environmental and Health, Institut Pasteur de Côte d'Ivoire (IPCI) 01 P.O. Box 490, Abidjan 01, Côte d'Ivoire
[4]Training and Research Unit of Biological Sciences, Péléforo Gon Coulibaly University, P.O. Box 1328, Poro, Côte d'Ivoire.
[5]Department of Public Health, Hydrology and Toxicology, Training and Research Unit of Pharmacological and Biological Sciences, Félix Houphouët-Boigny University, BP 34 Abidjan, Côte d'Ivoire.

Authors' contributions

This work was carried out in collaboration among all authors. Author KD designed the study, wrote the protocol, fitted the data and wrote the first draft of the manuscript. Author NGL and SD checked the first draft of the manuscript and achieved the submitted manuscript. Authors KY performed the statistical analysis and assisted the experiments implementation. Author BHM expertise the results interpretations. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Fungal pathogens are one of the main biological agents causing maize post-harvest loss and affect food security in the country. Thus, this study was conducted to assess fungal pathogens associated to post-harvest maize (*Zea mays* L.) with especial focus to mycotoxin-producing fungi at

**Corresponding author: E-mail: nbgleo@gmail.com;*

producer's storage condition in different regions of Côte d'Ivoire.
Study Design: A total of 1 500 samples of maize as grains, cobs and husks were collected at rate of 500 samples by region (Gbêkê, Poro, Hambol, Indénié-Djuablin and Gontougo) and sent to the laboratory in order to analyse their sanitary quality.
Place and Duration of Study: This study was carried out during March 2016 to January 2017. The analyses of the collected sample carried out at the Biotechnology, Agriculture and Valorisation of Biological Resources Laboratory of the Félix Houphouët-Boigny's University, Abidjan.
Methodology: Microbiological analysis was assessed by recording the number of colony in the plate. The contents of aflatoxin and ochratoxin A in the different samples were determined using standard methods.
Results: The total microbial species isolated ranged from 10^4 to 10^{11} cfu/g with thermotolerants coliforms ($10^3 – 10^4$) and fecal coliforms ($10^2 – 10^3$). The stored maize samples contained also high amount of yeast and molds (10^4 to 10^7 cfu/g). *Aspergillus* genus was the predominantly fungal isolated in all maize samples with tree species which are *A. flavus*, *A. niger* and *A. versicolor* potential producers of mycotoxins. More importantly, stored maize sample as grain, cobs and husks were affected by aflatoxins (B1, B2, G1 and G2) and ochratoxin A. Sixty per cent of the maize samples, mostly husks, showed aflatoxin B1 (from 12.73 to 130.31 µg/kg) and OTA (from 16.75 to 134.21 µg/kg) concentrations above the Maximum Authorized Limit of 5 µg/kg.
Conclusion: A significant variability from one region to another can be noticed at level of maize quality regardless the type of maize. The sanitary quality of maize seems to be tied to postharvest treatments (drying), type of storage (grains, cobs and husks) and structure of storage.

Keywords: Fungal contamination; aflatoxin, ochratoxin A, maize grains; cobs; husks; production region; Côte d'Ivoire.

1. INTRODUCTION

Maize is one of the most widely cultivated crops in the world [1]. World maize production is expected to reach 1.16 billion tonnes in 2020/21 [2]. The demand continues to increase and cannot be satisfied without strong technological interventions [3]. Cereal cultivation in Côte d'Ivoire is dominated by rice, maize, millet and sorghum, with several improved and traditional local varieties. Corn is the second cereal produced and consumed after rice [1]. Maize is used for human and animal food (poultry, pigs, cattle) and serves as a raw material in certain industries (brewing, soap and oil mill) [4]. Long considered a simple subsistence product, maize is now the subject of agricultural speculation which is intensifying in Côte d'Ivoire, due to the economic stakes of this crop which has become increasingly important. In 2018, its national production estimated at 1,025,000 tonnes, for a total area of 523,538 ha [5]. Despite the growth in its production and its socio-economic importance, post-harvest losses during storage remain a real challenge for farmers [6]. Storage practices and traditional storage structures can make maize susceptible to different types of damages including storage pests and disease [7]. Study conducted in Côte d'Ivoire to monitor the merchant quality of maize grains stored for 9 months in polypropylene bags and traditional granaries revealed respectively 47.40% and 60.42% mean grain damage caused both by weevil and mold [8]. The characterization of the fungal flora of maize grains (*Zea mays*) intended for the compound preparation of poultry has been reported by Dedi et al. [9]. Furthermore, little information's are available on the mycobiota in traditional storage structures. Moreover, studies highlighted the need for much attention for bio-deterioration which is caused by fungal pathogen particularly mycotoxin-producing fungal pathogens that leads to the loss of physical, nutritional qualities and health impact (grain unsuitable for human consumption) [10,11]. However, there is limited study conducted in major maize producing areas on fungal pathogens associated with stored maize in Côte d'Ivoire. Therefore, the objective of the current study is to investigate incidence of mold infection and mycotoxin (aflatoxin and ochratoxin A) concentrations in five regions of Côte d'Ivoire.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Biological material

The biological material is composed of dry maize in the form of grains, cobs and husks deriving the major region production of this resource in Côte d'Ivoire.

2.1.2 Study site

The samples were collected from the regions of Gbêkê (Center), Poro (North), Hambol (North - Center), Indénié-Djuablin (Northeast) and Gontougo (East). Each of these regions has a geographical specificity and climatic characteristics which influence the seasons of maize production. Indeed, the regions of Gbêkê (7º50'N 5º18'W), Hambol (8º10'N 5º40'W), Indénié-Djuablin (7º02'N 3º12'W) and Gontougo (8º30'N 3º20'W) are characterized by a humid tropical climate (Baouléen climate). It has four seasons including two rainy seasons favouring maize production twice a year and two dry seasons. Except the other four regions, the climate of Poro region (9º27'N 5º38'W) is of Sudanese type characterized by a rainy season favourable to maize production and a dry season [12,13].

2.2 Methods

2.2.1 Sampling of stored maize

The strategy adopted consisted of two phases. The first phase consisted in identifying the regions where maize cultivation constitutes the main subsistence activity. In each region, meetings were organized with the traditional chiefdom to present the study. Then, samples of 1 kg of maize as husks, cobs and grains were taken from the stocks of growers constituting the second phase. A total of 1500 samples were collected for each form of maize from March 2016 to January 2017 with 500 grains, 500 cobs and 500 husks, (Table 1). Maize samples were then taken to the laboratory in sterile plastic bags and kept at 4°C for the microbiological and mycotoxin analysis.

2.2.2 Microbiological analysis of maize stored

2.2.2.1 Enumeration of microorganisms

The culture dependent approach was performed as follow: 2100 mL of peptone water (Oxoid, Basingstoke, United Kingdom) was added to 100 g of maize grains in a sterile Stomacher bag that was vigorously shaken for 5 min in a Stomacher 400 (Seward, Worthington, United Kingdom) to obtain a uniform homogenate. Samples (1 mL) of the homogenate were serially diluted *10*-fold in peptone water, from which aliquots (0.1 mL) were spread-plated onto different selective agar media and incubated at different temperatures for 1 to 4 days for isolation and enumeration (by recording the number of CFU) *by using a colony counter (JP Selecta, Spain)* of specific groups of microorganisms [14] : plate count agar (PCA; Oxoid) for the total aerobic bacterial count (30 °C), yeast glucose chloramphenicol (YGC; Oxoid) agar for yeast and molds (30 °C); Baird Parker (BioRad) agar for *S. aureus* (37 °C); Violet Red Bile Lactose (VRBL, AES Laboratoire) agar for coliforms (30 °C for total coliforms and 44 °C for thermotolerant coliforms); Hektoen (BioRad) for Salmonella and Trypton Sulfite Neomycin (TSN, BioRad) agar for anaerobic sulphito-reducers (46 °C).

2.2.2.2 Fungal isolation

Frequency and relative percentage of maize fungi were determined by agar plating method with plating maize grains on potato dextrose agar (PDA, BioRad) medium [15]. Ten maize grains from each sample were surface sterilized with 3% sodium-hypochlorite solution for 3 min and rinsed twice with sterile distilled water. Samples were then plated on PDA plates at the rate of five seeds per plate (9 cm in diameter). The plates were incubated for 5 to 7 days at 37°C. Fungal isolates were sub-cultured on Malt Extract and Czapek Yeast medium agars (Oxoid, UK) and incubated for 5 to 7 days at 37°C for purification. Fungi were identified by using taxonomic schemes based on microscopic observation and culture appearance [15]. Fungi frequency and relative percentage of a particular species in a genus was calculated using the formula of Larone [16]:

Frequency (%) = number of samples infected with fungi x 100 / total number of samples analysed

Relative percentage (%) = number of fungal species isolated x 100 / total number of fungi isolated

2.2.3 Analysis of aflatoxins and ochratoxin A

2.2.3.1 Extraction and purification of aflatoxins

Aflatoxins (AFs) were extracted and purified from maize using the official guidelines of AOAC [17]. To 100 g of maize taken in an erlenmeyer flask, 100 mL of 80% methanol aqueous solution were added. The mixture was homogenized, put in darkness at room temperature for 12 h, and then filtered with a Whatman paper (Whatman N°4). Thereafter, 50 mL of the filtrate were added with 40 mL of a mixture deriving from phosphotungstic acid-zinc sulfate-water (5/15/980, w/w/v), and kept at room temperature for 15 min before filtration upon Whatman paper. Aflatoxins were extracted from the out coming

filtrate with 3 volumes of 10 mL of chloroform. The extract was collected into a 50 mL flask and processed with rotary evaporator (Buchi Rotavapor R-215) at 40 °C for evaporation of the chloroform reagent. Finally, 0.4 mL of hydrochloric acid and 4.6 mL of bidistillated water were added to the dry extract, and the solution was filtered through filter Rezist in a chromatographic tube then passed through an immunoaffinity column (column RiDA aflatoxin, Biopharm, Germany).

2.2.3.2 Extraction and purification of ochratoxin A

100 g of the sample of maize was crushed in a hammer mill to obtain a homogeneous fine grind. In a Nalgene jar containing 15 g of grind, 150 mL of aqueous methanol-bicarbonate 1% (m / v, 50:50) were added. The mixture was homogenized by Ultra-Turax for 3 min and the homogenate centrifuged at 5000 rpm for 5 min at 4°C. The supernatant was filtered through a Whatman paper (Whatman N°4) into tubes of 100 mL, 11 mL of filtrate were added 11 ml of saline phosphate buffered (PBS) at pH 7.3. Immunoaffinity columns brand Ochraprep and RBiopharm were conditioned with 10 mL of PBS. Purification of 20 mL of the mixture was made on immunoaffinity columns and OTA extraction was performed with two volumes of 1.5 mL of PBS at a flow rate of 5 mL/min. The resulting sample was packed in a chromatographic tube and the analysis of OTA was made by HPLC using the European community regulation [18].

2.2.3.3 Quantification of aflatoxins and ochratoxin A

Determination of AFs and OTA contents was achieved with high performance liquid chromatography column, using a Shimadzu liquid chromatograph (Kyoto, Japan) fitted with fluorescence detector (Table 2).

2.3 Statistical Analysis

All the analyses were carried out in three-fold test and data processed with software Statistical Product and Service Solutions, SPSS version 20.0. For each characteristic, results were expressed in averages followed by their standard deviations. A two-way analysis of variance (ANOVA 2) was also made in order to test the impact of region and the ways of preserving maize on assessed characteristics to 5% significant threshold statistical. For the statistically different averages, the Tukey's test served for the classification. Furthermore, the correlation between data and samples was estimated on basis of main components analysis (MCA), thanks to STATISTICA version 7.1 software.

Table 1. Number of samples collected according to maize variety and department

Regions	Grains	Epis	Spathes	Total
Gbêkê	100	100	100	300
Poro	100	100	100	300
Hambol	100	100	100	300
Indénié-Djuablin	100	100	100	300
Gontougo	100	100	100	300
Total	500	500	500	1500

Table 2. Conditions of aflatoxins and ochratoxin A analysis by HPLC

ITEM	Aflatoxins (AFB1, AFB2, AFG1, AFG2)	Ochratoxin A (OTA)
Pre-column	Shim-pack GVP-ODS 10 x 4,6 mm	
Column	Shim-pack GVP-ODS, 250 mm x 4,6 mm	
Detector fluorescence	λ excitation: 365 nm λ emission: 435 nm	λ excitation: 330 nm λ emission: 460 nm
Mobile Phase	Acetonitrile/Water/ Methanol (20/20/60)	Acetonitrile/Water/ Acetic acid (49/49/2)
Inject volume	20 µl	100µl
Flow rate	1 mL/min	
Column Temperature	40°C	
Rising solvent	Methanol	Acetonitrile

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Loads of spoilage and hygiene indicator microorganisms

The microbial load found in samples is shown in Table 3. Maize samples (grains, cobs and husks) show a varying load between 5.5 x 10^4 and 9.8 x 10^{11} cfu/g of total mesophilic aerobic bacteria count. Except the Hambol maize grains which has a lower load than the standard criteria which is 10^5 cfu/g, all of the other maize samples had higher loads than the standard. Gontougo and Indénié-Djuablin regions recorded the highest loads for values between 8.4 x 10^6 and 9.8 x 10^{11} cfu/g. For the total and thermotolerant coliforms, the counting varied between 1.8 x 10^3 - 9.9 x 10^4 cfu/g and 10 - 3.3 x 10^3 cfu/g, respectively. Samples of maize grains and maize cobs from Gbêkê, Poro and Hambol regions are free from thermotolerant bacteria, while the other samples show loads greater than the standard criteria which is 10 cfu/g. Yeast and molds were present on all maize samples regardless of the form and the region and represents the predominant flora of total microorganisms at 30 °C. The most significant loads were enumerated on maize husks samples from the Gontougo and Indénié-Djuablin regions. These values are estimated between 1.5 x 10^4 and 9 x 10^4 cfu/g for yeasts and between 5 x 10^6 and 3.9 x 10^7 cfu/g for molds. All of the maize samples (grains, cobs and husks) from the various regions showed no contamination due to pathogenic microorganisms such as *Salmonella*, *Staphylococcus aureus* and sulfite-reducing anaerobic microorganisms.

3.1.2 Fungal flora isolated

The fungal flora isolated from stored maize samples is shown in Table 3. In this research, mycological examination of maize samples revealed the occurrence of five genera: *Aspergillus*, *Fusarium*, *Penicillium*, *Mucor* and *Alternia* (Table 4). Genus *Aspergillus* represented a greater number of species isolated from stored maize samples. The percentage of contamination of *this genus* varies between 41 and 70.2%. The regions of Indénié-Djuablin and Gontougo record the highest percentages for the various maize forms with values ranging from 55.3 to 70.2%. The lowest percentages are recorded on maize cobs and grains from Hambol region (41 and 42.3%, respectively) and on maize grains from Poro region (42%). Occurrence due to *Fusarium* species was higher on grains, cobs and husks samples from Gontougo and Indénié-Djuablin regions with percentages ranging from 20 to 36 %. The lowest percentages are recorded from Gbêkê for maize grains sample (10%) and Poro respectively for maize cobs (12%) and husks (11%) samples. The contamination rate of *Penicillium* species is higher on maize husks samples from Gbêkê region, on maize grains and cobs samples from Hambol with values of 15.9%, 16% and 18% respectively. Occurrence due to *Mucor* species was higher on grains, cobs and husks samples from the five regions with values ranging between 12% and 31%. However, maize cobs and husks from Indénié-Djuablin region recorded the lowest values of 4%. With regard to the genus *Alternia*, the lowest contamination rates were recorded in Indénié-Djuablin and Gontougo regions regardless of maize forms sampled with percentages between 2% and 6%. The highest rates were determined in maize grains samples (10% to 13%) and cobs (14%) respectively for Gbêkê, Hambol and Poro regions.

3.1.3 Relative density of *Aspergillus* species

The relative density of *Aspergillus* species is shown in Fig. 1. Six species were identified. The regions of Gontougo and Indénié-Djuablin have a high percentage of *A. flavus* regardless of the shape of the sample compared to other areas. The highest percentages were recorded on husks (17%), followed by cobs with values ranging from 12 ± 0.50 to 14.6 ± 0.65%, in addition, high percentages were also determined on husks of Gbêkê, Poro and Hambol with respective values of 8.5 ± 0.45%, 9 ± 0.30% and 12 ± 1.50%. A predominance of *A. niger* is observed on Gbêkê husks (16 ± 0.15%), followed by samples of Gontougo husk and cobs maize with respective rates of 11 ± 0.50% and 12 ± 0.65%. The region of Hambol has the lowest occurrence rates of *A. niger* with values between 2 ± 0.17% and 4.8 ± 0.100%. As regards to the *A. fumigatus* species, a high occurrence is observed on all maize samples regardless the region with percentages varying between 9.6 ± 0.65% and 15 ± 0.50%. However, low percentages were recorded on maize grains samples from the regions of Poro and Gontougo with respective rates of 5.5 ± 0.40% and 5.8 ± 0.17%. The regions of Gbêkê and Poro have the highest levels of contamination of *A. terrus* on maize grains and cobs samples with percentages between 10.5 ± 0.50% and 14.40 ± 0.35%. Likewise, strong contaminations were recorded

Table 3. Hygienic and microbiological quality of stored maize samples from five collection regions

Regions	Maize forms	MAG	TC	FC	Yeasts	Mold	ASR	*Salmonella*	*S. aureus*
Gbêkê	Grains	5.0×10^5	1.8×10^3	<10	3.4×10^2	4.5×10^3	$<10^2$	<10	<10
	Cobs	8.1×10^8	5.6×10^3	<10	4.2×10^3	7.2×10^3	$<10^2$	<10	<10
	Husks	6.0×10^9	8.6×10^3	1.3×10^2	7.1×10^4	2.2×10^5	$<10^2$	<10	<10
Poro	Grains	2.6×10^5	2.1×10^3	<10	2.2×10^2	2.7×10^3	$<10^2$	<10	<10
	Cobs	6.7×10^8	4.7×10^3	<10	7.0×10^2	6.6×10^3	$<10^2$	<10	<10
	Husks	6.5×10^9	8.0×10^3	1.3×10^2	8.8×10^2	1.5×10^4	$<10^2$	<10	<10
Hambol	Grains	5.5×10^4	2.9×10^3	<10	2.7×10^2	3.6×10^3	$<10^2$	<10	<10
	Cobs	7.7×10^7	7.5×10^3	<10	5.6×10^2	8.4×10^3	$<10^2$	<10	<10
	Husks	4.5×10^{10}	7.6×10^3	1.9×10^2	7.7×10^3	1.8×10^4	$<10^2$	<10	<10
Indénié-Djuablin	Grains	8.4×10^6	6.3×10^3	1.1×10^2	5.0×10^2	7.5×10^4	$<10^2$	<10	<10
	Cobs	6.5×10^9	6.8×10^3	1.8×10^2	3.2×10^3	5.3×10^4	$<10^2$	<10	<10
	Husks	9.8×10^{11}	7.9×10^4	3.3×10^3	9.0×10^4	3.9×10^7	$<10^2$	<10	<10
Gontougo	Grains	9.2×10^6	7.5×10^3	1.5×10^2	7.2×10^2	5.5×10^4	$<10^2$	<10	<10
	Cobs	8.1×10^9	8.1×10^3	2.9×10^2	9.2×10^3	8.8×10^4	$<10^2$	<10	<10
	Husks	1.2×10^{11}	9.9×10^4	2.9×10^3	1.5×10^4	5.0×10^6	$<10^2$	<10	<10
Microbiological criteria		10^5 CFU/g	10^3 cfu /g	10 cfu /g	10^3 cfu /g	10^3 cfu /g	10^2 cfu /g	Not present	Not present

Each value is the average of the analysis of three tests; MAG: Mesophilic Aerobic Germs; TC: Total Coliforms; FC: Fecal Coliforms; ASR: Anaerobic Sulphito-Reducers

Table 4. Contamination levels of stored maize samples according to isolated fungi

Regions	Form of maize	*Aspergillus*	*Fusarium*	*Penicillium*	*Mucor*	*Alternia*
Gbêkê	Grains	50.5 ± 2.40^{d}	6 ± 0.100^{d}	10 ± 0.90^{b}	21 ± 0.45^{a}	12.5 ± 1.72^{bc}
	Cobs	48.8 ± 2.65^{d}	8.2 ± 0.84^{c}	11 ± 1.20^{b}	18 ± 1.70^{b}	14 ± 0.60^{b}
	Husks	58.1 ± 2.32^{c}	4 ± 0.80^{d}	15.9 ± 1.50^{a}	20 ± 1.94^{ab}	2 ± 0.10^{e}
Hambol	Grains	42.3 ± 2.15^{e}	5.2 ± 0.60^{d}	16 ± 1.10^{a}	23.5 ± 0.30^{a}	13 ± 0.50^{b}
	Cobs	41 ± 3.17^{e}	5 ± 0.33^{d}	18 ± 1.73^{a}	17 ± 2.100^{b}	19 ± 0.78^{a}
	Husks	48 ± 3.24^{d}	14 ± 0.90^{a}	4 ± 0.60^{d}	20 ± 1.60^{ab}	14 ± 1.15^{b}
Poro	Grains	42 ± 2.10^{e}	7 ± 0.50^{c}	14 ± 1.00^{b}	20 ± 1.50^{ab}	17 ± 0.50^{a}
	Cobs	58 ± 3.57^{c}	4.1 ± 0.76^{d}	6 ± 1.20^{c}	18 ± 1.80^{b}	14 ± 0.95^{b}
	Husks	59 ± 2.63^{c}	8 ± 0.20^{c}	11 ± 0.100^{b}	10 ± 1.50^{c}	12 ± 0.78^{bc}
Indénié-Djuablin	Grains	60 ± 3.75^{b}	16 ± 0.82^{a}	10 ± 0.56^{b}	10 ± 1.35^{c}	4 ± 0.03^{d}
	Cobs	62 ± 9.65^{b}	10.2 ± 0.10^{b}	11 ± 0.20^{b}	11 ± 0.56^{c}	6 ± 0.20^{d}
	Husks	67.5 ± 2.96^{a}	15.5 ± 0.56^{a}	8 ± 0.50^{c}	4 ± 0.05^{d}	5 ± 0.57^{d}
Gontougo	Grains	55.3 ± 2.41^{c}	12.4 ± 1.65^{b}	7.3 ± 0.15^{c}	13 ± 1.50^{c}	$12 \pm 0,75^{bc}$
	Cobs	60 ± 3.10^{b}	10.3 ± 1.15^{b}	9 ± 0.65^{bc}	10 ± 0.45^{c}	$10,9 \pm 0.56^{c}$
	Husks	70.2 ± 2.76^{a}	12.8 ± 0.95^{b}	7 ± 0.50^{c}	10 ± 0.56^{c}	0 ± 0.00

On the same column, the numbers followed by the same letter are statistically identical to the 5% threshold (Tukey's test mean ± standard deviation)

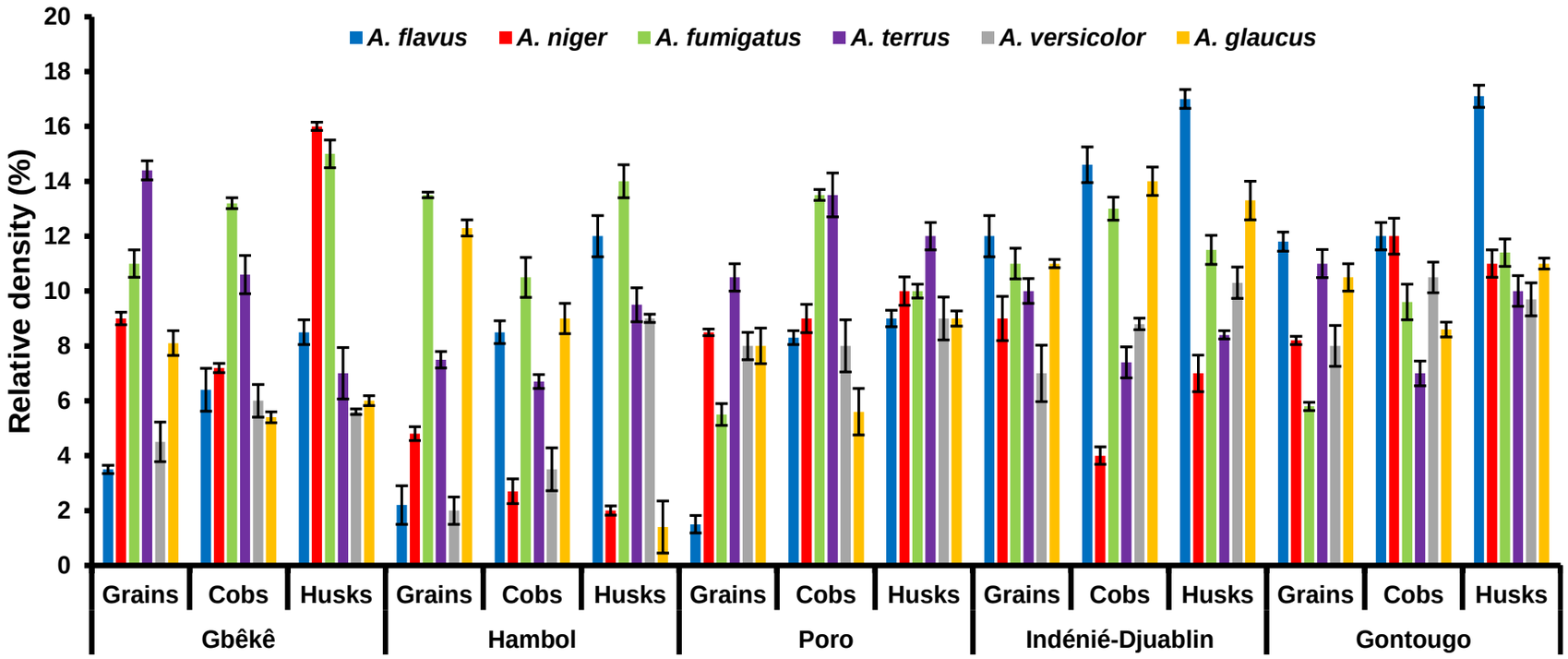

Fig. 1. Relative density of *Aspergillus* species isolated from stored maize samples

on maize husks samples from Poro (12%), on maize grains from Gontougo and Indénié-Djuablin and on maize husks from Gontougo. The Hambol region presented the lowest percentages with rates ranging between 6.7 ± 0.10% and 9.5 ± 0.60%. Of all the samples analysed, the regions of Poro, Indénié-Djuablin and Gontougo recorded the highest percentages of contamination in *A. versicolor*, with values between 8 ± 0.50% and 10.5 ± 0.56%, in addition, maize husks samples of Hambol showed high proportions reaching the value of 9 ± 0.55%. On the contrary, Gbêkê and Hambol regions have low percentages with an average proportion of 4.32%. The species of *A. glaucus* isolated from maize samples showed the strongest contamination in Indénié-Djuablin and Gontougo regions with percentages ranging from 11% to 14 ± 0.20%. The lowest levels were recorded from Gbêkê, Hambol and Poro regions with values ranging from 1.4 ± 0.15% to 6 ± 0.10%.

3.1.4 Aflatoxin concentrations in stored maize samples

The aflatoxin concentrations of the stored maize samples from the different regions are recorded in Table 5. Aflatoxin B1 contents vary from 0.80 ± 0.75 to 20.92 ± 27.63 µg/kg for maize grains, from 2.40 ± 2.67 to 32.22 ± 50.40 µg/kg for maize cobs and from 12.73 ± 26.26 to 130.31 ± 92.56 µg/kg for maize husks. Maize grains and cobs samples from Gbêkê, Hambol and Poro regions recorded the lowest concentrations below 5 µg/kg, representing the maximum limit reference value. The highest concentrations exceeding the standard were recorded on all maize husks samples which differ significantly from other forms of maize regardless of the region. Maize samples from Indénié-Djuablin and Gontougo regions show concentrations ranging from 9.30 ± 9.76 to 32.22 ± 50.40 µg/kg, above the normative value of 5 µg/kg. In total, 40% of the maize samples analysed has concentration below the maximum limit reference value. For aflatoxin B2 concentrations, the values vary between 0.10 ± 0.12 and 1.43 ± 2.04 µg/kg for maize grains, between 0.28 ± 0.28 and 3.21 ± 4.84 µg/kg for maize cobs and between 0.33 ± 0.35 and 3.26 ± 4.89 µg/kg for maize husks. Indénié-Djuablin region stands out significantly from other regions by the highest levels on maize cobs and husks samples. Regarding aflatoxin G1, the various maize samples recorded values ranging from 3.36 ± 4.52 to 3.90 ± 4.00 µg/kg for Gbêkê region, from 1.65 ± 1, 52 to 4.12 ± 4.57 µg/kg for Poro region, from 2.35 ± 2.33 to 8.71 ± 19.16 µg/kg for Hambol region, from 16.07 ± 17.45 to 32.31 ± 47.48 µg/kg for Indénié-Djuablin region and from 27.56 ± 51.44 to 37.11 ± 48.85 µg/kg for Gontougo region. With values varying from 0.10 ± 0.10 to 0.63 ± 1.21 µg/kg, maize grains, cobs and husks samples from Gbêkê, Hambol and Poro regions show the lowest concentrations of aflatoxin G2 unlike the other samples with concentrations ranging from 1.33 ± 1.89 to 3.35 ± 5.10 µg/kg. The total aflatoxin concentrations resulting from the sum of different aflatoxins differed significantly ($P < 0.05$) from maize form and region. The concentrations vary from 2.63 ± 2.36 to 60.79 ± 80.24 µg/kg for maize grains, from 7.04 ± 7.04 to 71.04 ± 91.59 µg/kg for maize cobs and from 17.66 ± 31.30 to 169.19 ± 150.13 µg/kg for maize husks (Table 4).

3.1.5 Concentrations of ochratoxin A in stored maize samples

Table 6 shows ochratoxin A (OTA) levels determined in the maize samples. All maize samples are contaminated regardless of the different regions. However, maize samples as grains and cobs from Gbêkê, Poro and Hambol regions show concentrations between 0.84 ± 0.78 and 2.61 ± 2.24 µg/kg, below the normative value set at 5 µg/kg. These samples represent 40% of the total samples conforming to the standard. With values varying from 16.75 ± 32.42 to 134.21 ± 77.24 µg/kg, maize husks samples differ significantly from other samples by contents greater value than to those recommended by European Union. Maize grains and cobs from Indénié-Djuablin and Gontougo regions have concentrations ranging from 5.58 ± 5.43 to 18.60 ± 18.16 higher than the normative value set at 5 µg/kg by the European Union.

3.1.6 Correlations between the parameters of the sanitary quality of the maize samples

Table 7 shows the existence of several significant positive correlations between mold loads and the mycotoxins levels. Indeed, an increase load of *Aspergillus flavus* strongly coincides with an increase in mycotoxin concentrations, r varying from 0.58 to 0.79. Likewise, the load of *Aspergillus versicolor* depends very significantly on the contents of mycotoxins (r ranging from 0.52 to 0.62). The level of aflatoxin B1 significantly influences the other mycotoxins (r varying from 0.71 to 0.95). Also, ochratoxin A concentration is proportional to those of the mold and the aflatoxin levels (r between 0.56 and 0.88).

3.1.7 Differentiation of maize samples in relation to the sanitary quality parameters studied

The principal components analysis was carried out using the F1 and F2 components which records an eigenvalue higher than 1, according to the Kaiser rule (Table 8). The projection of analysed variables in factorial design F1-F2 shows strong negative correlation between all the parameters studied (mold load, relative density of *Aspergillus* species, aflatoxin and ochratoxin A concentrations) with F1 factor (Fig. 1, A). Based on the projection of samples in the same design, they are organised in two groups. Group 1 is composed of two individuals presenting high levels of mold load, relative density of *Aspergillus* and mycotoxin level. It deals with maize husks coming from Indénié-Djuablin region. Group 2 includes individuals having low mold load, relative density of *Aspergillus* and mycotoxin level. It deals with all maize samples from Gbêkê, Poro, Hambol and Gontougo on the one hand and maize grains and cobs from Indénié-Djuablin on the other (Fig. 1, B).

3.2 Discussion

The results of the microbiological analyses indicate a significant level of contamination of the various forms of stored maize sample (grains, cobs and husks) in the five regions visited during this study. The flora of maize sample is composed of saprophytic germs which proliferate in parallel with an increase in the humidity level causing alterations. This high-water content could be a limiting factor for traditional maize storage. Indeed, it promotes the proliferation of microorganisms, capable, using their amylases, of hydrolysing starch and producing mycotoxins that are harmful to humans and animals. Similar remarks were noted by N'goran et al. [19] implying a high proportion of germs of hygienic interest in samples of maize flour marketed in nine communes of Abidjan. Microbiological analyses of maize samples show that all maize forms are predominantly contaminated by aerobic mesophilic germs regardless the region (of the order of 10^5 to 10^{11}). Maize cobs and husks samples are well above the standard which is 10^5 cfu/g recommended by the Codex Alimentarius. These microorganisms consist of pathogens and non-pathogenic germs for the most part not very demanding at the level nutritional [20]. Indeed, for this type of germs, temperature and humidity remain important criteria for their growth. These results are similar to those obtained by Ennadir et al. [21] which showed the large predominance of the aerobic mesophilic flora in the contamination of wheat flour from storage condition in Morocco. The count of thermo-tolerant coliforms indicates results well above the standard for almost all maize samples. The strong presence of these germs is justified by the ubiquitous nature of the latter. These bacteria, which are widespread in the environment and saprophytic in humans and warm-blooded animals, are found in maize after harvest and throughout the drying and storage period. Indeed, these germs are considered as hygiene indicators in the food manufacturing process [22]. Results of this study are in agreement with those of N'goran et al. [19] and N'guessan et al. [20] who enumerated these germs in the samples of maize flour collected in different markets of Abidjan with charges between 10^3 and 10^6 cfu/g. However, it should be noted that maize grain and cobs from Gbêkê, Poro and Hambol are free from faecal coliforms probably reflecting good post-harvest maize hygienic conditions practiced by producers in these regions. All maize samples analysed were free from pathogenic bacteria such as *Salmonella*, *S. aureus* and Sulfite-Reducing Anaerobes. This absence of pathogenic germs in maize stored could reflect the respect of good hygiene practices during storage by producers. Conservation of crops remains one of the key factors in a country's food security. Indeed, agricultural production is generally seasonal as consumer needs extend throughout the year. It is an art that requires the establishment of an adequate sanitary policy to spare populations from the risk of food shortages during the agricultural off-season. In this perspective, particular emphasis should be placed on the control of crop pests in stocks such as molds. Indeed, the damage caused by the latter can lead to financial losses, famines and risks of intoxication linked to the consumption of spoiled products [23,24]. Analysis of the results showed that all maize samples were contaminated with yeasts and molds. The highest loads were recorded on maize husks. This contamination could be due to a poor storage condition of maize. According to Tabuc [25], fungal contamination probably takes place before harvest, in the field, during drying and storage. In fact, molds have the property, under unfavorable conditions, of becoming spore-forming and of multiplying by germination when conditions become favorable. The most common spoilage fungi, and the most destructive of foods, belong

to the genera *Aspergillus*, *Penicillium* and *Fusarium*. Contamination of cereal grains by a multitude of molds, particularly in maize, has been documented in other studies [9,26,27,28], and results of this study also confirm this state of affairs. Five genera of molds (*Aspergillus*, *Fusarium*, *Penicillium*, *Mucor* and *Alternia*) were isolated and identified at varying percentages on all maize samples (grains, cobs, husks). The presence of fungal flora in maize samples can have serious consequences on the health of consumers. Indeed, these molds can produce mycotoxins which are toxic to humans and animals [29]. The frequency of isolation shows that the genus *Aspergillus* shows a greater number of species isolated on stored maize samples with a percentage varying between 40% and 70% with predominance on maize husks. The dominance of the genus *Aspergillus* in the contaminating flora of cereals has been reported in several studies [9, 10, 30, 31]. Furthermore, the incidence of *Aspergillus* results shows that *Aspergillus flavus* and *Aspergillus niger* are the fungi most present in maize samples. *Aspergillus* strains of the flavi and nigri section can be isolated from different farming systems, be it maize, rice or peanuts [32]. These are fungi that proliferate at high temperatures and support relatively low water activity. They are considered "storage fungi" although contamination frequently begins in fields [33]. Under optimal growth conditions, *Aspergillus* is able to produce a biologically significant amount of toxins within days. This same observation was made by Dedi et al. [9] on maize grains intended for the preparation of compound feed for poultry in Côte d'Ivoire. The frequencies of occurrence of these

Table 5. Aflatoxin concentrations in stored maize samples

Aflatoxins	Regions	Grains	Cobs	Husks
AFB1 (µg/kg)	Gbêkê	1.97 ± 2.70^{cB}	2.27 ± 2.40^{bB}	12.73 ± 26.26^{cA}
	Poro	0.80 ± 0.75^{cB}	2.53 ± 2.93^{bB}	18.28 ± 30.97^{cA}
	Hambol	1.31 ± 1.37^{cB}	2.40 ± 2.67^{bB}	55.41 ± 65.00^{bA}
	Indénié-Djuablin	9.30 ± 9.76^{bB}	32.22 ± 50.40^{aB}	130.31 ± 92.56^{aA}
	Gontougo	20.92 ± 27.63^{aA}	13.78 ± 24.46^{aA}	19.92 ± 32.50^{cA}
AFB2 (µg/kg)	Gbêkê	0.9 ± 0.28^{aA}	0.39 ± 0.37^{bA}	0.44 ± 0.42^{bA}
	Poro	0.10 ± 0.12^{aA}	0.28 ± 0.28^{bA}	0.33 ± 0.35^{bA}
	Hambol	0.19 ± 0.23^{aA}	0.34 ± 0.33^{bA}	0.56 ± 0.10^{bA}
	Indénié-Djuablin	0.59 ± 0.77^{aB}	3.21 ± 4.84^{aA}	3.26 ± 4.89^{aA}
	Gontougo	1.43 ± 2.04^{aA}	1.51 ± 2.57^{aA}	1.56 ± 2.62^{aA}
AFG1 (µg/kg)	Gbêkê	3.36 ± 4.52^{bA}	3.85 ± 3.95^{bA}	3.90 ± 4.00^{bA}
	Poro	1.65 ± 1.52^{bA}	4.07 ± 4.52^{bA}	4.12 ± 4.57^{bA}
	Hambol	2.35 ± 2.33^{bA}	3.96 ± 4.24^{bA}	8.71 ± 19.16^{bA}
	Indénié-Djuablin	16.07 ± 17.45^{aA}	32.31 ± 47.48^{aA}	31.37 ± 47.58^{aA}
	Gontougo	37.11 ± 48.85^{aA}	27.56 ± 51.44^{aA}	27.61 ± 51.49^{aA}
AFG2 (µg/kg)	Gbêkê	0.18 ± 0.26^{aA}	0.54 ± 0.57^{bA}	0.59 ± 0.62^{bA}
	Poro	0.10 ± 0.10^{aA}	0.50 ± 0.31^{bA}	0.55 ± 0.36^{bA}
	Hambol	0.16 ± 0.21^{aA}	0.52 ± 0.44^{bA}	0.63 ± 1.21^{bA}
	Indénié-Djuablin	0.51 ± 0.64^{aB}	3.30 ± 5.05^{aA}	3.35 ± 5.10^{aA}
	Gontougo	1.33 ± 1.89^{aA}	1.68 ± 2.68^{aA}	1.73 ± 2.73^{aA}
AFT (µg/kg)	Gbêkê	5.70 ± 7.68^{cB}	7.04 ± 7.04^{bB}	17.66 ± 31.30^{cA}
	Poro	2.63 ± 2.6^{cB}	7.39 ± 7.79^{bB}	23.28 ± 36.100^{cA}
	Hambol	4.01 ± 4.02^{cB}	7.22 ± 7.42^{bB}	65.31 ± 85.47^{bA}
	Indénié-Djuablin	26.46 ± 28.100^{bC}	71.04 ± 91.59^{aB}	169.19 ± 150.13^{aA}
	Gontougo	60.9 ± 80.24^{aA}	44.53 ± 80.88^{aA}	50.82 ± 89.34^{bA}

AFB1: Aflatoxin B1; AFB2: Aflatoxin B2; AFG1: Aflatoxin G1; AFG2: Aflatoxin G2; AFT: Total Aflatoxins; By columns and rows the averages with the same letters are statistically identical. The upper-case letters are representative of the lines and the lower-case letters are representative of the columns

Table 6. Ochratoxin A concentrations in stored maize samples

Regions	Grains	Cobs	Husks
Gbêkê	1.46 ± 2.14^{cB}	1.47 ± 1.89^{bB}	16.75 ± 32.42^{cA}
Poro	0.84 ± 0.78^{cC}	2.28 ± 2.86^{bB}	47.80 ± 88.13^{cA}
Hambol	1.69 ± 1.83^{cB}	2.61 ± 2.24^{bB}	68.40 ± 55.06^{bA}
Indénié-Djuablin	5.58 ± 5.43^{bC}	18.60 ± 18.16^{aB}	134.21 ± 77.24^{Aa}
Gontougo	11.35 ± 11.13^{aB}	13.11 ± 18.63^{aB}	45.51 ± 44.38^{Ca}

By columns and rows, the averages with the same letters are statistically identical. The upper-case letters are representative of the lines and the lower-case letters are representative of the columns.

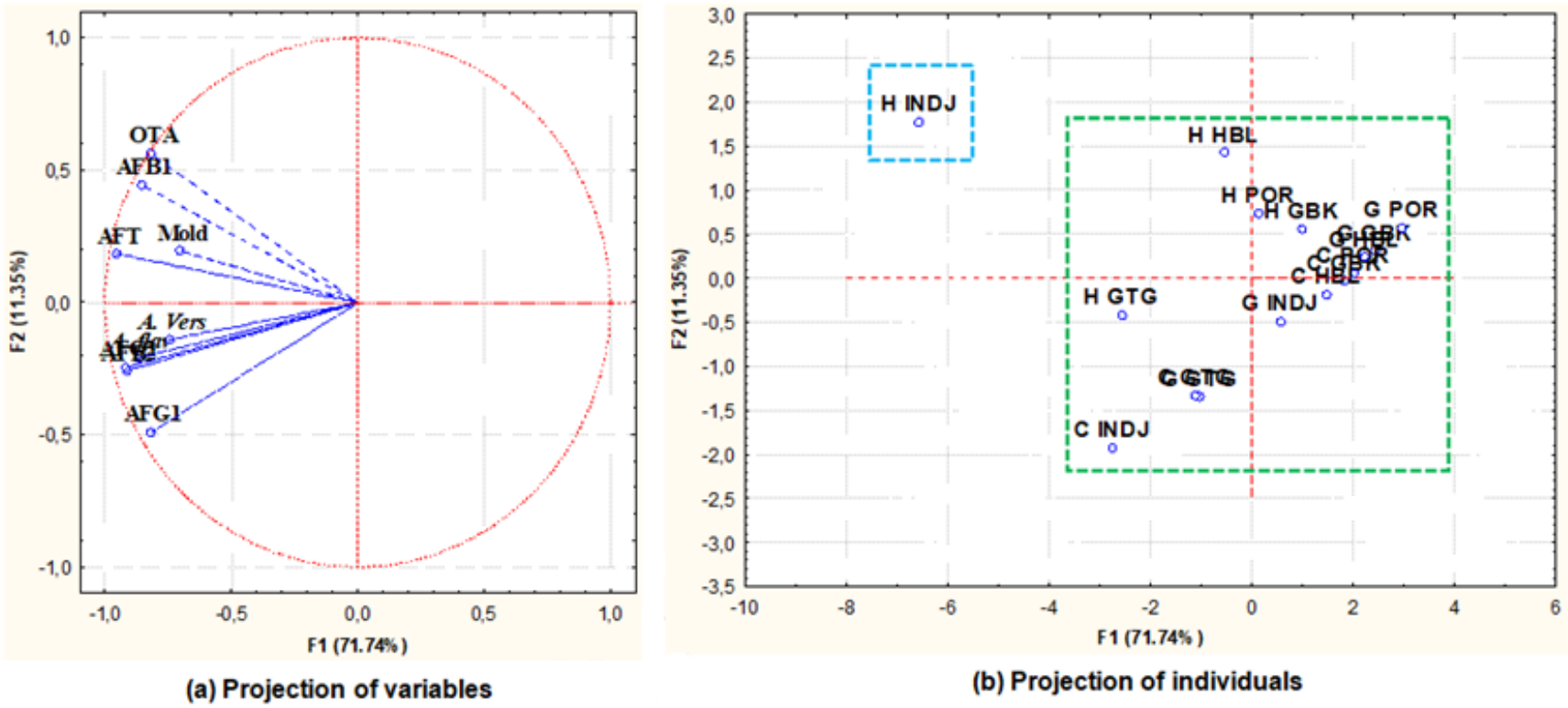

Fig. 1. Projection of the sanitary parameters (a) and individuals (b) of maize grains, cobs and husks in the factorial design 1-2 of the principal component analysis

Mold, mold loads; A. fla, relative density of A. flavus; A. vers, relative density of A. versicolor; AFB1, aflatoxin B1 content; AFB2, aflatoxin B2 content; AFG1, aflatoxin G1 content; AFG2, aflatoxin G2 content; AFT, total aflatoxin content; OTA, ochratoxin A content; GBK, Gbêkê; POR, Poro; HBL, Hambol; INDJ, Indénié-Djuablin; GTG, Gontougo; G, Grains; C, Cobs; H, Husks.

Table 7. Correlation matrix between sanitary parameters of maize samples

	Mold	*A. fla*	*A. Ver*	AFB1	AFB2	AFG1	AFG2	AFT	OTA
Mold	1.00								
A. fla	0.71	1.00							
A. Ver	0.47	0.69	1.00						
AFB1	0.53	0.58	0.52	1.00					
AFB2	0.50	0.76	0.60	0.71	1.00				
AFG1	0.47	0.78	0.62	0.50	0.86	1.00			
AFG2	0.53	0.79	0.61	0.71	0.99	0.83	1.00		
AFT	0.58	0.72	0.62	0.96	0.86	0.73	0.85	1.00	
OTA	0.65	0.60	0.56	0.95	0.59	0.39	0.60	0.88	1.00

In bold, significant correlation values. Mold: mold loads; A. fla: relative density of A. flavus; A. ver: relative density of A. versicolor; AFB1, aflatoxin B1 content; AFB2, aflatoxin B2 content; AFG1, aflatoxin G1 content; AFG2, aflatoxin G2 content; AFT, total aflatoxin content; OTA, ochratoxin A content

Table 8. Matrix of the eigenvalues of the factors resulting from the principal components analysis and correlation with the parameters of the sanitary quality of maize samples according to the regions

	Axis 1	Axis 2
Contribution of the axis		
Proper value	6.46	1.02
Variability (%)	71.74	11.35
% cumulated	71.74	83.09
Definition of axis and factor weights		
Molds	-0.70	0.19
A. flavus	-0.87	-0.21
A. versicolor	-0.74	-0.14
AFB1	-0.86	0.45
AFB2	-0.91	-0.26
AFG1	-0.82	-0.49
AFG2	-0.92	-0.25
AFT	-0.95	0.18
OTA	-0.82	0.56

In bold, significant correlation values

filamentous fungi, according to these authors, were 35.47% and 17.78% respectively for *A. flavus* and *A. niger*. In addition, the work of Dubale et al. [26] showed that these two species *A. flavus* and *A. niger* were the most present among the fungal flora isolated on maize grains stored in traditional structures such as Gombisa and polypropylene bags in Ethiopia with a frequency of 90% and 51%. According to Klich [34], *Aspergillus flavus* is an opportunistic pathogen of crops and has a cosmopolitan distribution. The presence of *Aspergillus flavus* in maize stored, which is either intended for human and animal consumption, will pose health concerns given that this fungus is likely to produce a dreaded toxin of aflatoxin in maize before and after harvest, in almost all stored foods and the latter is a potent carcinogen that is highly regulated in most countries [24]. *Aspergillus fumigatus*, *A. terrus*, *A. glaucus* and *A. versicolor*, also present on maize sample, are common contaminants on various substrates and frequently isolated in nuts and sun-dried products [35] as is the case with the maize samples analysed in our study. Some strains of *A. niger*, *A. versicolor* and *Penicillium* are producers of ochratoxin A (OTA), a carcinogen classified by the International Agency for Research on Cancer [10].

All analyzed maize samples in this study were also contaminated by *Fusarium* sp, *Mucor* sp and *Alternia* sp. The presence of these fungi may lead also to the mycotoxins formation, which are secondary fungal toxic metabolites to humans and animals, causing disorders like cancer, immune suppression or endocrine disruption [36]. Mycotoxins such as aflatoxins B1, B2, G1, G2 and OTA were detected in the different forms of maize (grains, cobs, husks) from the five regions. The presence of these toxins in maize samples could be explained by the fact that the maize storage conditions favored the growth of the molds responsible for the production of these toxins. In addition, the detection of these mycotoxins in maize is a public health concern in places, where this cereal is consumed as a staple food and is also used as an ingredient in

animal feed. In fact, in Côte d'Ivoire, as in other developing countries, maize is one of the staple foods of the population. It is eaten in the form of fresh boiled (kaba-belégué) or simply braised, porridge or baked pancakes made from maize flours, “kabatoh” or “akassa Boulle” [20]. Sixty per cent of maize samples, mostly maize husks, showed aflatoxin B1 and OTA concentrations above the Maximum Authorized Limit set at 5 µg/kg [37]. These results are in agreement with the work carried out by Sangare-Tigori et al. [38] who reported a high level of aflatoxin B1 and ochratoxin A contamination on maize from the Abidjan markets with concentrations ranging from <1.5 µg/kg to 20 µg/kg. Substantially equal results were also reported by Worku et al. [39]. These authors detected concentrations between 6.3 - 150 µg/kg and 2 - 186.5 µg/kg for total aflatoxins and ochratoxin A in maize samples stored in five producing regions of Ethiopia. This study is similar to Jager et al. [40] in Brazil who reported a consumption of foods contaminated with aflatoxins. The determination of aflatoxins in maize showed 42% positive samples (ranging from 0.05 to 8.3 µg/kg), with a greater incidence in maize flour. Similar results on the presence of OTA in breakfast cereals have been obtained in Canada. To this end, 30% of the samples analysed were contaminated, with low contamination levels ranging from 0.01 to 0.38 µg/kg [41].

However, low concentrations of aflatoxin B1 (0.80 to 2.53 µg / kg) and OTA (0.84 to 2.61 µg/kg) were detected in maize grains and cobs from Gbêkê, Poro and Hambol regions, representing 40% compliance of maize samples with respect to the normative value. These results are similar to those obtained by Fofana-Diomandé et al. [24]. These authors found aflatoxin B1 contents of 0.92 µg/kg in maize flours collected from the North-West region of Côte d'Ivoire. This low value can encourage maize producers in these regions to better promote good production and storage methods. This same observation is also made for total aflatoxins representing the total sum of aflatoxins (B1, B2, G1 and G2). The lowest concentrations of total aflatoxins, below the reference value which is 10 µg/kg [37], were recorded on all maize grains and cobs samples from Gbêkê, Poro and Hambol regions with levels varying from 1.75 to 4.06 µg/kg.

4. CONCLUSION

The evaluation of the microbiological parameters revealed an absence of pathogenic microorganisms in the maize samples regardless to the region. However, significant contamination levels of maize hygiene and spoilage indicator germs were detected in almost all of the maize samples. With regard to the quality criteria specified, the husks are not of good microbiological quality. Regarding the isolation and identification of molds, the genus *Aspergillus* was the most prominent with an occurrence of three species which are *Aspergillus flavus*, *Aspergillus niger* and *Aspergillus versicolor* potential producers of mycotoxins. Mycotoxin contaminants revealed the presence of aflatoxins B1, B2, G1, G2 and ochratoxin A in all forms of maize. With the exception of maize grains and cobs from the regions of Gbêkê, Poro and Hambol, all other samples showed concentrations above the Maximum Allowable Limit for aflatoxins B1, total aflatoxins and ochratoxin A as regulated by the European Union.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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