

**ASSESSMENT OF THE HEALTH QUALITY RELATED TO THE PRESENCE OF
OCHRATOXIN A, FUMONISIN B1 AND ZEARALENONE IN MAIZE (*ZEA MAYS*
L.) PRODUCED IN CÔTE D'IVOIRE**

Abstract

Aims : The aim of this work is to take stock of the level of ochratoxin A (OTA), fumonisin B1 (FB1) and zearalenone (ZEA) contamination in maize produced in Cote d'Ivoire in order to help improve its quality.

Study Design : Maize samples (375) were taken in five producing regions (Poro, Hambol, Gontougo, Gbêkê, Indénié-Djuablin).

Place and Duration of Study : the collection was carried out on maize in grain, on the cob and in spathe from February 2016 to January 2017. Then, the analyzes were carried out at the Biotechnology Laboratory, Agriculture and Development of Biological Resources of the Félix HOUPHOUËT-BOIGNY University.

Methodology : The determination of ochratoxin A, fumonisin B1 and zearalenone was carried out according to the methods of regulation No. 401/2006/EC, AFNOR, Miraglia and Brera.

Results : The results indicate the presence of ochratoxin A, fumonisin B1 and zearalenone in all forms of maize (grains, cob, spathes) and the five regions visited. However, the average concentrations of fumonisin B1 and zearalenone are respectively 27.46 µg/kg-1999.22 µg/kg and 8.48 µg/kg-341.84 µg/kg and are lower than the prescribed reference standards (2000 µg/kg ; 500 µg/kg). For ochratoxin A, the average concentrations vary from 0.83 µg/kg to 14.38 µg/kg ; 1.92 µg/kg to 18.60 µg/kg and 2.21 µg/kg to 134.89 µg/kg respectively for grains, cob and spathes.

Samples from the Regions of Poro, Gbêkê and Hambol have mean concentrations below the maximum reference limit of 5µg/kg. Thus, variability in the sanitary quality of maize was demonstrated from one region to another, regardless of the form of the maize. Based on the principal component analysis, spathes represent the form of maize most prone to high contamination regardless of mycotoxin and région.

Conclusion : The searching alternative storage methods and the right form of maize storage could be a solution to the high mycotoxin contamination of marketed maize.

Keywords : Maize, ochratoxin A, fumonisin B1, zearalenone, Côte d'Ivoire.

1. INTRODUCTION

In Cote d'Ivoire, maize ranks seventh in food crop production and second in cereal production after rice [1-2]. It is a staple food for a large part of the Ivorian population. Unfortunately, the efforts to produce this foods tuff are far from meeting the needs of the said population [3-5]. Added to this are the conservation constraints which most often reduce the quality and quantity of productions [3].

In fact, maize is subject to several biotic and abiotic constraints during storage, which make it difficult to preserve [6-7]. Among these constraints, biotic factors pose the greatest threat to deteriorating maize quality [8]. One of the main biotic factors is fungal contamination because it can alter the marketability, health and nutritional qualities of the grains. Fungal contamination can occur both in the field and during storage. Thus, from germination to harvest, maize is attacked by insects which create entry points that favorits contamination by phytopathogenic molds. In addition, when post-harvest activities, in particular drying and storage conditions, are inefficient there is an amplification of insect activity and the development of molds responsible for the production of mycotoxins in stocks [8-10].

Produced mainly by the genera *Aspergillus*, *Penicillium* and *Fusarium*, ochratoxins, fumonisins and zearalenone are groups of mycotoxins that affect maize [11-13]. It is quite obvious that two or more mycotoxins can coexist in the same food stuff increasing the health risk for consumers. Indeed, the co-contamination of crops by these toxins is a major concern due to the combined risks of exposure to the toxic effects of these toxins [13-14].

A survey carried out in three major maize producing regions in Côte d'Ivoire revealed that the majority of maize producers (97%) use traditional storage methods. This study also indicates that these methods expose stored grains to attack by mycotoxin-producing molds such as ochratoxin A, fumonisin B1, and zearalenone [15]. This study was initiated to assess the level of ochratoxin A, fumonisin B1 and zearalenone contamination in maize produced and marketed in Côte d'Ivoire in order, on the one hand, to make reliable data available to populations and decision-makers. On the other hand, it could help improve the quality of maize produced and marketed in the Côte d'Ivoire.

2. MATERIAL AND METHODS

2.1. Material

2.1.1. Biological material

The biological material consists of grain, cob and spathe of dried maize from the North, East, Center, Center-North and North-East areas of Côte d'Ivoire.

2.1.2. Chemical materiel

Reagents (acetonitrile and methanol) and standards (OTA, FB1 and ZEA) were used for the study. Reagents were purchased from Carlo Erba (Spain) with analytical grade, while standards OTA, FB1 and ZEA were provided from Sigma (Sigma, St Louis, MO, USA).

2.1.2. Study sites

The samples were collected from the régions of Gbêkê (Center), Poro (North), Hambol (Center-North), Indénié-Djuablin (East) and Gountougo (Northeast). The specifics of these Regions were given by Bamba et al. [16].

2.2. Methods

2.2.1. Sampling

The strategy adopted has consisted of two phases. The first phase consisted of identifying regions where maize is the main food crop. In each region, meetings were organized with the chiefdom to present the study. Then, samples of 1 kg of maize (in spathes, cobs and grains) were taken from the stocks of the producers constituting the second phase. The collection of samples were taken from February 2016 to January 2017. A total of 375 samples were collected for each form of maize (125 on grains, 125 on cobs and 125 on spathes, Table 1). Then, the received samples were sent to the laboratory in order to determine contamination level in ochratoxin A, fumonisin B1 and zearalenone.

Table 1 : Number of samples collected according to maize variety and Region

Regions	Grain	Cob	Spathe	Total
Gbêkê	25	25	25	75
Poro	25	25	25	75
Hambol	25	25	25	75
Indénié-Djuablin	25	25	25	75
Gontougo	25	25	25	75
Total	125	125	125	375

2.2.2. Determination of water activity of maize

The water activity was measured with a HygroLab Rotronic hygrometer according to indications of McCormick (1995). Prior to assays, the hygrometer was calibrated with specific water activity salts. Then, samples of 5 g of ground maize were put into standard dry empty containers for the Aw analysis. The water activity digital measures were directly displayed by the hygrometer [17].

2.2.3. Determination of mycotoxins

Validation of the ochratoxin A, fumonisin B1 and zearalenone method analysis

The method validation was conducted using the method of the French Association for Standardization [18]. This procedure includes the study of the linearity of the calibration range (0 µg/L et 2.0 µg/L), the determination of the limits of detection and quantification, the calculation of the coefficient of variation for the tests of repeatability and reproducibility, and the calculation of the recovery percentage for testing accuracy (4.5 ng/kg, 10 ng/kg et 20 ng/kg). The reference material was used to compare the concentration of OTA, FB1 et ZEA obtained at the certified value.

Extraction of ochratoxin A

The entire sample was crushed in a hammer mill to obtain a homogeneous fine grind. In a Nalgene jar containing 15 g of homogenate, 150 mL of aqueous methanol-bicarbonate 1% (m/v; 50:50) were added. The mixture was homogenized by Ultra-Turax for 3 minutes and the homogenate was centrifuged at 5000 rpm for 5 minutes at 4°C. The supernatant was filtered through filter paper into tubes of 25 mL. To 11 mL of filtrate were added 11 mL of saline phosphate buffered (PBS) at pH 7.3. Immunoaffinity columns brand Ochraprep 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 solvent (methanol/acetic acid ; 98:2; v/v) at a flow rate of 5 mL/minute. The resulting sample was packed in a chromatographic tube and the analysis for OTA was made by HPLC using the European community regulation [19].

Extraction of fumonisin B1

Twenty-five grams of maize sample were extracted with 50 mL of water blending for 2 min with a hammer mill blender. At five grams of ground maize, 25 mg of NaCl were added and the mixture was shaken on a horizontal mechanical shaker for 120 minutes at 300 rpm, and then centrifuged for 15 minutes at 2500 g. The supernatant was recovered and degreased by 4 mL of hexane. The organic phases were removed by centrifugation for 5 minutes at 2500 g.

The aqueous layer was recovered and diluted with 16 mL of phosphate buffered saline (PBS) at pH 7.3, filtered through Whatman No. 4 filter paper and then applied to a column immunoaffinity Fumoniprep (R BiopharmRhône Ltd, Glasgow, Scotland) at a flow rate of 1–2 drops/s. The column was washed with 10 mL of the same buffer to 1-2 drops/s for removal of residues. Fumonisin B1 was eluted with 1.5 mL of methanol (HPLC grade) and then 1.5 mL of water. The eluate was collected and evaporated, protected from light in a nitrogen stream. The dry extract was taken up in 200 µL acetonitrile/water (50: 50, v/v) and then sonicated for 5 minutes. Then, 50 µL of extract was diluted into 50 µL of a solution of ortho-phthalaldehyde (OPA 40 mg, 1 mL methanol, 5 mL of 0.1 M sodium tetraborate and 50 µL of 2- mercaptoethanol). The resulting sample was packed in a chromatographic tube and the analysis of FB1 was made by HPLC using AFNOR methods [20].

Extraction of Zearalenone

Twenty-five grams of maize sample were extracted with 50 mL of 125 mL of acetonitrile : water (94:31) blending for 2 min with a hammer mill blender. After filtration through Whatman No 4 filter paper, 20 mL of the filtrate were diluted with 80 mL of double distilled water. Then, 25 mL of the diluted filtrate was applied to an immunoaffinity column (Easi-Extract® zearalenone, R-Biopharm Rhône Ltd, Glasgow) containing a monoclonal antibody specific for the zearalenone. The column was washed with 10 mL of double distilled water. Zearalenone was eluted by applying 1.5 mL of methanol. The eluate was diluted with 1.5 mL of bidistilled water and mixed by vortexing. The resulting sample was packed in a chromatographic tube and the analysis of ZEA was made by HPLC using the method of AOAC [21] and Miraglia and Brera [22].

OTA, FB1 and ZEN quantification

Quantification of OTA, FB1 and ZEA contents was achieved with high performance liquid chromatography column, using a Shimadzu liquid chromatograph (Kyoto, Japan) fitted with fluorescence detector. The operating conditions are described in Table 2.

Table 2 : HPLC analytical conditions

ITEM	Ochratoxin A	Fumonisin B1	Zearalenone
Pre-column	Shim-pack GVP-ODS 10 x 4.6 mm		
Column	Shim-pack GVP-ODS, 250 mm x 4.6 mm		
	Fluorescence,	Fluorescence,	Fluorescence,
Detector	$\lambda_{\text{excitation}}$: 330 nm, $\lambda_{\text{émission}}$: 460 nm	$\lambda_{\text{excitation}}$: 335 nm, $\lambda_{\text{émission}}$: 440 nm	$\lambda_{\text{excitation}}$: 274 nm, $\lambda_{\text{émission}}$: 440 nm
Mobile phase	Acetonitrile/Water/ Aceticacid (99/99/2)	Acetonitrile/Water (50/50)	Acetonitrile/Water/Methanol (46/46/8)
Inject volume	100 μ l	100 μ l	100 μ l
Flow rate	1 mL/min in isocratic		
Column temperature	40 °C		
Rising solvent	Acetonitrile		
Analysis duration	12 minutes	6 minutes	9.5 minutes

2.2.4. Statistical analysis

The homogeneity of the means was assessed using the Student-Newman-Keuls test at risk 0.05 using SPSS (Statistical Product and Service Solutions) version 20.0 software. The percentages of non-compliant samples made it possible to assess the occurrence of OTA, FB1 and ZEA in the various regions. Finally, the correlation between the data and the samples was estimated using principal component analysis (PCA), using the STATISTICA 7.1 software.

3. RESULTS AND DISCUSSION

3.1. Results

3.1.1. Water activity of maize

The water activity values are shown in Table 3. They are all greater than 0.65 and range from 0.78±0.01 to 0.93±0.03 for maize grains. They vary from 0.81±0.02 to 0.88±0.05 for the cobs. Regarding the values for the spathes, they are between 0.84±0.05 and 0.93±0.05. Statistical analysis revealed the presence of a significant difference (p=0.001) between the means. Samples from the Gbêkê and Poro Regions show low water activity while those from Indénié-Djuablin and Gontougo show high values.

Table 3 : Average values of the water activity of the maize samples in the different regions

Average water activity					
Regions	Grains	Cobs	Spathes	F-value	P-value
Gbêkê	0.80±0.02 ^{Bb}	0.81±0.02 ^{Bc}	0.84±0.05 ^{Ac}	5.97	<0.001
Poro	0.78±0.01 ^{Cc}	0.81±0.03 ^{Bc}	0.85±0.05 ^{Ac}	19.53	<0.001
Hambol	0.81±0.01 ^{Cb}	0.84±0.02 ^{Bb}	0.89±0.06 ^{Ab}	29.29	<0.001
Indénié-Djuablin	0.93±0.03 ^{Aa}	0.88±0.06 ^{Ba}	0.93±0.05 ^{Aa}	9.91	<0.001
Gontougo	0.92±0.05 ^{Aa}	0.85±0.05 ^{Bb}	0.84±0.04 ^{Bc}	15.84	<0.001
F-value	95.84	95.84	13.22	nd	nd
P-value	<0.001	<0.001	<0.001)	nd	nd

nd : not determined

By column and row, the means bearing the same letters are statistically identical. The lower case letters are representative of the columns and the upper case letters represent the lines.

3.1.2. Validation of determination method of OTA, FB1 and ZEA

The Pearson coefficients (R^2) determined for the linearity vary between 0.98 and 0.99. The detection limits for OTA, FB1 and ZEA are 5 ng/kg, 12.5 ng/kg and 2.6 ng/kg, respectively. Regarding the limits of quantification, the values are 20 ng/kg, 50.5 ng/kg and 7.43 ng/kg respectively for OTA, FB1 and ZEA.

The coefficients of variation vary between 0.26% and 3.75% for the repeatability tests and between 0.89% and 5.67% for the reproducibility tests. The extraction yields obtained are $86.92\pm 0.39\%$, $82.90\pm 2.19\%$ and $93.20\pm 4.21\%$ respectively for OTA, FB1 and ZEA (Table 4).

Table 4 : Results of the validation of the method for determination of OTA, FB1 and ZEA

Designation	OTA	FB1	ZEA
Linearity (R^2)	0.99	0.98	0.99
Limit of detection (LOD, ng/kg)	5	12.5	2.6
Limit of quantification (LOQ, ng/kg)	20	50.5	7.43
Repeatability (CV, %)	0.26 ± 0.00	3.75 ± 0.25	1.34 ± 0.00
Reproducibility (CV, %)	5.67 ± 0.45	4.21 ± 0.23	0.91 ± 0.10
Extraction efficiency (RE, %)	86.92 ± 0.39	82.90 ± 2.19	93.20 ± 4.21

3.1.3. OTA concentrations in samples

The contamination levels of ochratoxin A, of the different forms of maize, are reported in Table 5.

OTA concentrations are between $0.83\pm 0.07 \mu\text{g/kg}$ and $14.38\pm 2.22 \mu\text{g/kg}$ for maize grains. The Fischer test indicates the presence of a significant difference ($p=0.001$) between the contents. Low levels of OTA have been detected in the Regions of Poro, Gbêkê and Hambol. These contents are less than $5\mu\text{g/kg}$, maximum reference limit. Levels above this norm are observed at Gontougo and Indénié-Djuablin with a contamination frequency of 60% and 32% respectively.

Concerning the cobs, the concentrations obtained vary between $1.92\pm 0.64 \mu\text{g/kg}$ and $18.60\pm 2.16 \mu\text{g/kg}$. Statistical analysis revealed a significant difference ($p = 0.001$) between the means.

Average OTA concentrations below the maximum reference limit are observed at Gbêkê, Poro and Hambol. The proportions of samples with OTA contents greater than 5 µg/kg are 60% and 32% respectively for the localities of Indénié-Djuablin and Gontougo.

As for the spathes, they have average OTA contents higher than 5 µg/kg except those coming from the Region of Gbêkê. These average concentrations vary from 2.21±0.84 µg/kg to 134.89±9.10 µg/kg. The analysis of variance indicated the presence of a significant difference ($p = 0.001$) between the rates. The low and high concentrations are obtained respectively at Gbêkê and Indénié-Djuablin. In addition, concentrations above 5µg / kg have been determined in all areas and the proportions vary between 20% and 96%.

3.1.4. Fumonisin B1 content in samples

The fumonisin B1 contents and the percentages of samples not conforming to the standard are given in table 6. A clear variability of the average concentrations is observed as well between the forms of maize as the regions. The average concentrations are all lower than 2000 µg/kg whatever the région and the type of maize. They vary between 27.46±2.70 µg/kg and 1023.73±28.50µg/kg, 153.43±19.79 µg/kg and 1206.46±20.00 µg/kg, 476.21±13.03 µg/kg and 1999.22±24.03 µg/kg respectively for grains, Cobs and spathes.

According to statistical analysis, the Region of Indénié-Djuablin has high levels of FB1 regardless of the maize type, while the Regions of Poro and Gbêkê have low levels. On the other hand, the proportions of maize Cobs samples, which do not comply with the standard, vary from 8% to 28% and the regions concerned are Hambol, Indénié-Djuablin and Gontougo. Regarding maize in spathe, the proportions of non-compliance vary between 12% and 32% and all Regions are concerned.

Table 5 : Average concentrations of OTA and proportion of non-compliant samples with the standard according to the Regions and type of maize

OTA CONCENTRATIONS ($\mu\text{g}/\text{kg}$)					
Regions	Grains	Cobs	Spathes	F-value	P-value
Gbêkê	0.98±0.084 ^{Bd}	1.99±0.77 ^{Ac}	2.21±0.84 ^{Ae}	6.11	< 0.003
Poro	0.83±0.078 ^{Cd}	2.16±0.83 ^{Bc}	9.46±2.43 ^{Ad}	26.02	< 0.001
Hambol	1.89±0.74 ^{Bc}	1.92±0.64 ^{Bc}	73.22±7.15 ^{Ab}	50.42	< 0.001
Indénié-Djuablin	5.78±1.76 ^{Cb}	18.60±2.16 ^{Ba}	134.89±9.10 ^{Aa}	61.91	< 0.001
Gontougo	14.38±2.22 ^{Ba}	10.99±1.67 ^{Cb}	25.35±4.79 ^{Ac}	7.78	< 0.001
F-value	317.25	30.76	35.01	nd	nd
P-value	< 0.001	< 0.001	< 0.001	nd	nd
NON-COMPLIANT SAMPLES WITH THE STANDARD (%)					
Regions	Grains	Cobs	Spathes		
Gbêkê	0	0	20		
Poro	0	0	60		
Hambol	0	0	96		
Indénié-Djuablin	32	60	96		
Gontougo	60	32	92		
MAC ($\mu\text{g}/\text{kg}$)		5			

nd : not determined ; **MAC**: Maximum Authorized Concentration

By column and row, the means bearing the same letters are statistically identical. The lower case letters are representative of the columns and the upper case letters represent the lines.

Table 6 : Average fumonisin B1 concentrations and proportions of non-compliant samples

FB1 CONCENTRATIONS ($\mu\text{g}/\text{kg}$)					
Regions	Grains	Cobs	Spathes	F-value	P-value
Gbêkê	172.46 \pm 10.96 ^{cB}	153.43 \pm 19.79 ^{eB}	476.21 \pm 13.03 ^{eA}	2.51	0.001
Poro	27.46 \pm 2.70 ^{eC}	374.98 \pm 14.00 ^{dB}	639.60 \pm 16.20 ^{dA}	7.49	<0.001
Hambol	136.28 \pm 18.19 ^{dC}	488.22 \pm 14.05 ^{cB}	1226.26 \pm 19.44 ^{bA}	15.61	<0.001
Indénié-Djuablin	1023.73 \pm 28.50 ^{aB}	1206.46 \pm 20.00 ^{aB}	1999.22 \pm 24.03 ^{aA}	7.42	<0.001
Gontougo	802.73 \pm 20.50 ^{bB}	710.39 \pm 11.75 ^{bB}	1177.82 \pm 18.31 ^{cA}	2.96	<0.001
F-value	26.18	5.30	15.15	nd	nd
P-value	< 0.001	< 0.001	< 0.001	nd	nd
NON-COMPLIANT SAMPLES WITH THE STANDARD (%)					
Regions	Grains	Cobs	Spathes		
Gbêkê	0	0	12		
Poro	0	0	12		
Hambol	0	8	12		
Indénié-Djuablin	0	28	32		
Gontougo	0	12	16		
MAC ($\mu\text{g}/\text{kg}$)		2000			

nd : not determined ; **MAC**: Maximum Authorized Concentration

By column and row, the means bearing the same letters are statistically identical. The lower case letters are representative of the columns and the upper case letters represent the lines.

3.1.5. Zearalenone content in samples

Average zearalenone concentrations are all below 500 µg / kg, the maximum reference limit. They are between 8.48±0.25 µg/kg and 173.10±7.75 µg/kg, 38.61±4.02 µg/kg and 234.87±12.05 µg/kg, 94, 54±5.50 µg/kg and 341.84±9.15 µg/kg respectively for grains, Cobs and spathes (Table 7). Statistical analysis indicates that the high average concentrations are obtained in the Regions of Poro (grains) and Gbêkê (cobs, spathes) while the strongones are obtained in Gontougo (grains) and Indénié-Djuablin (Cobs, spathes). All the samples taken have concentrations conform to the standard of 500 µg/kg except 12% of samples of maize in Cobs and 8% of samples of maize in spathes from Indénié-Djuablin (Table 7).

3.1.6. Variability of contamination of different forms of maize

Figure 1 shows that analysis of F1 and F2 factors of main components reveal 99.34% of total variability of studied parameters. The F1 factor with a proper value of 2.74 expresses 91.34% of total variability while F2 factor with proper value of 0.24 reveals 8.00%. The projection of analysed variables in factorial design F1-F2 shows strong negative correlation between ochratoxin A, zearalenone and fumonisin B1 content of maize with the F1 factor (Figure 1). Based on the projection of samples in the same design, they are organised in two groups. Group 1 is composed of four individuals presenting high ochratoxine A, zearalenone and fumonisin B1 contents. It deals with spathes and cobs maize coming from Hambol, Indénié-Djuablin and Gontougo.

Table 7 : Average concentrations of ZEA and proportion of non-compliant samples with the standard according to the Regions and type of maize

ZEA CONCENTRATIONS ($\mu\text{g}/\text{kg}$)					
Regions	Grains	Cobs	Spathes	F-value	P-value
Gbêkê	23.00 \pm 2.25 ^{cC}	38.61 \pm 4.02 ^{eB}	94.54 \pm 5.50 ^{eA}	4.09	<0.001
Poro	8.48 \pm 0.25 ^{dC}	86.68 \pm 7.17 ^{dB}	120.09 \pm 10.02 ^{dA}	7.50	<0.001
Hambol	22.81 \pm 1.75 ^{cC}	115.11 \pm 10.02 ^{cB}	255.70 \pm 8.30 ^{bA}	19.60	<0.001
Indénié-Djuablin	145.95 \pm 8.50 ^{bB}	234.87 \pm 12.05 ^{aB}	341.84 \pm 9.15 ^{aB}	8.66	<0.001
Gontougo	173.10 \pm 7.75 ^{aA}	165.10 \pm 9.75 ^{bA}	226.07 \pm 8.20 ^{cB}	5.66	<0.001
F-value	22.56	5.46	15.75	nd	nd
P-value	< 0.001	< 0.001	< 0.001	nd	nd
NON-COMPLIANT SAMPLES WITH THE STANDARD (%)					
Regions	Grains	Cobs	Spathes		
Gbêkê	0	0	0		
Poro	0	0	0		
Hambol	0	0	0		
Indénié-Djuablin	0	12	8		
Gontougo	0	0	0		
MAC ($\mu\text{g}/\text{kg}$)		500			

nd : not determined ; **MAC**: Maximum Authorized Concentration

By column and row, the means bearing the same letters are statistically identical. The lower case letters are representative of the columns and the upper case letters represent the lines.

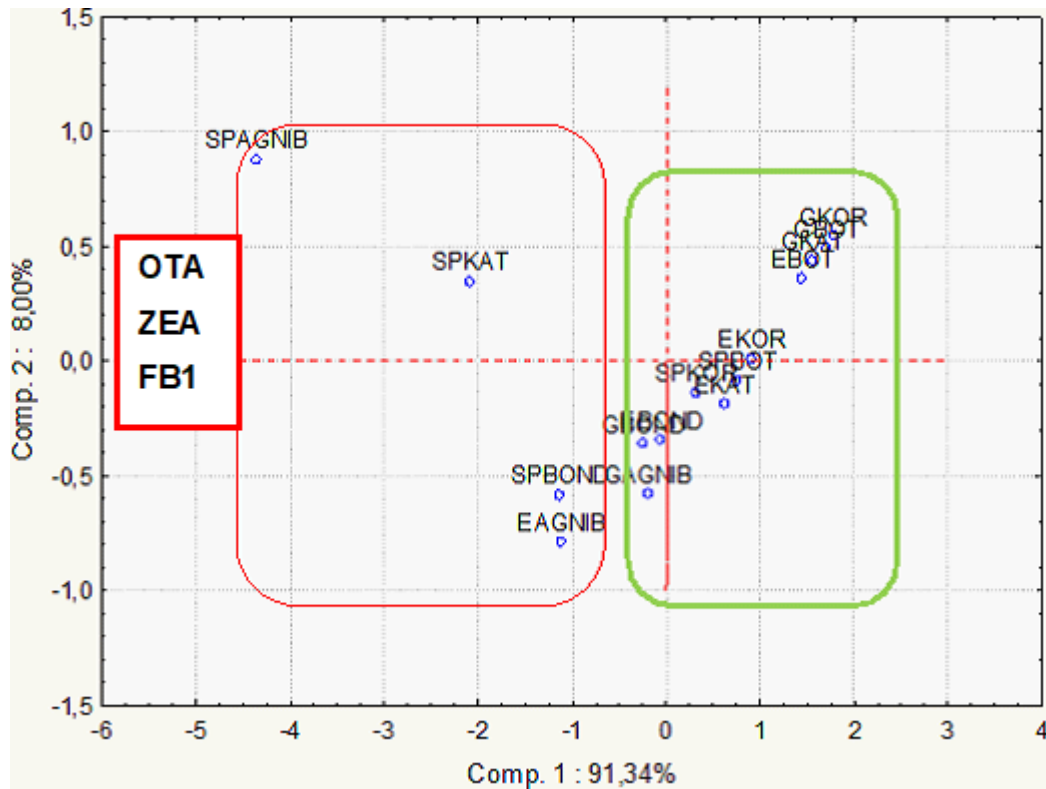


Figure. 1. Projection of ochratoxin A, zearalenone, and fumonisin B1 content and the individuals of grains, epis and spathes of maize in factorial plan 1-2 of the analysis of main components

BOT : Gbêkê ; KOR : Poro ; KAT : Hambol ; AGN : Indénié-Djuablin ; BON : Gontougo ; G : Grains ; E : Cobs ; SP : Spathes ; OTA : Ochratoxin A ; ZEA : Zearalenone ; FB1 : Fumonisin B1

3.2. Discussion

The average values of the water activity are higher than 0.65 whatever the region and the form of corn. This parameter refers to the availability of water in the food. According to Adebajo and *al.*[23], whatever the nature of the food, no microorganism can develop at a water activity below 0.65. On the other hand, according to the AFSSA [13] and the Codex Alimentarius [24], toxigenic molds can develop in all climates, on all solid or liquid supports as soon as there are nutrients, humidity and water activity greater than 0.65. Because the availability of water is one of the important factors in the growth of mold and the production of mycotoxins.

Thus these strong water activities, observed in the different forms of maize and production regions, could be a source of contamination of the maize in ochratoxin A, fumonisin B1 and zearalenone.

The validation of the methods made it possible to demonstrate the reliability of the different methods for determining ochratoxin A, fumonisin B1 and zearalenone in maize samples. All the Pearson coefficients (R^2) determined are close to 1, which indicates a good correlation between the quantity of the analyte and the response of the apparatus [25-26]. The detection and quantification limits are 2.6 ng/kg-12.5 ng/kg and 7.43 ng/kg-50.5 ng/kg respectively, which indicates good sensitivity of the dosing device used. In addition, the coefficients of variation determined for repeatability and reproducibility are all less than 6% and all the extraction rates are greater than 82% regardless of the mycotoxin considered. This situation indicates that the extraction and dosage methods of the various mycotoxins are reliable [25-26].

Ochratoxin A, fumonisin B1 and zearalenone were detected and quantified in all the maize samples regardless of the regions (Gbêkê, Poro, Hambol, Indénié-Djuablin and Gontougo) and the type of maize (grains, Cobs, spathes). The presence of several mycotoxins in corn samples has been reported by several authors worldwide and in Côte d'Ivoire [5,27-28]. However, the average concentrations are below the maximum authorized limits (OTA, 5 $\mu\text{g}/\text{kg}$; FB1, 2000 $\mu\text{g}/\text{kg}$; ZEA, 500 $\mu\text{g}/\text{kg}$) except those of OTA. A similar study by Hadjeba, [29] had already mentioned low concentrations (6.5 $\mu\text{g}/\text{kg}$ to 15.4 $\mu\text{g}/\text{kg}$) of FB1 in rice. Concerning OTA, average concentrations higher than 5 $\mu\text{g}/\text{kg}$ are obtained with maizes in spathes (Poro, Hambol, Indénié-Djuablin and Gontougo), in grains (Indénié-Djuablin and Gontougo) and in Cobs (Indénié-Djuablin and Gontougo). The work of Sangaré-Tigori *et al.* [12] mentioned high levels (3 $\mu\text{g}/\text{kg}$ to 1738 $\mu\text{g}/\text{kg}$) of OTA in maize taken from Abidjan markets.

All maize forms contain substandard samples. However, the high proportions (12-96%) were obtained with maize in spathes regardless of the mycotoxin. So the spathes seem to be poor protectors of the maize grains during storage although they are natural protectors of the cobs against insects. This situation is confirmed by the projection in principal component indicating that 3 of 4 samples strongly contaminated, in ochratoxin A, fumonisin B1 and zearalenone, are in spathes. According to the FAO [9], spathes constitute a barrier to the direct action of insecticide and fungicide treatments and to air-grain exchanges which take place during drying. Thus the shape of the maize is very decisive for the success of their storage. In addition, the mycotoxins were detected and quantified in all the regions visited. However, the regions of Hambol, Indénié-Djuablin and Gontougo have been severely affected by the multi-contamination as indicated by the principal component analysis. The forms of maize concerned are maize spathes (Poro, Hambol, Indénié-Djuablin and Gontougo) and maize cobs (Indénié-Djuablin). Niamketchi [28] had shown, in his study on storage equipment (granary), that post-harvest treatments had an impact on the level of mycotoxin contamination of maize. Other authors have indicated that all neglect in the post-harvest stages can lead to the appearance of poor quality grains [7,30].

However, maize from Gbêkê appears to be less contaminated regardless of the form of maize and the type of mycotoxin. These results could help encourage stakeholders in the maize sector, in the Gbêkê region, to better promote good production and storage methods. Thus, taking into account the carcinogenic, mutagenic, immunosuppressive and teratogenic characteristics of these mycotoxins demonstrated by IARC [14], this low contamination of maize could be a source of danger for Ivorian consumers. Because these mycotoxins (OTA, FB1 and ZEA) have been found in other foods, in particular cowpea [31], cocoa [32] and green coffee [33]. Thus, the search for alternative storage methods, as indicated by Niamketchi [28], could be a solution to the strong mycotoxin contamination of market maize.

4. CONCLUSION

In this study, the concentrations and frequencies of contamination of maize samples with ochratoxin A, fumonisin B1 and zearalenone were determined. The results showed the presence of these three toxins in almost all samples and in all study regions. However, the concentrations of FB1 and ZEA are below the maximum reference limits whatever the region and the form of the maize. The high frequencies of contamination were obtained in Indénié-Djuablin and Gontougo with OTA contents exceeding the normative value. The results of these regions show a need to monitor their productions. These contamination levels should motivate actions up stream, through the application of good agricultural practices, in order to subsequently reduce these different levels.

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