Original Research Article

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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 thisworkis to take stock of the level of ochratoxin A (OTA), fumonisin B1 (FB1) and zearalenone (ZEA) contamination in maizeproduced in Cote d'Ivoire in order to help improveitsquality.

Study Design: Maizesamples (375) weretaken in five producingdepartmentsRegions (Poro, Hambol, Gontougo, Gbêkê, Indénié-Djuablin).

Place and Duration of Study: the collection wascarried out on corn in grain, on the cob and in spathe fromFebruary 2016to January 2017. Then, the analyzeswerecarried out at theBiotechnologyLaboratory, Agriculture and Development of BiologicalResources of the Félix HOUPHOUËT-BOIGNYUniversity.

Methodology: Thedetermination of ochratoxin A, fumonisin B1 and zearalenonewascarried out according to the methods of regulation No. 401/2006/EC, AFNOR,Miraglia and Brera.

Results: Theresultsindicate the presence of ochratoxin A, fumonisin B1 and zearalenone in all forms of maize (grains, cob, spathes) and the five departmentsvisited.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 lowerthan the prescribedreference standards (2000 µg/kg; 500 µg/kg).For ochratoxin A, the average concentrations varyfrom 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.Samplesfrom the Regions of Poro, Gbêkê and Hambol have mean concentrations below the maximum referencelimit of 5µg/kg.Thus, variability in the sanitaryquality of

maizewasdemonstratedfrom one locality to another, regardless of the form of the maize.Based on the principal component analysis, spathes represent the form of maizemostprone to high contamination regardless of mycotoxin and department.

Conclusion: Theresearching alternative storagemethods and the right form of maizestoragecouldbe a solution to the high mycotoxin contamination of marketedmaize.

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

1. INTRODUCTION

In Cote d'Ivoire, maizeranksseventh in foodcrop production and second in cereal production afterrice[1-2].It is a staplefood for a large part of the Ivorian population. Unfortunately, the efforts to producethisfoodstuff are far from meeting the needs of the said population [3-5].Added to this are the conservation constraintswhichmostoftenreduce the quality and quantity of productions [3].

In fact, maizeissubject to severalbiotic and abioticconstraintsduringstorage, whichmakeitdifficult to preserve[6-7].Amongtheseconstraints, bioticfactors pose the greatestthreat to deterioratingmaizequality[8].One of the main bioticfactorsisfungal contamination becauseit can alter the marketability, health and nutritionalqualities of the grains. Fungal contamination can occurboth in the field and duringstorage.Thus, from germination to harvest, maizeisattacked by insectswhichcreate entry points thatfavorits contamination by phytopathogenicmolds.In addition, when post-harvestactivities, in particular drying and storage conditions, are inefficient, thereis an amplification of insectactivity and the development of moldsresponsible for the production of mycotoxins in stocks [8-10].Producedmainly by the genera *Aspergillus*, *Penicillium* and *Fusarium*, ochratoxins, fumonisins and zearalenone are groups of mycotoxinsthat affect maize[11-13].It isquiteobviousthattwo or more mycotoxins can coexist in the samefoodstuffincreasing the

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healthrisk for consumers.Indeed, the co-contamination of crops by thesetoxinsis a major concern due to the combinedrisks of exposure to the toxiceffects of thesetoxins[13-14].

A surveycarried out in three major maizeproducingRegions in Côte d'Ivoire revealedthat the majority of maizeproducers (97%) use traditionalstoragemethods.This studyalsoindicatesthatthesemethods expose stored grains to attack by mycotoxin-producingmoldssuch as ochratoxin A, fumonisin B1, and zearalenone[15].This studywasinitiated to assess the level of ochratoxin A, fumonisin B1 and zearalenone contamination in maizeproduced 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, itcould help improve the quality of maizeproduced and marketed in the Côte d'Ivoire.

2. MATERIAL AND METHODS

2.1.Material

2.1.1. Biologicalmaterial

The biologicalmaterialconsists of grain,cob and spathe of driedmaizefrom the North, East, Center, Center-North and North-East areasof Côte d'Ivoire.

2.1.2. Study sites

The sampleswerecollectedfrom the localities of Gbêkê (Center),Poro (North),Hambol (Center-North),Indénié-Djuablin(East)and Gountougo(Northeast).The specifics of theseRegionsweregiven by Bamba et *al.*[16].

2.2. Methods

2.2.1. Sampling

The sampleswerecollectedin 5Regions of production areas includingPoro, Hambol, Gontougo, Gbêkê, Indénié-Djuablin. Theise collection of sampleswerewascarried out on grain,cob and spathe maizefromFebruary 2016 to January 2017. A total of 375 sampleswerecollected for

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eachform of maize (125 on grain, 125 on cob and 125 on spathe, Table 1).Then, the receivedsampleswere sent to the laboratory in order to determine contamination level in ochratoxin A, aflatoxins and zearalenone.

Table 1 :Number of samplescollectedaccording to maizevariety 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

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2.2.2. Determination of water activity of maize

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The water activity of the maizeweredetermined as reported by Bamba et *al.*[16].

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2.2.3.Determination of mycotoxins

Extraction ofochratoxin A

The entiresamplewascrushed in a hammermill to obtain a homogeneous fine grind. In a Nalgene jar containing 15 g of homogenate, 150 mL of aqueousmethanol-bicarbonate 1% (m/v; 50:50) wereadded. The mixture washomogenized by Ultra-Turax for 3 minutes and the homogenatewascentrifuged at 5000 rpm for 5 minutes at 4°C. The supernatantwasfilteredthroughfilterpaperinto tubes of 25 mL. To 11 mL of filtratewereadded 11 mL of saline phosphate buffered (PBS) at pH 7.3. Immunoaffinitycolumns brand Ochraprepwereconditionedwith 10 mL of PBS. Purification of 20 mL of the mixture was made on immunoaffinitycolumns and OTA extraction wasperformedwithtwo volumes of 1.5 mL of solvent (methanol/aceticacid; 98:2; v/v) at a flow rate of 5 mL/minute. The

resultingsamplewaspacked in a chromatographic tube and the analysis for OTA was made by HPLC using the Europeancommunityregulation[17].

Extraction of fuminosin B1

Twenty-five grams of maizesamplewereextractedwith 50 mL of water blending for 2 min with a hammermill blender. At five grams of groundmaize, 25 mg of NaClwereadded and the mixture wasshaked on a horizontal mechanical shaker for 120 minutes at 300 rpm, and thencentrifuged for 15 minutes at 2500 g. The supernatantwasrecovered and degreased by 4 mL of hexane. The organic phases wereremoved by centrifugation for 5 minutes at 2500 g. The aqueous layer wasrecovered and dilutedwith 16 mL of phosphate buffered saline (PBS) at pH 7.3, filteredthroughWhatman No. 4 filterpaper and thenapplied to a columnimmunoaffinityFumoniprep (R BiopharmRhone Ltd, Glasgow, Scotland) at a flow rate of 1–2 drops/s. The columnwaswashedwith 10 mL of the same buffer to 1-2 drops/s for removal of residues. Fumonisin B1 waselutedwith 1.5 mL of methanol (HPLC grade) and then 1.5 mL of water.Theeluatewascollected and evaporated, protectedfrom light in a nitrogenstream. The dry extractwastaken up in 200 µL acetonitrile/water (50: 50, v/v) and thensonicated for 5 minutes. Then, 50 µL of extractwasdilutedinto 50 µL of a solution of ortho-phthalaldehyde (OPA 40 mg, 1 mLmethanol, 5 mL of 0.1 M sodium tetraborate and 50 µL of 2- mercaptoethanol). The resultingsamplewaspacked in a chromatographic tube and the analysis of FB1 was made by HPLC using AFNOR methods[18-19].

Extraction of Zearalenone

Twenty-five grams of maizesamplewereextractedwith 50 mL of 125 mL of acetonitrile: water (94:31) blending for 2 min with a hammermill blender. After filtration throughWhatman No 4 filterpaper, 20 mL of the filtrateweredilutedwith 80 mL of double distilled water. Then, 25 mL of the dilutedfiltratewasapplied to an immunoaffinitycolumn (Easi-Extract® zearalenone,

R-BiopharmRhone Ltd, Glasgow) containing a monoclonal antibodyspecific for the zearalenone. The columnwaswashedwith 10 mL of double distilled water. Zearalenonewaseluted by applying 1.5 mL of methanol. The eluatewasdilutedwith 1.5 mL of bidistilled water and mixed by vortexing. The resultingsamplewaspacked in a chromatographic tube and the analysis of ZEA was made by HPLC using the method of AOAC [20] and Miraglia and Brera [21].

OTA, FB1 and ZEN quantification

Quantification of OTA, FB1 and ZEA contents wasachievedwith high performance liquidchromatographycolumn, using a Shimadzuliquidchromatograph (Kyoto, Japan) fittedwith fluorescence detector. The operating conditions are described in Table 2.

Table 2 :HPLC analytical conditions

ITEM	Ochratoxin A	Fuminosin B1	Zearalenone
Pre-column	Shim-pack GVP-ODS 10 x 4,6 mm		
Column	Shim-pack GVP-ODS, 250 mm x 4,6 mm		
Detector	Fluorescence, λexcitation : 330 nm, λémission : 460 nm	Fluorescence, λexcitation : 335 nm, λémission : 440 nm	Fluorescence, λexcitation : 274 nm, λémission : 440 nm
Mobile phase	Acetonitrile/Water / Aceticacid (99/99/2)	Acetonitrile/Water (50/50)	Acetonitrile/Water/Methanol (46/46/8)
Injectvolume	100 µl	100 µl	100 µl

Flow rate	1 mL/min in isocratic		
Columntemperature	40 °C		
Rising solvent	Acetonitrile		
Analysis duration	12 minutes	6 minutes	9.5 minutes

Validation of the ochratoxin A, fumonisinB1 and zearalenonemethodanalysis

The method validation wasconductedusing the method of the French Association for Standardization[22]. This procedureincludes 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 testingaccuracy (0,10 ng/kg, 4,5 ng/kg, 10 ng/kg et 20 ng/kg). The referencematerialwasused to compare the concentration of OTA obtainedat the certified value.

2.2.4. Statisticalanalysis

The tests werecarried out in triplicate and the averageswerecalculated in order to assess the level of ochratoxin A, fumonisin B1 and zearalenone contamination in the samples.The homogeneity of the meanswasassessedusing 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 ochratoxin A,fumonisin B1 and zearalenone in the variouslocalities.Finally, the correlationbetween the data and the sampleswasestimatedusing 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 greaterthan 0.65 and range from 0.78±0.01 to 0.93±0.03 for maize grains.Theyvaryfrom 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.Statisticalanalysisrevealed the presence of a significantdifference (p=0.001) between the means.Samplesfrom the Gbêkê and PoroRegions show low water activitywhilethosefromIndénié-Djuablinand Gontougo show high values.

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

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

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3.1.2. Validation of determinationmethod of OTA, FB1 and ZEA

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The Pearson coefficients (R^2) determined for the linearityvarybetween 0.98 and 0.99.The detectionlimits 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 varybetween 0.26% and 3.75% for the repeatability tests and between 0.89% and 5.67% for the reproducibility tests.The extraction yieldsobtained are 86.92±0.39%, 82.90±2.19% and 93.20±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	Ochratoxin A	Fumonisin B	Zearalenone
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

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3.1.3. OTA concentrations in samples

The contamination levels of ochratoxin A, of the differentforms of maize, are reported in Table 5.OTA concentrations are between 0.83±0.07 µg/kg and 14.38±2.22 µg/kg for maize grains.The Fischer test indicates the presence of a significantdifference (p=0.001) between the contents. Low levels of OTA have been detected in the Regions of Poro, Gbêkê and Hambol.These contents are lessthan 5µg/kg, maximum referencelimit. Levels abovethisnorm are observed at Gontougo and Indénié-Djuablinwith a contamination frequency of 60% and 32% respectively.

Concerning the epis, the concentrations obtainedvarybetween 1.92±0.64 µg/kg and 18.60±2.16 µg/kg.Statisticalanalysisrevealed a significantdifference (p = 0.001) between the means. Average OTA concentrations below the maximum referencelimit are observed at Gbêkê,PoroandHambol. The proportions of sampleswith OTA contents greaterthan 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 higherthan 5 µg/kg exceptthosecomingfrom the Region of Gbêkê.Theseaverage concentrations varyfrom 2.21±0.84 µg/kg to 134.89±9.10 µg/kg.The analysis of variance indicated the presence of a significantdifference (p = 0.001) between the rates. The low and high concentrations are obtainedrespectively at Gbêkê and Indénié-Djuablin.In addition, concentrations above 5 µg / kg have been determined in all areas and the proportions varybetween 20% and 96%.

Table 5 : Average concentrations of OTAandproportion of non-compliant sampleswith the standardaccording to the Regions and type of maize

OTA CONCENTRATIONS (µg/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

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Gbêkê	0	0	20
Poro	0	0	60
Hambol	0	0	96
Indénié-Djuablin	32	60	96
Gontougo	60	32	92
MAC (µg/kg)		5	

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

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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 clearvariability of the average concentrations isobserved as wellbetween the forms of corn as the departments.The average concentrations are all lowerthan 2000 µg/kg whatever the department and the type of maize. Theyvarybetween 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 statisticalanalysis, the Region of Indénié-Djuablin has high levels of fumonisin B1 regardless of the maize type, while the Regions of Poro and Gbêkê have lowlevels.

No sample of maize grains has a fumonisin B1 content above the standard of 2000 µg/kg.On the other hand, the proportions of maizeCobs samples, which do not complywith the standard, varyfrom 8% to 28% and the Regionsconcerned are Hambol, Indénié-Djuablin and Gontougo.Regardingmaize in spathe, the proportions of non-compliance varybetween 12% and 32% and all Regions are concerned.

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

FB1 CONCENTRATIONS (µg/kg)					
Regions	**Grains**	**Cobs**	**Spathes**	**F_{-value}**	**P_{-value}**
Gbêkê	172.46±10.96^{cB}	153.43±19.79^{eB}	476.21±13.03^{eA}	2.51	0.001
Poro	27.46±2.70^{eC}	374.98±14.00 ^{dB}	639.60±16.20^{dA}	7 .49	<0.001
Hambol	136.28±18.19^{dC}	488.22±14.05^{cB}	1226.26±19.44^{bA}	15.61	<0.001
Indénié-Djuablin	1023.73±28.50^{aB}	1206.46±20.00^{aB}	1999.22±24.03^{aA}	7.42	<0.001
Gontougo	802.73±20.50^{bB}	710.39±11.75^{bB}	1177.82±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 (µg/kg)		2000	

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

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3.1.5. Zearalenone content in samples

Averagezearalenone concentrations are all below 500 µg / kg, the maximum referencelimit.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).Statisticalanalysisindicatesthat the high average concentrations are obtained in the Regions of Poro (grains) and Gbêkê (ears, spathes) while the strongones are obtained in Gontougo (grains) and Indénié-Djuablin (Cobs, spathes).

All the samplestaken 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 fromIndénié-Djuablin (Table 8).

Table 7 : Average concentrations of ZEA and proportion of non-compliant sampleswith the standardaccording to the Regions and type of maize

ZEA CONCENTRATIONS (µg/kg)					
Regions	**Grains**	**Cobs**	**Spathes**	**F_{-value}**	**P_{-value}**
Gbêkê	$23.00±2.25^{cC}$	$38.61±4.02^{eB}$	$94.54±5.50^{eA}$	4.09	<0.001
Poro	$8.48±0.25^{dC}$	$86.68±7.17^{dB}$	$120.09±10.02^{dA}$	7.50	<0.001
Hambol	$22.81±1.75^{cC}$	$115.11±10.02^{cB}$	$255.70±8.30^{bA}$	19.60	<0.001
Indénié-Djuablin	$145.95±8.50^{bB}$	$234.87±12.05^{aB}$	$341.84±9.15^{aB}$	8.66	<0.001
Gontougo	$173.10±7.75^{aA}$	$165.10±9.75^{bA}$	$226.07±8.20^{cB}$	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 (µg/kg)		500	

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

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3.1.6. Variability of contamination of differentforms of maize

Figure 1 shows thatanalysis of F1 and F2 factors of main components reveal 99.34% of total variability of studiedparameters. The F1 factor with a proper value of 2.74 expresses 91.34% of total variabilitywhile F2 factor withproper value of 0.24reveals8.00%. The projection of analysed variables in factorial design F1-F2 shows strongnegativecorrelationbetweenochratoxin A, zearalenone and fumonisin B1content of maizewiththe F1 factor (Figure 1). Based on the projection of samples in the same design, they are organised in two groups. Group 1 iscomposed of fourindividualspresentinghigh ochratoxine A, zearalenone and fumonisin B1 contents. It deals with spathes and cobs maizecomingfromHambol, Indénié-Djuablinand Gontougo. Group 2 includesindividualshavinglowochratoxin A, zearalenone and fumonisin B1 content. It deals with spathes (Gbêkê, Poro), Cobs (Gbêkê, Poro, Gontougo and Hambol) and grains (Poro, Gbêkê, Gontougo, Indénié-Djuablin and Hambol)maize (Figure 1).

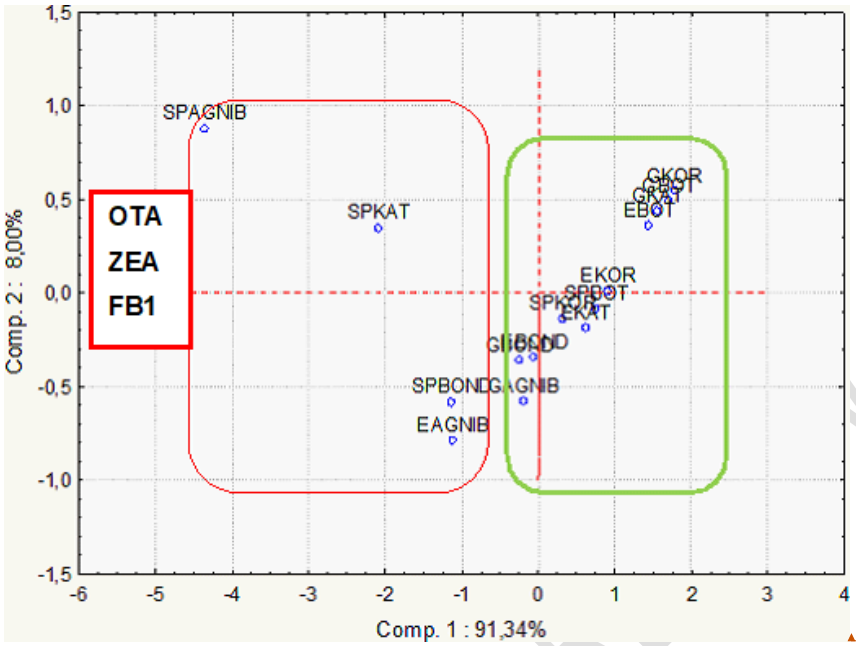

Figure. 1. Projection of ochratoxin A, zearalenone, and fumonisin B1 content and the individuals of grains, epis and spathes of maize in factorial plan 1-2 of the analysis of main components

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 higherthan 0.65 whatever the Region and the form of corn.This parameterrefers to the availability of water in the food.According to Adebajoet *al.*[23], whatever the nature of the food, no microorganism can develop at a water activitybelow 0.65.On the other hand, according to the AFSSA [13]and the Codex Alimentarius [24], toxigenicmolds can develop in all climates, on all solid or liquid supports as soon as there are nutrients, humidity and water activitygreaterthan 0.65.Because the availability of water is one of the important factors in the growth of mold and the production

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of mycotoxins.Thusthesestrong water activities, observed in the differentforms of maize and production Regions, couldbe 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 differentmethods for determiningochratoxin A, fumonisin B1 and zearalenone in maizesamples.All the Pearson coefficients (R^2) determined are close to 1, whichindicates a good correlationbetween 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, whichindicates good sensitivity of the dosingdeviceused.In addition, the coefficients of variation determined for repeatability and reproducibility are all lessthan 6% and all the extraction rates are greaterthan 82% regardless of the mycotoxinconsidered.This situation indicatesthat the extraction and dosage methods of the variousmycotoxins are reliable [25-26].

Ochratoxin A, fumonisin B1 and zearalenoneweredetected and quantified in all the maizesamplesregardless of the regions (Gbêkê, Poro, Hambol, Indénié-Djuablin and Gontougo) and the type of maize (grains, Cobs, spathes).The presence of severalmycotoxins in corn samples has been reported by severalauthorsworldwide and in Côte d'Ivoire [5,27-28].However, the average concentrations are below the maximum authorizedlimits (ochratoxin A, 5 µg/kg; fumonisin B1, 2000 µg/kg; zearalenone, 500 µg/kg) exceptthose of ochratoxin A.A similarstudy by Hadjeba, [29]hadalreadymentionedlow concentrations (6.5 µg/kg to 15.4 µg/kg) of fumonisin B1 in rice.Concerningochratoxin A, average concentrations higherthan 5 µg/kg are obtainedwithmaizes 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 µg/kg to 1738 µg/kg) of ochratoxin A in maizetakenfrom Abidjan markets.

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All maizeformscontain substandard samples. However, the high proportions (12-96%) wereobtainedwithmaize in spathes regardless of the mycotoxin.So the spathes seem to bepoorprotectors of the maize grains duringstoragealthoughthey are naturalprotectors of the cobs againstinsects.This situation isconfirmed by the projection in principal component indicatingthat 3 of 4 samplesstronglycontaminated, 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 fungicidetreatments and to air-grain exchanges whichtake place during drying.Thus the shape of the maizeisverydecisive for the success of theirstorage.In addition, the mycotoxinsweredetected and quantified in all the regions visited. However, the regions of Hambol, Indénié-Djuablin and Gontougohave been severelyaffected by the multi-contamination as indicated by the principal component analysis.The forms of maizeconcernedare maize spathes (Poro, Hambol, Indénié-Djuablin and Gontougo) and maize cobs (Indénié-Djuablin). Niamketchi[28]hadshown, in hisstudy on storageequipment (granary), that post-harvesttreatmentshad an impact on the level of mycotoxin contamination of maize.Otherauthors have indicatedthat all neglect in the post-harvest stages can lead to the appearance of poorquality grains [7,30].

However, maizefromGbêkêappears to lesscontaminatedregardless of the form of maize and the type of mycotoxin.Theseresultscould help encourage stakeholders in the maizesector, in the Gbêkê region, to betterpromote good production and storagemethods.Thus, takingintoaccount the carcinogenic, mutagenic, immunosuppressive and teratogeniccharacteristics of thesemycotoxinsdemonstrated by IARC [14], thislow contamination of maizecouldbe a source of danger for Ivorianconsumers.Becausethesemycotoxins (OTA, FB1 and ZEA) have been found in otherfoods, in particularcowpea[31], cocoa[32] and green coffee [33].Thus, the search for

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alternative storagemethods, as indicated by Niamketchi[28], couldbe a solution to the strongmycotoxin contamination of marketmaize.

4. CONCLUSION

In thisstudy, the concentrations and frequencies of contamination of maizesampleswithochratoxin A, fumonisin B1 and zearalenoneweredetermined.The resultsshowed the presence of thesethreetoxins in almost all samples and in all study regions.However, the concentrations of FB1 and ZEA are below the maximum referencelimitswhatever the region and the form of the maize.The high frequencies of contamination wereobtained in Indénié-Djuablin and Gontougowith OTA contents exceeding the normative value.The results of theselocalities show a need to monitor their productions. These contamination levelsshouldmotivate actions upstream, through the application of good agricultural practices, in order to subsequentlyreducethesedifferentlevels.

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